Global Change and Land Use Effects on Carbon Turnover in Tidal Wetlands

Dissertation

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Peter Müller, M.Sc.

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Erstgutachter: Prof. Dr. Kai Jensen

Zweitgutachter: Prof. Dr. Lars Kutzbach

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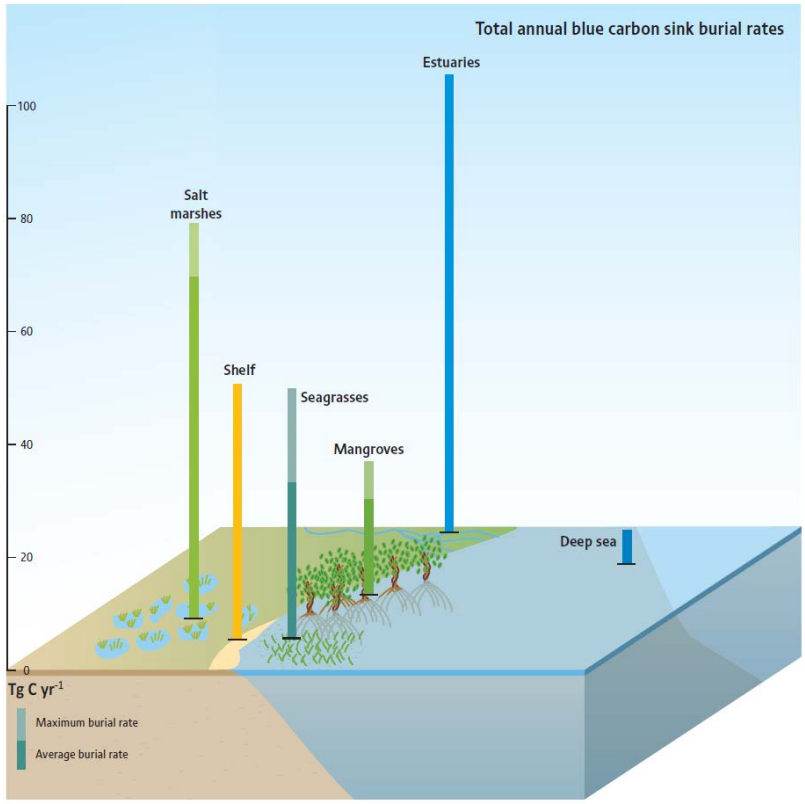
1 General introduction

Tidal wetlands, such as mangroves and marshes, are among the most threatened and vulnerable ecosystems on Earth, experiencing dramatic loss due to direct anthropogenic impacts, such as conversion to agricultural land or aquaculture ponds, urbanization, and deforestation (Green and Short 2003, Duarte et al. 2008, Gedan et al. 2009), and accelerated sea level rise (SLR) (Craft et al. 2009, Crosby et al. 2016). For instance, during the last century about 25% of the total global salt-marsh area and up to 50% of the mangrove area have been lost (Mcleod et al. 2011). Furthermore, 20-45% of the current salt-marsh area is predicted to be lost to open water through accelerated SLR until the end of the century following mean and maximum estimates of SLR by the *Intergovernmental Panel on Climate Change* (Craft et al. 2009, IPCC 2013).

At the same time, tidal wetlands provide a wide array of critical ecosystem services, making them some of the most economically important ecosystems, that are valued at approximately US$ 10,000 per hectare (Barbier et al. 2011, Kirwan and Megonigal 2013). Tidal wetlands support coastal fisheries by serving as reproductive habitats and nursery grounds for juvenile fish (Boesch and Turner 1984, Zimmerman et al. 2000, MacKenzie and Dionne 2008), and they provide feeding grounds for livestock by sustaining high rates of primary production (Bakker et al. 2002, Yang et al. 2008, Gedan et al. 2009, Di Bella et al. 2014). Tidal wetlands contribute to coastal protection through wave attenuation and erosion control via sediment stabilization (Feagin et al. 2009, Moeller et al. 2014). Furthermore, tidal wetlands purify coastal and riverine waters, retaining pollutants and nutrients (Mitsch and Gosselink 2010). However, the recently most high-lighted ecosystem service of tidal wetlands is probably the sequestration of carbon (C) through burial and preservation of organic matter in soils (Chmura et al. 2003).

The preservation of organic matter in soils is not only relevant for C sequestration, it is also the primary process for many tidal wetlands to gain elevation and keep pace with rising sea level (Kirwan and Megonigal 2013). The surface elevation of a tidal wetland system is affected by the different components of soil volume, namely mineral matter, organic matter, and pore space. However, due to its lower density, organic matter contributes 2–5 times more to soil

volume than mineral matter of the same mass (Neubauer 2008, Kirwan and Megonigal 2013). Organic matter preservation or C sequestration is therefore a key ecosystem service that maintains ecosystem stability and thereby facilitates the collectivity of other ecosystem services provided by tidal wetlands.



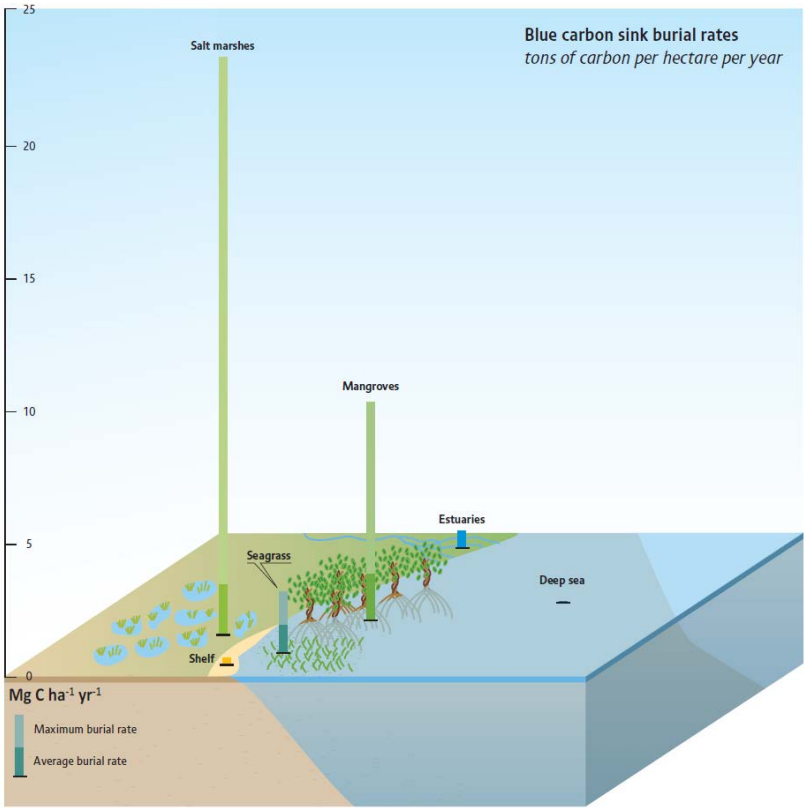


Figure 1 [A] Total annual C sequestration in blue C and associated systems; data from Cebrián and Duarte (1995) and Duarte et al. (2005)

[B] Rates of C sequestration in blue C and associated systems; data from Cebrián and Durate (1995) and Duarte et al. (2005), modified after Nellemann et al. (2009)

Vegetated coastal ecosystems as hot spots of C sequestration

In the context of climate change mitigation, the UN framework of convention on climate change (UNFCCC) describes C sequestration as "the process of removing C from the atmosphere and depositing it in a reservoir" (IPCC 2013). In the same manner, ecosystems can contribute to climate change mitigation by assimilating CO_2 from the atmosphere and transforming it to organic matter that is stored and cycled in biomass, soils, and sediments. Depending on the residence time of C in the ecosystem – the inverse of the C turnover rate – C can be removed from the atmosphere and stored and cycled in different organic matter pools of the ecosystem for periods between hours and millennia (Megonigal et al. 2004, Mcleod et al. 2011). While the C turnover within the biomass pool can be rapid (9 years mean residence time of C in plant biomass across biomes; Schlesinger 1997), soils and sediments yield the potential to store C over centuries and millennia (Duarte et al. 2005, Limpens et al. 2008).

The C sequestered in the soils and sediments of marshes, mangroves, and seagrass beds has been termed "blue C". These vegetated coastal ecosystems occupy only <0.5% of the ocean's surface, however, account for ~50% of the total marine C sequestration (Duarte et al. 2013; Figure 1A). As a comparison, long-term C sequestration (millennial time scale) in blue C systems is with 84-234 Tg C yr^{-1} roughly as high as in the entity of terrestrial forest ecosystems (181 Tg C yr^{-1}). On an area basis, long-term C sequestration in blue C systems is >10 times higher than in terrestrial ecosystems, with marshes and mangroves characterized by the highest mean sequestration rates of ~220 g C m^{-2} yr^{-1} (Mcleod et al. 2011; Figure 1B). Tidal wetlands thereby act as powerful C sinks and exert a strong influence over the global C cycle.

Mechanisms of long-term C sequestration in tidal wetlands

Rates of C sequestration in tidal wetlands exceed those of most other ecosystem types because slow rates of organic matter decomposition slow down C turnover and co-occur with high rates of organic matter input through both autochthonous primary production and import of allochthonous organic matter. Slow C turnover under water-saturated and anoxic conditions (Freeman et al. 2001, Megonigal et al. 2004) is a feature that tidal and non-tidal wetlands share. However, in contrast to non-tidal wetlands (i.e. bogs, fens), C sequestration in tidal wetlands is driven by the constant burial of organic matter through SLR (Bridgham et al. 2006). Furthermore, tidal wetlands are among the most productive ecosystems on Earth. Net primary production (NPP) of tidal wetlands is comparable to tropical-rainforest NPP and commonly exceeds 1000 g C m^{-2} yr^{-1} (Duarte and Cebrián 1996, Huston and Wolverton 2009, Duarte et al.

2013). In comparison, average NPP of *Sphagnum*, the dominant plant species of northern peatlands, is only ~150 g C m^{-2} yr^{-1} (Gunnarsson 2005; converted from [g dry mass m^{-2} yr^{-1}], factor = 0.58).

Additionally to the high rates of autochthonous plant production, organic matter input into tidal wetlands can be strongly increased by external or allochthonous organic matter inputs (Middelburg et al. 1997, Bouillon et al. 2003). The proportion of allochthonous vs. autochthonous contributions to the total organic matter pool varies considerably across sites and ecosystem types. For instance, organogenic, peat-forming tidal wetland systems (e.g. *Spartina* marshes on the US East coast; also marshes of the Baltic Sea) may experience only negligible inputs of allochthonous organic matter, whereas soil organic matter of minerogenic systems (e.g. temperate European salt marshes of the Atlantic coast) easily consists of >20% allochthonous materials (Middelburg et al. 1997, Boschker et al. 1999, Allen 2000). By sequestering C from both internal and external sources, tidal wetlands provide a C sink for a larger area than they occupy (Mcleod et al. 2011).

Aerobic vs. anaerobic decomposition in tidal wetland soils

Because oxygen (O_2) diffusion in water is almost 10,000 times slower than in air (e.g. Cussler 1984), O_2 availability for aerobic decomposition is both spatially and temporally variable in tidal wetland soils and may depend on the mean water-table depth or relative elevation, the inundation frequency and time, the drainage capacity and porosity of the soil, and the amount of organic matter input and decomposition rate (Boelter 1969, Seybold et al. 2002, Sundby et al. 2003, Megonigal and Rabenhorst 2013, Kirwan et al. 2013).

A wide held assumption in wetland ecosystem ecology is that flooding, water-logging, and consequently anaerobia leads to slow decomposition (Davidson and Janssens 2006). Clearly, there is abundant evidence to support this notion, including the fact that tidal wetlands store vast amounts of soil organic matter (SOM) (Chmura et al. 2003, Mcleod et al. 2011), and the fact that anaerobic conditions can suppress decomposition (Freeman et al. 2001). However, the difference in decomposition rate between aerobic and anaerobic conditions certainly depends on a number of other factors, such as availability of alternative (other than O_2) terminal electron acceptors (TEAs) or type and quality of the organic matter. For instance, the decomposition of lignin, a terrestrial derived, structural, and recalcitrant organic polymer, is indeed slower and incomplete in the absence of O_2 (Colberg and Young 1982, Benner et al. 1984, 1985, 1986, Young and Frazer 1987). Marine sediments, however, experience higher

inputs of less structural and recalcitrant organic matter than terrestrial systems. For these systems, Cowie and Hedges (1993) could show that O_2 concentrations indeed have little effect on the decomposition rate of deposited materials. Generally, the decomposition of aged and relatively recalcitrant organic matter can be several-fold faster under aerobic conditions, while fresh and more labile organic matter decomposes equally fast in anaerobic and aerobic conditions (Kristensen et al. 1995, Hulthe et al. 1998).

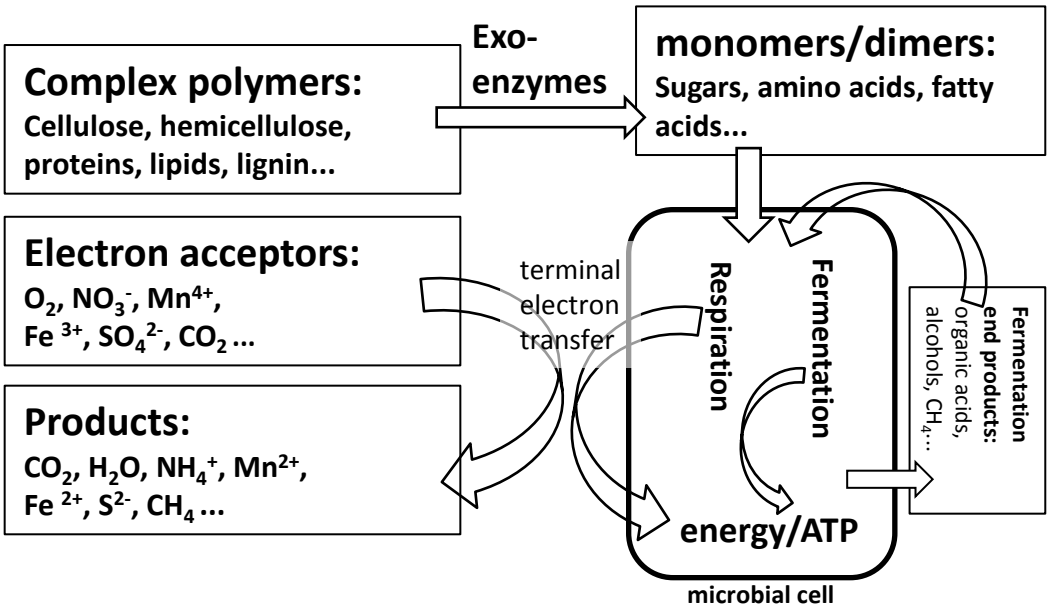


Figure 2 Schematic diagram of microbial organic matter decomposition Modified after Reddy and DeLaune (2008)

Aerobic and anaerobic decomposition share the first and rate-limiting step of decomposition, which is the degradation of organic polymers by extracellular (exo) enzymes into dimers or monomers (Figure 2) because larger molecules (>600 Da) cannot be taken up by the microbial cell (Weiss et al. 1991). The main difference between aerobic and anaerobic decomposition of organic matter is probably the high energy yield of aerobic metabolism, which allows single microorganisms to decompose and completely oxidize complex organic matter to CO_2, while this is not possible under anaerobic conditions (Megonigal et al. 2004). Anaerobic decomposition of organic polymers requires a series of steps conducted by different and often specialized groups of a larger microbial consortium. The potential free energy of the original substrate is thereby shared in several steps by several participating organisms during

this syntrophic process. For instance, not all anaerobic microorganisms produce exo-enzymes to break down organic polymers, and consequently they strongly rely on substrates provided by other microorganisms during decomposition (Megonigal et al. 2004). After cell uptake of organic monomers and dimers, primary and secondary fermentation steps often follow under anaerobic conditions (Figure 2).

Table 1 Thermodynamic sequence for reduction of inorganic substances as TEAs by organic matter[a]. Presented are values for the redox potential of the reactions (Eh in mV) and Gibbs free energy (ΔG)

Reaction	Eh [mV]	ΔG
Reduction of O_2		
$O_2 + 4H^+ + 4e^- <=> 2H_2O$	812	-29.9
Reduction of NO_3^-		
$2NO_3^- + 6\ H^+ + 6e^- <=> N_2 + 3H_2O$	747	-28.4
Reduction of Mn^{4+} to Mn^{2+}		
$MnO_2 + 4H^+ + 2\ e^- <=> Mn^{2+} + 2H_2O$	526	-23.3
Reduction of Fe^{3+} to Fe^{2+}		
$Fe(OH)_3 + 3H^+ + e^- <=> Fe^{2+} + 3H_2O$	-47	-10.1
Reduction of SO_4^{2-} to H_2S		
$SO_4^{2-} + 10H^+ + 8\ e^- <=> H_2S + 4H_2O$	-221	-5.9
Reduction of CO_2 to CH_4		
$CO_2 + 8\ H^+ + 8\ e^- <=> CH_4 + 2\ H_2O$	-244	-5.6

a) Units are kcal mol^{-1} e^{-1} assuming coupling to the oxidation reaction 1/4 CH_2O + ¼ H_2O → ¼ CO_2 + H^+ + e^-. ΔG = -RT ln (K) at pH 7.0 and 25°C. **Source: Schlesinger 1997**

Fermentation is an intracellular process that involves organic molecules as both electron donors and acceptors. Although fermentation yields little energy compared to the final respiratory steps of anaerobic decomposition (Table 1), it is a crucial part in the total process of anaerobic organic matter decomposition as the majority of non-fermentative microorganisms cannot use the initial substrates generated by exo-enzymic break down of polymers. At the same time, as fermentation is inhibited by its end-products (organic acids and alcohols), fermenters rely on the consumption of their end-products by non-fermentative microorganisms (Hansson and Molin 1981, Megonigal et al. 2004). During the final steps of organic matter decomposition, electrons are shuttled from an electron donor (organic matter) to a TEA. When

O_2 as TEA is not available, decomposition relies on anaerobic microbial metabolism and alternative TEAs (Table 1).

Alternative TEAs are abundant in tidal wetland soils; however, depending on the chemical species that are available as alternative TEAs, the energy yield of anaerobic metabolism is considerably lower than that of aerobic metabolism (Table 1). The energy yield of a reaction is determined by the affinity of a TEA to acquire electrons (and thus get reduced) and is reflected in the redox potential, which describes the balance between abundance and strength of electron donors and electron acceptors (Eh; Table 1). While nitrate reduction can provide a similar energy yield than O_2 reduction, the reduction of other relatively abundant TEAs provides considerably less energy and declines in the order of manganic manganese (MnIV) reduction, > ferric iron (FeIII) reduction, > sulfate reduction, and > carbon dioxide reduction (Table 1).

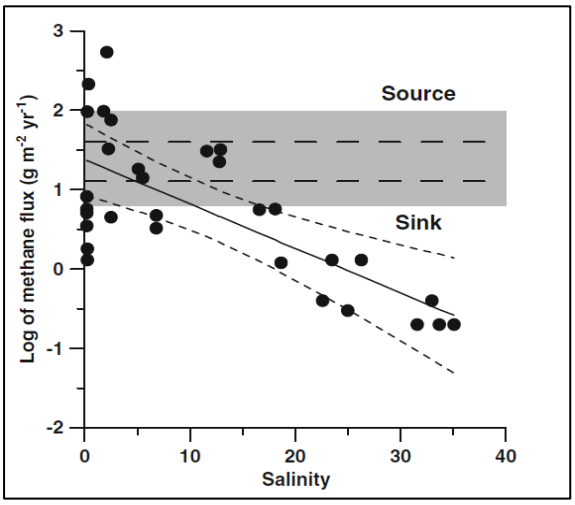


Figure 3 Tidal marsh CH_4 emissions versus salinity. The curve is a linear fit of salinity against log-transformed CH_4 emission data with 95% confidence intervals (fine dashed lines). The horizontal gray band represents the CH_4 emission equivalents of the 5 and 95% quantiles of tidal marsh carbon sequestration rates reported by Chmura et al. (2003); the horizontal dashed lines are the 25 and 75% quantiles of this data set (CH_4 equivalence based on a global warming potential of 25) Source: Poffenbarger et al. (2011)

The availability and abundance of alternative TEAs strongly affects the energy flow in tidal wetland soils, at the same time, however, it also has a strong influence on the production of greenhouse gases (GHGs), thereby affecting the net ecosystem effect on radiative forcing. For instance, stepwise anaerobic nitrate (NO_3^-) reduction to molecular nitrogen can leak nitrous oxide (N_2O), a powerful GHG with a global-warming potential (GWP) over 100 years of 263-times that of CO_2 (Neubauer and Megonigal 2015). In turn, the availability of sulfate (SO_4^{2-}), which has a high abundance in seawater, can efficiently suppress the production of the strong GHG methane (CH_4) in brackish and saline tidal wetland systems. Because sulfate reduction is energetically more favorable than methanogenesis (Table 1), mathanogens are outcompeted

when sulfate concentrations are high enough. In fact, from a GWP perspective, the salinity of a tidal wetland (as proxy for sulfate availability) allows to estimate whether a system acts as a net sink or a net source for GHGs (Figure 3). Based on the CH_4-CO_2 balance of tidal marshes, Poffenbarger et al. (2011) suggest that systems with a porewater salinity ≥18 are likely to act as net GHG sinks, systems with salinity of 10-18 either act as net sink or source of GHGs, and systems with a salinity ≤10 often act as net GHG sources despite high rates of CO_2 uptake and sequestration.

Plant controls on decomposition processes

Primary producers naturally affect decomposition by supplying the microbial substrate as litter or rhizodeposits, such as root exudates. For instance, nitrogen and lignin contents of plant litter can exert strong control over decomposition rate across terrestrial ecosystems (Prescott 2010) and in tidal wetlands (Hemminga and Buth 1991). A related point is the tight temporal coupling between assimilation, root exudation, and CH_4 production in wetland soils (Minoda and Kimura 1994, Megonigal et al. 1999, 2004).

However, besides determining the quality and quantity of the organic matter input as microbial substrate, certain plant-physiological and morphological traits can also affect the decomposition of other organic matter pools such as SOM. Plants deliver labile organic compounds to the rhizosphere, where they stimulate microbial activity and lead to accelerated mineralization of recalcitrant SOM, a phenomenon known as rhizosphere priming effect, or short: priming (Blagodatskaya and Kuzyakov 2008). Besides positive priming effects, SOM decomposition can also be reduced in the rhizosphere and lead to negative priming. This can be induced by preferential microbial utilization of the rhizodeposits without stimulating also the mineralization of SOM (Kuzyakov 2002). While substrate-induced (positive) priming is a well-established concept in terrestrial systems, the few studies on plant stimulation of SOM decomposition in wetland systems are focused on the effects of greater TEA availability inside vs. outside rhizospheres. Vascular wetland plants can support microbial respiration in their rhizosphere by supplying TEAs via root oxygen loss (ROL) to water-saturated and otherwise anaerobic soil environments (Figure 4).

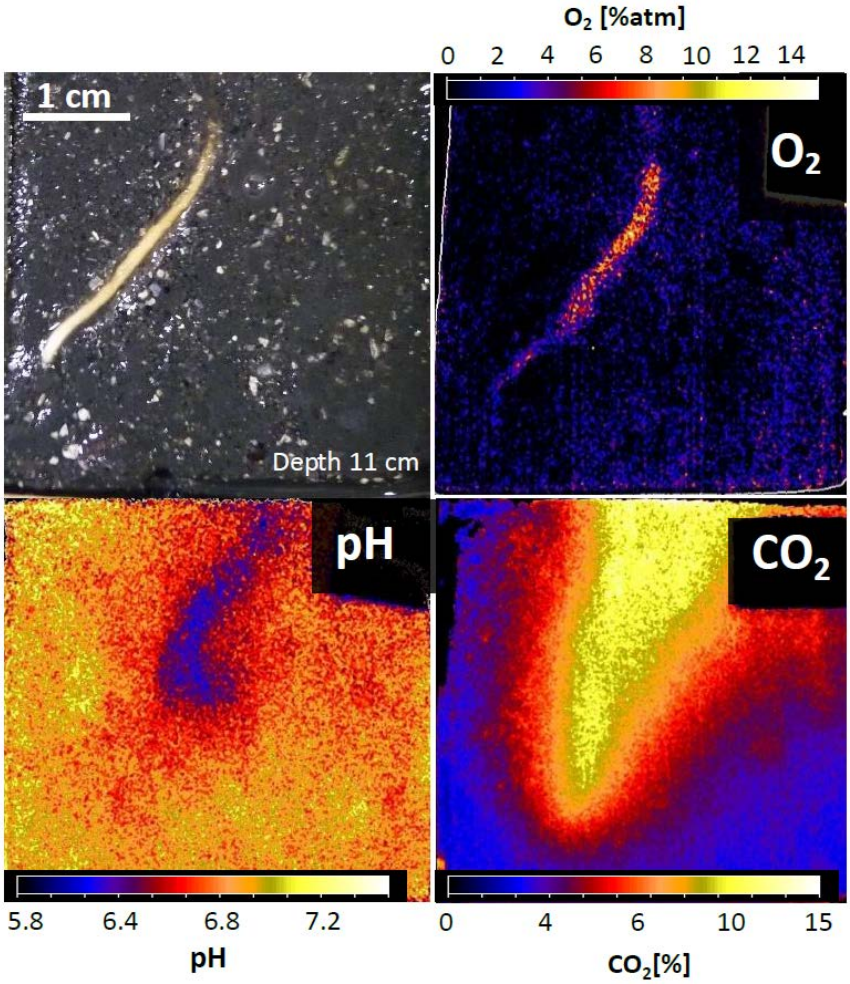


Figure 4 Spatial variation of O_2, pH, and pCO_2 around a single root of *Spartina anglica*. Oxygen release, CO_2 enhancement, and pH decline is clearly visible in the vicinity of the root. Optode images of the spatial distribution of O_2, pH, and pCO_2 in a 4x4 cm area around a single root. Position of root (photo) may change slightly between optode images due to root growth and movement during exchange of optode foils. Measurements were conducted using a novel planar-optode system (VisiSens TD; PreSens, Precision Sensing GmbH, Regensburg, Germany). The VisiSens TD system is a ratiometric fluorescence imaging-based optode system with integrated hardware for image acquisition and software (VisiSens Analytical) for image processing.

K. Koop-Jakobsen and P. Mueller, in prep.

Global change and land use effects on C turnover in tidal wetlands: thesis outline

Organic matter production and decomposition are the two most important factors determining the rate at which C accumulates in tidal wetlands (Kirwan et al. 2013), and changes in their balance will determine the net effect of global change on C sequestration. Global changes, such as increasing temperatures and atmospheric CO_2 concentrations, eutrophication, land use, encroachment of invasive species, and accelerated SLR, are expected to influence decomposition processes and C turnover in tidal wetland soils with potentially strong feedbacks on ecosystem stability (Kirwan and Megonigal 2013).

In the following, I will address the effects of numerous global change factors on decomposition processes and C turnover in tidal wetlands. Besides studying direct effects of the different global changes on such processes, I am particularly interested in plant mediated and thus indirect effects. Plant-mediated effects on ecosystem functions are often governed by specific plant traits, such as quality or quantity of primary production, biomass allocation, root morphology and depth, and vegetation structure (Cornwell et al. 2008, Lavorel 2013, Moeller et al. 2014). Although plant-mediated global change effects on ecosystem functions are often difficult to assess, recent studies with implications to tidal wetland stability (Kirwan and Megonigal 2013) and C turnover (Wolf et al. 2007, Mozdzer and Megonigal 2013, Bernal et al. 2017) have taught us that often these indirect effects are critical to understand the net effect of change. Therefore, the central hypothesis of my work is that plant-trait responses to global change are the main drivers of change in C turnover and GHG fluxes from tidal wetland soils.

The main part of the work presented here was conducted in brackish marsh systems of the Chesapeake Bay, US East coast (Chapters 2 and 3) and in the salt marshes of the European Wadden Sea (Chapters 4 and 5). In **chapter 2**, I assess the potential of a deep rooting and highly productive invasive plant species to change CH_4 emissions from a brackish marsh and discuss implications on the net GHG balance of the ecosystem. In **chapter 3**, I present findings on direct and plant-mediated effects of accelerated SLR on SOM decomposition in brackish marshes. In **chapter 4**, I describe the C-sequestration potential of artificial salt marshes along the German mainland coast, and I illustrate the importance to track soil C turnover over decades in order to avoid overestimation of long-term C sequestration. In **chapter 5**, I focus on land-use effects on C turnover by describing the direct and plant-mediated effects of livestock grazing on the microbial function and structure in salt marsh soils. Concluding in **chapter 6**, I present the results of a global-scale comparison of litter decomposition and stabilization rates in 25 marsh and mangrove sites, stretching a large temperature gradient, in order to improve our process understanding of temperature and sea level effects on C turnover.

2 Complex invader-ecosystem interactions and seasonality mediate the impact of non-native *Phragmites* on CH_4 emissions

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Peter Mueller*, Rachel N. Hager*, Justin E. Meschter, Thomas J. Mozdzer, J. Adam Langley, Kai Jensen, and J. Patrick Megonigal

*first two authors contributed equally to the manuscript

Abstract

Invasive plants can influence ecosystem processes such as greenhouse gas (GHG) emissions from wetland systems directly through plant-mediated transfer of GHG to the atmosphere or through indirect modification of the environment. However, patterns of plant invasion often covary with other environmental gradients, so attributing ecosystem effects to invasion can be difficult in observational studies. Here, we assessed the impact of *Phragmites australis* invasion into native shortgrass communities on CH_4 emissions by conducting field measurements of CH_4 emissions along transects of invasion by *Phragmites* in two neighboring brackish marsh sites and compared these findings to those from a field-based mesocosm experiment. We found remarkable differences in CH_4 emissions and the influence of *Phragmites* on CH_4 emissions between the two neighboring marsh sites. While *Phragmites* consistently increased CH_4 emissions dramatically by 10.4 ± 3.7 μmol m^{-2} min^{-1} (mean ± SE) in our high-porewater CH_4 site, increases in CH_4 emissions were much smaller (1.4 ± 0.5 μmol m^{-2} min^{-1}) and rarely significant in our low-porewater CH_4 site. While CH_4 emissions in *Phragmites*-invaded zones of both marsh sites increased significantly, the presence of *Phragmites* did not alter emissions in a complementary mesocosm experiment. Seasonality and changes in temperature and light availability caused contrasting responses of CH_4 emissions from *Phragmites* versus native zones. Our data suggest that *Phragmites*-mediated CH_4 emissions are particularly profound in soils with innately high rates of CH_4 production. We demonstrate that the effects of invasive species on ecosystem processes such as GHG emissions may be predictable qualitatively but highly variable quantitatively. Therefore, generalizations cannot be made with respect to invader-ecosystem processes, as interactions between the invader and local abiotic conditions that vary on the order of meters in wetland ecosystems can have a stronger impact on GHG emissions than the invader itself.

Introduction

Wetland ecosystems as landscape sinks are thought to be particularly susceptible to plant invasions (Zedler and Kercher 2004). Invasive wetland plants often form monocultures with cascading consequences for wetland biodiversity, food webs, biogeochemistry, and entire habitat structure (Zedler and Kercher 2004). Given the increasingly recognized function of tidal wetlands as a long-term carbon sink (Duarte et al. 2005, Mcleod et al. 2011), there is a growing body of research focused on the impact of invasive species on tidal wetland carbon sequestration and greenhouse gas (GHG) emissions, particularly with respect to changes in the balance of sequestration and methane (CH_4) emissions (e.g. Valéry et al. 2004, Emery and Fulweiler 2014, Yuan et al. 2015). Under anoxic conditions and depending on the availability of alternative electron acceptors (e.g. nitrate, iron, or sulfate) large amounts of GHGs such as CH_4 and nitrous oxide can be produced in tidal wetland soils. Previous work has shown that particularly CH_4 emissions have the potential to offset even high rates of carbon sequestration in low salinity systems that experience low inputs of sulfate through seawater intrusion (Poffenbarger et al. 2011). Besides the availability of electron acceptors, also plant activity and certain plant traits (like aerenchyma formation) can greatly influence methane emissions by affecting the three processes that contribute to net CH_4 emissions: microbial CH_4 production, microbial CH_4 oxidation, and plant-supported ventilation of CH_4 directly to the atmosphere, bypassing soil surface CH_4 oxidation (Armstrong et al. 1996, Colmer 2003, Sutton-Grier and Megonigal 2011).

Perhaps the most comprehensive research on the impact of an invasive wetland species on GHG emissions comes from the invasion of non-native *Spartina alterniflora* in East Chinese coastal wetlands where native plant communities are dominated by *Phragmites australis, Suaeda salsa,* or *Scirpus mariqueter*. Here, *in situ* closed-chamber quantification of CH_4 emissions (Tong et al. 2012, Yuan et al. 2015) and mesocosm experiments (Cheng et al. 2007, Zhang et al. 2010) suggest *S. alterniflora* invasion increases CH_4 emissions in comparison to the native community.

North American wetlands have been rapidly invaded by a non-native lineage of the common reed, *P. australis* (hereafter *Phragmites*). In tidal wetlands, the introduced Eurasian lineage outcompetes the native lineage (Mozdzer and Zieman 2010, Mozdzer et al. 2013), increases CH_4 emissions (Mozdzer and Megonigal 2013), and also expands into historically novel habitats (Chambers et al. 1999). Examples of native plant communities susceptible to *Phragmites* invasion in North America are high marshes dominated by *Spartina patens*

(Chambers et al. 1999, Windham and Lathrop 1999, Mozdzer et al. 2013), low marshes dominated by *Spartina alterniflora* (Chambers et al. 1998), and brackish marshes dominated by *Schoenoplectus americanus* (McCormick et al. 2010). Replacement of native communities by *Phragmites* results in altered soil properties (redox potential, nitrogen pools), porewater chemistry (salinity, nitrogen fluxes), and biomass production (Chambers et al. 1998, Windham and Lathrop 1999, Windham-Myers 2005, Mozdzer et al. 2010). Recent studies comparing *in situ* emissions of CH_4 from different native communities and *Phragmites* in North America have reported contrasting outcomes: Emery and Fulweiler (2014) found no differences in CH_4 emissions from low marsh *Spartina alterniflora* and *Phragmites* stands in a New England salt marsh, while Martin and Moseman-Valtierra (2015) found consistently higher CH_4 emissions from *Phragmites* stands compared to *S. patens*-dominated high marsh communities in three New England tidal marshes along a salinity gradient.

Previous studies that investigated invader-ecosystem effects on GHG emissions have generally used the approach of space-for-time substitution which assumes that areas supporting invasive species would be ecologically and biogeochemically identical to areas that presently support native communities if they had not been invaded. Although this approach is insightful, knowledge of the effect of invasion on GHG emissions (and other processes) is limited by the fact that conclusions drawn from field measurements are rarely coupled to experimental support.

Invasive-species establishment commonly requires distinct environmental conditions within an ecosystem, e.g. low-salinity or high-elevation environments as suggested for the establishment of *Phragmites* (Bart et al. 2006, Mozdzer et al. 2016). Patterns of invasion are likely to follow gradients in environmental variables like salinity, elevation, and flooding frequency that have an impact on GHG emissions irrespective of the dominant plant community (Megonigal et al. 2004, Poffenbarger et al. 2011, Olsson et al. 2015). Furthermore, contrasts between sites in spatial patterns of invasion, establishment histories (Bart et al. 2006), or pre-invasion biogeochemical cycles may result in different responses of GHG emissions upon invasion. In order to develop conceptual models that explain invasion effects on GHG emissions, it is necessary to assess the extent to which we can generalize across study sites.

In this study, we assess the impact of *Phragmites* invasion on CH_4 emissions using a space-for-time substitution approach along transects of invasion by *Phragmites*. We conducted the study in two neighboring Chesapeake Bay brackish marsh sites that differ in geomorphic

setting, porewater chemistry, invasion history, and *Phragmites* stature, assuming that these differences will indicate the extent to which our results can be generalized. Finally, we compare our field results to those from a field-based mesocosm experiment in order to isolate the factors that influence *Phragmites*-driven changes in CH_4 emission from other invasion-independent factors that may co-occur along natural transects of *Phragmites* invasion.

Material and methods

Field study

The field study was conducted in two *Phragmites*-invaded brackish tidal wetland systems, Fox Creek Marsh and Kirkpatrick Marsh, home to the Smithsonian Global Change Research Wetland (GCREW) operated by the Smithsonian Environmental Research Center (38°53'N, 76.33'W) in Edgewater, Maryland, USA. The study took place between June 2013 and October 2014. Both sites are located within the Rhode River, a sub-estuary of the Chesapeake Bay, but are separated by the Muddy Creek and a distance of approximately 800 m. These sites were selected based on aerial photos and remote sensing (unpublished data) that indicated they were uniformly covered by native grasses (dominated by *S. patens* with small contributions of *Distichlis spicata, Panicum virgatum,* and *S. americanus*) and *Iva frutescens* until at least 1972. However, these two sites differ with respect to mechanisms of *Phragmites* establishment and therefore represent different invasion patterns: At Fox Creek Marsh, *Phragmites* first established at the wetland-upland interface and has been spreading towards the marsh edge. In contrast, at the Kirkpatrick Marsh, *Phragmites* established on the creek bank and has been spreading along the marsh edge and toward the marsh interior. In both sites, three vegetation zones representing different stages of invasion by *Phragmites* were classified: (1) a dense, monotypic *Phragmites* stand, (2) a mixed zone of native grasses (>90% cover) and few clonally integrated shoots of *Phragmites* (approx. 10 per plot), and (3) a native zone dominated by *S. patens*. Replicated 50 x 50 cm aluminum collars (n = 4) were inserted 20 cm into the soil surface in each of the three zones at each site, and small boardwalks were installed to minimize disturbances during repeated sampling over two years.

CH_4-flux measurements

To examine the effect of *Phragmites* invasion on CH_4 emissions, CH_4 fluxes in *Phragmites*-, mixed-, and native-vegetation zones were measured approximately monthly between June and October of 2013 and 2014 (Figure 1) using closed chambers. Measurements were conducted

during day light under non-inundated conditions. As the main focus of this work is the relative comparison of CH_4 emissions between different vegetation zones and sites, we used an easily replicable closed-chamber design without temperature control. Depending onvegetation zone and site, absolute values of CH_4 emission presented here may be overestimated and should therefore be interpreted with caution (see "Results" for detail). Flux chambers were constructed of an aluminum frame and covered with transparent polyester film (Melinex 071, ICI, DE, USA) with topside sampling tubing from which to extract gas samples. Chambers were equipped with thermometers on the inside to record chamber-temperature development during measurements. Chamber volumes were manipulated to accommodate the three vegetation-zone heights by using stackable 70 and 120 cm sections, resulting in a chamber headspace between 200 to 1025 L. During each flux measurement, 20 ml gas samples were collected from the chamber headspace every 30 min for 2 h and transferred to 12 mL gas sampling vials (Labco Ltd, High Wycombe, UK). Gas samples were analyzed on a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (Shimadzu Corporation, Kyoto, Japan) during measurements 1-3 in 2013; all subsequent gas analyses were performed on a Varian 450 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). CH_4 fluxes were calculated by linear regression analysis of the change in headspace gas concentration in the chamber versus time following the ideal gas law, assuming ambient pressure and chamber temperature at a given measuring point. Only fluxes with $r^2 \geq 0.80$ were used (applied to >95% of measurements). Annual CH_4 emissions for each experimental unit were calculated by linear interpolation between emission of single sampling dates and integrating those over time. Annual CH_4 emissions are likely overestimated because measurements were only conducted during daytime (van der Nat et al. 1998) and no flux measurements were conducted during winter and early growing season (Nov-May) when CH_4 emissions were presumably lower.

Solar radiation and air temperature data corresponding to the dates and times of our field measurements were acquired from a nearby (<2 km) meteorological tower (Photobiology and Solar Radiation Lab, SERC, MD, USA) on the campus of the Smithsonian Environmental Research Center. Solar radiation (W/m^2) was measured using an Eppley model PSP precision spectral pyrometer (The Eppeley Lab, Inc., New Port, RI, USA) which measures radiation from 285 to 2800 nm. Temperature was measured with a Vaisala HMP45AC probe (Vaisala, Helsinki, Finland).

Biomass assessment

Biomass was assessed in order to characterize differences in biomass between sites and vegetation zones that have previously been shown to influence CH_4 emissions (e.g. Mozdzer and Megonigal 2013). In June, August, and October 2014, we measured the density and height of *Phragmites* ramets within the *Phragmites*-zone flux collars. *Phragmites* biomass within the flux collars was estimated with an exponential allometric equation derived from 30 plants collected from outside the collars in June and August, corresponding to the gas flux sampling dates (June: $y=0.6318e^{0.0151x}$; $R^2=0.945$, August: $y=1.0596e^{0.0126x}$; $R^2= 0.948$). In October 2014, *Phragmites* biomass and dead standing mass was estimated using the August allometric equation. Native vegetation biomass was assessed sampling three replicate clip plots (100 cm²) within each collar. Samples were separated into dead and live material, dried to constant weight at 60°C, and weighed.

Porewater analyses

To evaluate the influence of porewater chemistry on CH_4 emissions, porewater was extracted from 30 cm below the soil surface at both sites in all three vegetation zones (n = 5) using "sipper" wells (Keller et al. 2009). To directly compare the two sites, porewater for the different analyses (described below) was always sampled at low tide and within a 2-h period at both sites in all three vegetation zones. On 13 July 2013 porewater was extracted for dissolved sulfide, dissolved CH_4, and salinity analyses. Sulfide and dissolved CH_4 were analyzed within 4 h of sampling following Keller et al. (2012). Briefly, 3 mL of porewater were added to 3 mL of sulfide antioxidant buffer, and the solution was measured with an ion-selective electrode connected to a millivolt meter (ORION Research, Beverly, MA, USA). For dissolved CH_4 analysis, 15 mL of porewater was stripped by introducing a headspace of 15 mL of ambient air and vigorously shaking the syringe for 30 s. The water was then expelled and the the 15 mL headspace was analyzed for CH_4 using a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (Shimadzu Corporation, Kyoto, Japan). Porewater salinity was measured using a hand-held refractometer. On 22 October 2015, porewater was extracted for sulfate and chloride analyses, following Keller et al. (2009). Porewater samples were fixed with 5% zinc acetate and filtered through preleached syringe filters (0.45 µm). Filtered samples were measured for SO_4^{2-} and Cl^- on a Dionex ICS-2000 RFIC ion chromatography system (Dionex Corporation, Sunnyvale, CA, USA) with an auto sampler. Sulfate depletion was calculated based on the porewater SO_4^{2-}:Cl^- ratio (Keller et al. 2009).

Mesocosm experiment

In order to isolate *Phragmites*-driven changes in CH_4 emission from other invasion-independent factors, CH_4 emissions were also measured on mesocosms situated in a tidal creek of Kirkpatrick Marsh that were planted with *Phragmites* and native vegetation. The design of the mesocosm experiment has previously been described in Langley et al. (2013) and Mozdzer et al. (2016). The mesocosm experiment was originally designed to assess effects of elevation relative to the marsh platform (as a proxy for relative sea level) and treatments of elevated CO_2 and nitrogen (N) fertilization on plant growth. All plants used in this experiment were planted in spring 2010 into 70 cm-deep mesocosms constructed from 10 cm-diameter PVC, filled with commercial sedge peat (Baccto, Michigan Peat Company, Houston, TX, USA). For the purpose of this study, we only compared CH_4 emissions between *Phragmites*- and *S. patens*-dominated mesocosms set at the highest relative elevation (reference marsh platform (GCREW) +35 cm), which matches best the species composition in the native zones of our two high marsh field sites. For the comparison of CH_4 emissions between native- and *Phragmites*-planted mesocosms, we evaluated CH_4 emissions in the mesocosms grown at ambient CO_2 and N only (n = 4). After two seasons of growth, CH_4 flux measurements were conducted in July 2011. Mesocosms were removed from the tidal creek and placed into 120 L containers filled with tidal creek water corresponding to the mean water level of the respective elevation treatment and left to equilibrate overnight. Clear acrylic chambers (10.2 cm i.d. acrylic tube) were put onto the mesocosms and sealed with a rubber fitting. Headspace gas samples were taken every 20 min over a period of 2 h and analyzed for CH_4 on a Varian 450 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA).

To assess the impact of light on CH_4 emissions, the clear flux chambers were covered in aluminum foil and measurements were made in the dark. The effect of the presence or absence of light was tested on four *S. patens*-dominated mesocosms from the highest elevation (two of which were ambient CO_2/ambient N treatments and two of which were ambient CO_2/N fertilized treatments), and on four *Phragmites*-planted mesocosms, all of which were ambient CO_2/ambient N treatments. Our N treatment did not influence CH_4 emissions from native mesocosms (unpublished data).

Statistical analyses

Factorial repeated measures ANOVAs were used to test for effects of site, vegetation zone, and time on CH_4 emissions and to test for effects of site and time on *Phragmites* biomass. Factorial

ANOVAs were conducted to test for effects of vegetation zone and site on October 2014 biomass (biomass and dead standing mass), and on porewater CH_4, sulfide, sulfate, sulfate depletion, and salinity. One-way ANOVA was used to test for the effect of vegetation type on CH_4 emission from the experimental mesocoms. Tukey's HSD tests were conducted for pairwise comparisons, and data were log-transformed if Levene's test indicated heterogeneous variance (*in situ* CH_4 emission, porewater CH_4, and porewater sulfide data). A two-tailed, paired t-test was used to test for a light/dark effect on CH_4 emissions from the experimental mesocosms. To test for the effect of radiation and temperature on CH_4 emissions from the different vegetation zones and sites, the Homogeneity-of-Slopes GLM procedure was conducted. Linear and non-linear regression was used to further explore significant relations between radiation and CH_4 flux. Statistical analyses were conducted in STATISTCA 10, (StatSoft Inc., Tulsa, OK, USA).

Results

In situ CH_4 emissions

CH_4 emissions significantly differed between Fox Creek and Kirkpatrick Marsh ($p < 0.01$). Emissions were an order of magnitude greater from the *Phragmites* zone of Fox Creek versus Kirkpatrick Marsh ($p < 0.01$; Figure 1). However, there were no significant differences in CH_4 emissions between the sites when comparing the mixed- and the native zones ($p > 0.1$ and $p > 0.4$, respectively). CH_4 emissions were higher from *Phragmites* versus native zones in both sites (Figure 1). However, this difference in CH_4 emissions in the *Phragmites* versus native zones was far greater at Fox Creek Marsh than Kirkpatrick Marsh (Figure 1): At Fox Creek Marsh, CH_4 emissions varied significantly between vegetation zones with emissions from the *Phragmites* stand often exceeding those from the mixed and the native zone by an order of magnitude ($p < 0.001$; Figure 1). CH_4 emissions from the *Phragmites* zone were significantly greater every sampling date except June of 2013, whereas CH_4 emissions from the mixed zone were significantly higher than from the native zone only at three sampling dates (Figure 1). In contrast, at Kirkpatrick Marsh, although CH_4 emissions were also higher in the *Phragmites* zone, this effect of *Phragmites* was only significant at one sampling date (October 2014; Figure 1).

Sampling date had a significant effect on CH_4 emissions from both marsh sites ($p < 0.001$), and was significantly more pronounced for CH_4 emissions from the *Phragmites* zones of both sites ($p < 0.001$; Figure 1).The Homogeneity-of-Slopes (HOS) model indicated

that solar radiation had a significant effect on CH_4 emissions ($p < 0.05$) which differed between vegetation zones (*zone x radiation*, $p < 0.05$), but did not differ between sites (*site x radiation*, $p > 0.8$; *site x zone x radiation*, $p > 0.1$). Solar radiation significantly increased CH_4 flux from *Phragmites* zones (exponential $R^2 = 0.425$; $p < 0.001$), but had no effect on CH_4 flux from the mixed zones ($p > 0.05$) and the native zones ($p > 0.4$) of both sites. Also chamber temperature had a significant effect on CH_4 emissions ($p < 0.001$) which differed between vegetation zones (*zone x temperature*, $p < 0.001$) and also between sites (*site x temperature*, $p < 0.001$; *site x zone x temperature*, $p < 0.001$). Chamber temperature increased CH_4 flux from the *Phragmites* zone (exponential $R^2 = 0.709$; $p < 0.001$) and the mixed zone (exponential $R^2 = 0.589$; $p < 0.001$) of Fox Creek Marsh, but neither influenced CH_4 flux from the native zone of Fox Creek Marsh ($p > 0.8$), nor CH_4 emissons from any vegetation zone of Kirkpatrick Marsh (all $p > 0.2$).

Chamber temperature was significantly correlated with radiation ($R^2 = 0.577$; $p < 0.001$) and weakly but still significantly correlated with outside temperature as acquired from the meteorological tower ($R^2 = 0.062$; $p < 0.01$). At most sampling dates, temperature was considerably higher than outside temperature ($\Delta T = 8.8 \pm 0.7$ K) than outside of the chambers. However, differently sized chambers, as used for the three vegetation zones, showed no significant difference in ΔT ($p > 0.4$).

Table 1 Aboveground biomass (g m^{-2}) within *Phragmites* gas-flux measuring collars in June and August 2014 as well as aboveground biomass and dead standing mass (g m^{-2}) within *Phragmites* and native collars in October 2014

Site	Veg. zone	June	August	October live	October dead
Kirkpatrick	Native			61 ± 21 [a]	64 ± 15 [a]
	Phragmites	379 ± 184 [a]	380 ± 149 [a]	140 ± 149 [ab]	628 ± 200 [b]
Fox Creek	Native			92 ± 35 [a]	129 ± 24 [a]
	Phragmites	1122 ± 280 [b]	565 ± 120 [a]	338 ± 76 [b]	600 ± 166 [b]

Lowercase letters represent results of a Tukey's HSD test. Values within one column not connected by the same letter are significantly different ($p < 0.05$). Presented are mean values ± standard deviations ($n = 4$)

Biomass

Phragmites aboveground biomass was significantly greater at Fox Creek than at Kirkpatrick Marsh ($p < 0.05$; Table 1). Differences in biomass were evident in the height of the plants which were up to 3.85 m at Fox Creek Marsh versus 2.85 m at Kirkpatrick Marsh. At both sites, end of season aboveground biomass was significantly greater in the *Phragmites* zone than the

native zone; however, in Kirkpatrick Marsh this result applied to standing dead biomass but not live biomass (Table 1).

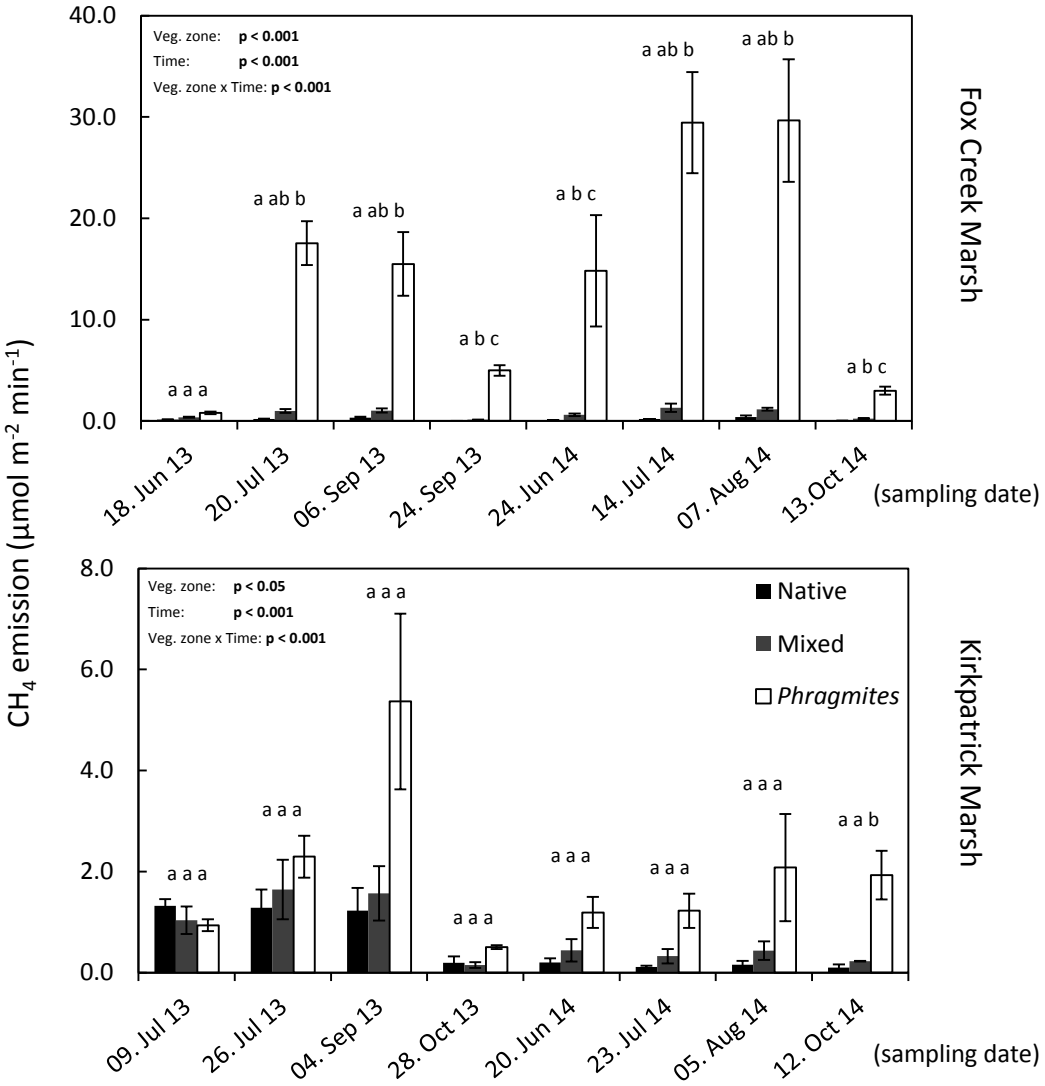

Figure 1 CH_4 emissions (µmol m^{-2} min^{-1}) from three vegetation zones representing different stages of invasion by *Phragmites australis* into native shortgrass communities measured on eight sampling dates at Fox Creek Marsh (top) and Kirkpatrick Marsh (bottom). P-values represent results of repeated measures ANOVA. Lowercase letters represent results of a Tukey's HSD test at the given sampling date within a marsh site. Bars not connected by the same letter are significantly different ($p < 0.05$). Presented are mean values ± standard errors; n = 4

Porewater chemistry

Dissolved porewater CH_4 was an order of magnitude higher in Fox Creek than in Kirkpatrick Marsh in all three vegetation zones (Figure 2). Similarly, porewater sulfide was significantly higher in Fox Creek Marsh (Figure 2). At both sites, porewater sulfide decreased significantly from the native to the *Phragmites* zone (Figure 2). Porewater sulfate was higher and sulfate-depletion was marginally lower in Kirkpatrick than at Fox Creek Marsh with no differences

between vegetation zones (Figure 2). Porewater salinity in October 2015 was higher at Kirkpatrick (10.4 ± 0.3) than at Fox Creek Marsh (9.5 ± 0.3; $p < 0.05$) with no significant differences between vegetation zones ($p > 0.2$). Porewater salinity in July 2013 was 9.7 ± 0.2 and did not differ between sites ($p > 0.6$) and vegetation zones ($p > 0.7$).

Experimental-mesocosm CH_4 emissions

CH_4 emissions from *Phragmites* mesocosms were higher, but not significantly, than those from native mesocosms ($p > 0.2$; Figure 3). CH_4 emissions decreased in each case after darkening ($n = 4$) in *Phragmites* (-65 ± 9%; $p < 0.05$), whereas the opposite was true in native, where CH_4 emissions increased in each case ($n = 4$) after darkening of the flux chamber by 35 ± 15% ($p < 0.1$).

Discussion

Spatial variability of CH_4 emissions

Our study demonstrates that the effects of invasive species on ecosystem processes such as GHG emissions may be predictable qualitatively but highly variable quantitatively. While *Phragmites* invasion tended to increase CH_4 emissions at both sites, the difference in emissions between native and invaded zones ranged from dramatic (an order of magnitude) at one site to occasionally significant at a site located nearby. The data illustrate that generalizations cannot always be made with respect to invader-ecosystem processes, as interactions between invader and local abiotic conditions that vary on the order of meters in wetland ecosystems can have a stronger impact on GHG emissions than the invader itself. Porewater data did not provide evidence that contrasting pre-existing biogeochemical gradients (e.g. in salinity or [sulfate]) exist between our two sites (Figure 2) that represent different invasion patterns of *Phragmites* into native shortgrass communities. Consequently, different invasion patterns do not explain the large differences in CH_4-emission change after *Phragmites* invasion between our two sites. Greater *Phragmites* biomass at Fox Creek than Kirkpatrick Marsh could help explain the observed differences in CH_4 emissions and CH_4-emission change after invasion between sites (Table 1). However, the fact that differences in biomass were only significant in the early growing season, as opposed to order of magnitude differences in CH_4 emissions throughout the season, does not support differences in plant biomass as the main driver of the observed effects.

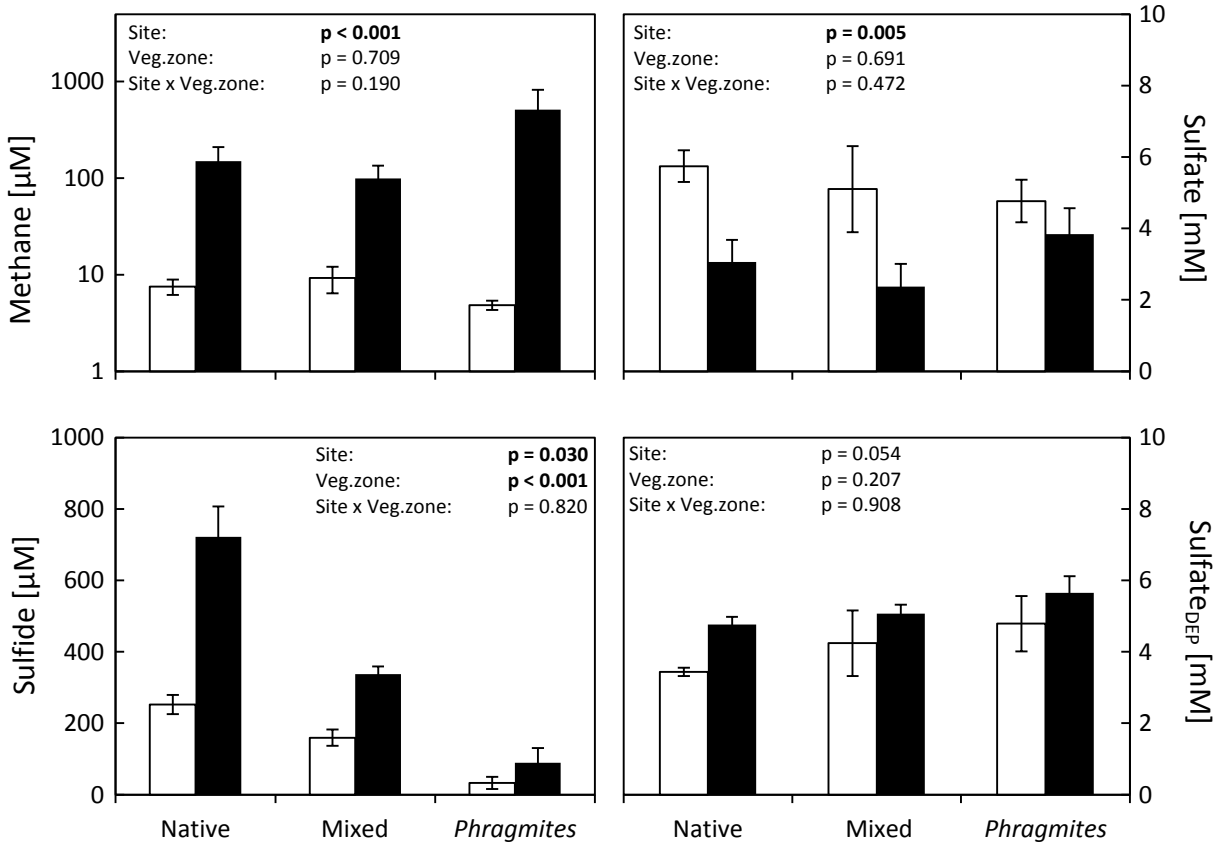

Figure 2 Porewater CH_4 (µM), sulfide (µM), sulfate (mM), and sulfate depletion ($Sulfate_{DEP}$, mM) sampled from 30 cm soil depth in three vegetation zones representing different stages of invasion by *Phragmites australis* into native shortgrass communities at Fox Creek Marsh (black bars) and Kirkpatrick Marsh (white bars). Presented are mean values ± standard errors; n = 5

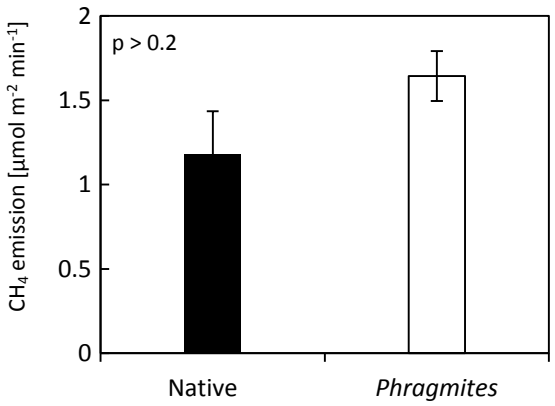

Figure 3 CH_4 emissions (µmol m^{-2} min^{-1}) from native- and *Phragmites*-planted experimental field mesocosms. Presented are mean values ± standard errors; n = 4

Clear and consistent effects of *Phragmites* on CH_4 emissions were found in our high-porewater CH_4 site only, where dissolved porewater CH_4 concentrations exceeded those at Kirkpatrick Marsh by more than an order of magnitude in all three vegetation zones. Although we measured porewater CH_4 only once, our findings suggests that an increase in CH_4 emissions after *Phragmites* invasion is particularly important in soils with high rates of microbial CH_4

production. In such sites, CH_4 emissions may be enhanced at the earliest stages of *Phragmites* invasion, as illustrated in our mixed zone that contained only few plants clonally integrated into the native zone, accounting for relatively little *Phragmites* biomass (Figure 1). Thus, the influence of *Phragmites* on CH_4 emissions is likely to vary depending on salinity and other factors that influence tidal marsh CH_4 biogeochemistry.

This conclusion – that relative increase in CH_4 emissions caused by *Phragmites* invasion increases with soil CH_4 production rates – is consistent with results from three New England marsh sites arranged across a salinity gradient: As expected from the well-established inverse relationship between salinity and CH_4 emissions (Poffenbarger et al. 2011), emissions from the native zones increased in the order of polyhaline < mesohaline < oligohaline (Martin and Moseman-Valtierra 2015). In each salinity zone, *Phragmites* invasion into native (*S. patens* or *D. spicata* dominated) systems increased CH_4 emissions; however, the relative increase in CH_4 emission at the oligohaline site, where CH_4 production was presumably higher, was considerably larger than the relative increase at the polyhaline site, where CH_4 production was presumably lower (Martin and Moseman-Valtierra 2015).

The CH_4-emission rates we measured from the *Phragmites* zone at Fox Creek Marsh are similar to their oligohaline site, while our Kirkpatrick Marsh site is similar to their meso-polyhaline site (Martin and Moseman-Valtierra 2015). Differences in salinity and porewater sulfate (Figure 2) between our sites could explain certain features of our data, including the relatively high CH_4 emissions and high porewater CH_4 concentrations at Fox Creek Marsh versus Kirkpatrick Marsh. However, particularly between the *Phragmites* zones, differences in salinity (<1) and porewater sulfate (Figure 2) were small, suggesting that mechanisms other than site salinity per se are responsible for differences in the effect of *Phragmites* invasion on CH_4 emissons in the present study.

A potential weakness of any study that uses space-for-time substitution is the possibility that the sites were not identical before the change in question occurred. In both the present study and that of Martin and Moseman-Valtierra (2015) it is possible that *Phragmites* invaded areas of relatively high CH_4 emissions. Such a conclusion is consistent with our mesocosm study results in which *Phragmites* seedlings did not increase CH_4 emissions compared to native species planted in the same soils (Figure 3). Evidence that higher CH_4 emissions in the *Phragmites* zones were due to the presence of *Phragmites* and not differences in pre-invasion soil biogeochemistry is the fact that porewater CH_4 concentrations were an order of magnitude higher in all Fox Creek Marsh plots compared to all Kirkpatrick Marsh plots. This is a clear

example of pre-existing soil biogeochemical differences that might cause different emissions even in the absence of *Phragmites*. However, differences in CH_4 emissions between Fox Creek Marsh and Kirkpatrick Marsh were only present between the *Phragmites* zones of the two sites, and not the native zones. Because both sites have supported the same native plant species since at least 1972 and CH_4 emissions from native zones of both sites were similar despite order of magnitude differences in porewater CH_4, we conclude that higher emissions in the *Phragmites* zones are due to changes induced by *Phragmites* itself.

While we demonstrated a plant-species effect on CH_4 emissions in both marsh sites (Figure 1), this result was not confirmed by our experimental data in a mesocosm experiment (Figure 3). However, there were similarities between the mesocosm and Kirkpatrick Marsh results that suggest that a longer term mesocosm study would have produced significant effects of *Phragmites* on CH_4 emissions. Namely, mesocosm CH_4 emissions were within range of *in situ* emissions from native marshes with similar vegetation in Kirkpatrick Marsh, and likewise *Phragmites* effects on CH_4 emissions in Kirkpatrick Marsh were only occasionally statistically significant.

Temporal variability of CH_4 emissions

Seasonality and the changing influence of plant-mediated effects on ecosystem processes also influence CH_4 emissions. Sampling date had a significant impact on CH_4 emissions from the *Phragmites* zone at both sites, but a less pronounced influence in the native zones (*zone x time* interaction at both sites, $p < 0.001$). This result could be interpreted as a diverging impact of seasonality, such as the time course of biomass development, on CH_4 emissions from different vegetation zones. Similar effects were reported by Martin and Moseman-Valtierra (2015). Although greater *Phragmites* biomass at Fox Creek Marsh could help explain differences in CH_4 emissions between sites, we do not have sufficient biomass data over the course of the season to determine if changes in biomass parameters explain changes in CH_4 emissions over time.

Light-driven convective gas flow significantly influenced CH_4 emissions in *Phragmites* only. The effects of solar radiation differed between vegetation zones, increasing CH_4 emissions from *Phragmites* but not the native zones. The relationships between radiation and CH_4 emissions we observed in the field were even clearer in the experimental mesocosms planted with *Phragmites*. We interpret the contrasting responses of CH_4 emission from *Phragmites* and native grasses such as *S. patens* to light as a result of differences in

mechanisms of gas transport through the different plant species, as previously demonstrated in studies comparing *Phragmites* with species of the genus *Scirpus* (van der Nat et al. 1998, Arkebauer et al. 2001). *Phragmites* is one of few wetland plants known to support light-enhanced convective gas flow, which increases O_2 delivery to the rhizosphere and emissions of CH_4 and H_2S (hydrogen sulfide) to the atmosphere (Mitsch & Gosselink 1993; Brix et al. 1996). Given the lack of convective gas flow, CH_4 emissions are insensitive to light in the *S. patens* dominated community.

As the flux chambers used in this study were not equipped with a cooling system, radiation also led to increases in chamber temperature. CH_4 emissions increased with chamber temperature; however, this was only true in the presence of *Phragmites* (mixed and *Phragmites* zone) and only at our high-porewater CH_4 site. Different responses of CH_4 emissions between vegetation zones to temperature are consistent with the responses to radiation and can be attributed to the temperature sensitivity of convective gas flow in *Phragmites* (e.g. Brix et al. 1996; Minke et al. 2014). Along with the large differences in relative CH_4-emission change after invasion between our two sites (discussed above), contrasting responses between the sites to temperature indicate that pre-existing biogeochemical site conditions determine the extent to which temperature- and light-sensitive plant traits like convective gas flow can influence GHG emissions from wetland soils.

At most sampling dates, chamber temperature was considerably higher than outside temperature. However, because outside temperature was measured at a nearby meteorological tower and not on site, we cannot precisely determine the extent to which chamber temperature actually differed from outside temperature of the surrounding. Further, we cannot account for potential microclimatic differences between vegetation zones, although those differences are likely to be expected (Báldi 1999). Therefore, it is possible that *Phragmites*-induced changes of the microclimate have an impact on temperature- and humidity-sensitive CH_4 emissions from *Phragmites* stands.

The design of our closed-chamber system limits our ability to separate temperature- from light-induced changes in CH_4 emissions. Further studies applying improved methodology (as for instance presented in Minke et al. (2014) or Martin and Moseman-Valtierra (2015)) are needed to evaluate the role of seasonal changes in light availability, diurnal (light) effects, and temperature in order to assess the net impact of plant invasions on GHG emissions from wetlands.

Mechanisms influencing CH_4 emissions after Phragmites invasion

Changes in CH_4 emissions after plant invasion in wetland ecosystems must be caused by one of three processes that contribute to net CH_4 emissions: (1) microbial CH_4 production, (2) microbial CH_4 oxidation, and/or (3) plant-supported ventilation of CH_4 directly to the atmosphere, bypassing soil surface CH_4 oxidation. However, there is a small existing literature on the mechanisms by which invasive plants change CH_4 cycling that can be used to develop hypotheses about the mechanisms that are likely responsible for increased CH_4 emissions after *Phragmites* invasion (Mozdzer and Megonigal 2013).

An increase in plant-supported CH_4 production (mechanism 1) does not seem to be an important driver of increased CH_4 emissions in our study. Otherwise we would have expected a stronger plant species effect on CH_4 emissions from our low-porewater CH_4 site and also from our mesocosm experiment. We do not have soil redox profiles, O_2 profiles, or CH_4-oxidation rate data to address the possibility that *Phragmites* changed CH_4 oxidation rates at the soil surface (mechanism 2). However, such an effect may occur if *Phragmites* invasion increases soil surface elevation as suggested by Rooth et al. (2003), or *Phragmites* increases root O_2 loss in the soil profile (Tanaka et al. 2007). Evidence of increased root O_2 loss is the fact that porewater sulfide concentrations were lower in *Phragmites*-invaded zones (Figure 2). Increased sulfide oxidation rates are one possible explanation for this pattern because there were no corresponding patterns in porewater sulfate concentration or sulfate depletion across vegetation zones (Figure 2). However, lower porewater sulfide concentrations in the *Phragmites*-invaded zones can also result from H_2S removal through increased plant ventilation of gases to the atmosphere (mechanism 3).

Higher concentrations of dissolved porewater CH_4 and sulfide, and higher sulfate depletion (Figure 2) at Fox Creek versus Kirkpatrick Marsh indicate higher rates of anaerobic decomposition in Fox Creek Marsh. As discussed above, the dramatic difference in porewater CH_4 concentrations between the two sites did not translate into differences in CH_4 emissions in the native zone. In contrast, the presence of *Phragmites* produced dramatic differences in emissions that reflect porewater CH_4 concentrations. Thus, we hypothesize that *Phragmites* stimulates CH_4 emissions primarily via mechanism 3, in which increased plant ventilation routes soil CH_4 past oxidizing soil layers. *Phragmites* has vastly deeper root systems (>1 m deep in our study sites; J. Meschter et al., unpublished data) than the native grasses it is replacing, and can more efficiently transport CH_4 via pressurized ventilation.

Table 2 Annual estimates of CH_4 emissions and CO_2-equivalents calculated based on a CH_4 global warming potential (GWP) of 32 and a sustained global warming potential (SGWP) of 45 over a 100-year time horizon (Neubauer and Megonigal 2015) from three vegetation zones representing different stages of invasion by *Phragmites australis* into native shortgrass communities measured in two brackish Chesapeake Bay marsh sites

		annual CH_4 emission [g m^{-2} yr^{-1}]				GWP-CO_2eq [Mg ha^{-1} yr^{-1}]	SGWP-CO_2eq [Mg ha^{-1} yr^{-1}]
Site	Type	mean	SD	min	max	mean	mean
Kirkpatrick	native	3.1	1.8	0.4	5.5	1.0	1.4
	mix	4.5	2.7	1.2	8.3	1.4	2.0
	Phragmites	13.5	4.6	6.4	18.7	4.3	6.1
Fox Creek	native	1.0	0.4	0.6	1.5	0.3	0.5
	mix	5.2	2.2	3.2	8.8	1.7	2.3
	Phragmites	105.5	39.3	66.7	164.8	33.8	47.5

GHG-balance implications

If the reported changes in CH_4 emissions from our field sites are primarily *Phragmites*-induced, as our data suggest, *Phragmites* invasion increased GHG emissions by 34 (Global Warming Potential) or 47 (Sustained Global Warming Potential; Neubauer and Megonigal 2015) Mg CO_2-equivalents ha^{-1} yr^{-1} in our high-porewater CH_4 site, and by 3.3 or 4.7 Mg CO_2-equivalents ha^{-1} yr^{-1} in our low-porewater CH_4 site (Table 2). However, it is not possible to interpret the impact of *Phragmites* invasion on radiative forcing without also determining whether soil carbon sequestration rates have also increased as one study suggests (Rooth et al. 2003). An increase in soil carbon sequestration of 47 and 4.7 Mg CO_2eq ha^{-1} yr^{-1} at Fox Creek Marsh and Kirkpatrick Marsh, respectively, would relate to 254% of the peak aboveground biomass (18.5 Mg CO_2eq ha^{-1} yr^{-1}) at the Fox Creek Marsh and 75% at the Kirkpatrick Marsh (Table 1). Even under the assumption of a 3:1 ratio of belowground to aboveground production, it seems unlikely that soil carbon sequestration rates have entirely offset the increase in CH_4 emissions. Future research needs to show if higher rates of biomass production, carbon sequestration and potential reduction in N_2O in *Phragmites* versus native shortgrass communities can counterbalance increases in CH_4 emissions.

Conclusion

In contrast to previous studies that have focused on invader-ecosystem effects on GHG emissions, we found that invader-ecosystem effects are site-specific. We found remarkable differences in total CH_4 emissions and the influence of the invader on CH_4 emissions at two adjacent marsh sites of similar salinity and species composition. Furthermore, seasonality and changes in temperature and light availability can cause contrasting responses of CH_4 emissions

from different vegetation types. Therefore, we suggest that generalizations with respect to invader-ecosystem processes should be interpreted with caution, as interactions between invader and local abiotic conditions that vary both spatially as temporally on the order of meters and hours, respectively, can have a stronger impact on GHG emissions than the invader itself.

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3 Plants mediate soil organic matter decomposition in response to sea level rise

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Peter Mueller, Kai Jensen, and J. Patrick Megonigal

Abstract

Tidal marshes have a large capacity for producing and storing organic matter, making their role in the global carbon budget disproportionate to land area. Most of the organic matter stored in these systems is in soils where it contributes 2-5 times more to surface accretion than an equal mass of minerals. Soil organic matter (SOM) sequestration is the primary process by which tidal marshes become perched high in the tidal frame, decreasing their vulnerability to accelerated relative sea level rise (RSLR). Plant growth responses to RSLR are well understood and represented in century-scale forecast models of soil surface elevation change. We understand far less about the response of SOM decomposition to accelerated RSLR. Here we quantified the effects of flooding depth and duration on SOM decomposition by exposing planted and unplanted field-based mesocosms to experimentally manipulated relative sea level over two consecutive growing seasons. SOM decomposition was quantified as CO_2 efflux, with plant- and SOM-derived CO_2 separated via $\delta^{13}CO_2$. Despite the dominant paradigm that decomposition rates are inversely related to flooding, SOM decomposition in the absence of plants was not sensitive to flooding depth and duration. The presence of plants had a dramatic effect on SOM decomposition, increasing SOM-derived CO_2 flux by up to 267% and 125% (in 2012 and 2013, respectively) compared to unplanted controls in the two growing seasons. Furthermore, plant stimulation of SOM decomposition was strongly and positively related to plant biomass and in particular aboveground biomass. We conclude that SOM decomposition rates are not directly driven by relative sea level and its effect on oxygen diffusion through soil, but indirectly by plant responses to relative sea level. If this result applies more generally to tidal wetlands, it has important implications for models of SOM accumulation and surface elevation change in response to accelerated RSLR.

Introduction

Relative sea level rise (RSLR) is expected to alter the accumulation and cycling of soil organic matter (SOM) in tidal marshes, with important consequences for tidal marsh stability and ecosystem services such as carbon sequestration (Kirwan and Megonigal 2013). Feedbacks between sea level and marsh surface elevation alter the hydrological and biogeochemical conditions that control carbon inputs via plant production (Morris et al. 2002, Kirwan and Guntenspergen 2012) and carbon outputs via decomposition (Kirwan et al. 2013). Indeed, organic matter production and decomposition are the two most important factors that control the rate at which SOM accumulates in soils, a process that confers stability to tidal marshes experiencing increased inundation due to accelerated rates of RSLR (Kirwan and Megonigal 2013) and contributes to high rates of carbon sequestration by coastal marine ecosystems. However, presently much more is known about factors that regulate plant production than decomposition.

A dominant paradigm in wetland ecosystem ecology is that flooding promotes soil carbon accumulation (Davidson and Janssens 2006). There is abundant evidence to support this notion, including the fact that tidal wetlands accumulate large pools of SOM (Mcleod et al. 2011) and the fact that anaerobic conditions suppress microbial respiration (Freeman et al. 2001, Megonigal et al. 2004). However, there is a surprising lack of empirical data on *in situ* decomposition of SOM, the soil carbon pool that decays most slowly and contributes the most to soil elevation change (Rybzcyk and Callaway 2009). Studies on decomposition processes in tidal marshes typically focus on recently senesced, labile plant material using classic litter bag techniques; they rarely measure the decomposition of the recalcitrant, stable fraction of SOM. Likewise, most studies of SOM decomposition are performed in laboratory incubations where the soil has been separated from plant sources of O_2 and labile carbon. The general lack of research on SOM decomposition in tidal wetlands limits a process-level understanding of soil carbon sequestration, elevation change, and refinement of numerical forecast models (Kirwan and Megonigal 2013).

There is evidence that flooding does not always preserve organic matter. Some fresh-litter decomposition studies in tidal wetlands have reported negligible effects of flooding (Hackney, 1987; Blum, 1993; Blum & Christian, 2004). More relevant to the issue of marsh elevation change is evidence that flooding may actually enhance SOM decomposition, although the relationship was weak (Kirwan et al. 2013). Thus, the effects of flooding on decomposition are equivocal, and the mechanisms causing weak or unexpected positive responses of SOM

decomposition to RSLR remain elusive. In this context, it is important to adopt a nuanced approach to studying SOM decomposition that addresses the complexity of RSLR effects on the process.

Relative sea level affects decomposition both directly by regulating the O_2 supply across the air-soil interface and indirectly by governing plant species composition, plant biomass, and primary production. SOM decomposition rates in all ecosystems are generally faster in the presence of plants than in plant-free soils (Guenet et al. 2010), a phenomenon known as *priming* (Kuzyakov et al. 2000). Priming effects in terrestrial systems are often positively related to plant biomass or size (Fu and Cheng 2002, Dijkstra et al. 2006) and driven by the input of labile organic compounds via rhizodeposition as substrate for microbial respiration (Blagodatskaya and Kuzyakov 2008). In addition, vascular wetland plants can affect SOM decomposition through changes in the supply of O_2 via root oxygen loss (Wolf et al. 2007) and the supply of alternative terminal electron acceptors for microbial respiration (Neubauer et al. 2005, Sutton-Grier and Megonigal 2011).

In this study, we quantified the effects of relative sea level on recalcitrant SOM decomposition rates using a design that captured both the direct effects of O_2 supply via atmospheric exchange across the soil surface and the indirect effects of plant processes. Soil-filled mesocosms were either left unplanted to account for direct effects of flooding onlyor planted to also account for flooding effects that operate indirectly through plant-induced priming. The mesocosms were then positioned at different elevations in a tidal creek to manipulate relative sea level. SOM decomposition was quantified using a carbon stable isotope technique that allowed to distinguish between plant- and SOM-derived CO_2 production. Methane flux was also assessed as a minor product of microbial respiration in brackish systems. We hypothesized that SOM decomposition rates would decrease with increasing flooding depth and duration in both planted and unplanted mesocosms as the supply of O_2 decreased. Based on the mechanisms discussed above, we further hypothesized that planted mesocosms would support higher rates of SOM decomposition than unplanted mesocosms of the corresponding elevation.

Material & methods

Site description, relative sea level manipulation, and soil redox potential

We conducted a field-based mesocosm experiment over two consecutive growing seasons (May-October 2012, May-August 2013) at the Global Change Research Wetland, located at the

Smithsonian Environmental Research Center, Maryland, USA (38°53′N, 76°33′W). The facility is situated in Kirkpatrick Marsh on Rhode River, a brackish subestuary of the Chesapeake Bay with a mean tidal range of 44 cm (Langley et al. 2013).

Mesocosms were deployed in a tidal creek of Kirkpatrick Marsh in order to test our hypothesis that SOM decomposition would decrease with increasing flooding depth and duration as the supply of O_2 decreased. We manipulated relative sea level by deploying the mesocosms at three elevations, -15 cm (low), ±0 cm (mid), and +20 cm (high), relative to the soil surface of the adjacent reference marsh platform. This general experimental design has been previously described as a "marsh organ" (Morris 2007). Relative elevation therefore served as a proxy for relative sea level. The original goal of this design is to determine the optimum flooding level for plant production, a goal that also applied to the present study in the sense the plants influence decomposition rate.

The effect of relative sea level on the oxidation-reduction state of the soil environment (as a proxy for O_2 supply) was quantified prior to carbon (CO_2+CH_4) flux measurements in both years. Redox potential measurements were made on three planted and three unplanted mesocosms per elevation at low tide by inserting three platinum-tipped electrodes (Megonigal and Rabenhorst 2013) to a depth of 10 cm below the soil surface. Readings were taken after 45 min of equilibration. Redox potential was referenced to a calomel electrode (Fisher Scientific accumet) pressed carefully to a soil depth of 1 cm in each mesocosm. Reference and redox electrodes were connected to a high-impedance, portable conductivity meter (Fisher Scientific accumet), allowed to stabilize for 3 min, then recorded. The three readings per mesocosm were averaged and corrected to the redox potential of the standard hydrogen electrode (+244 mV).

Mesocosm design: soils and plant species

A total of 30 mesocosms, 10 cm in diameter and 72 cm deep, were made from PVC pipe. A PVC cap was placed on the bottom of each mesocosm pipe and perforated at the bottom with five 1-cm-diameter holes to allow for vertical water exchange (see Langley et al. (2013) for additional details). There were no holes in the sides of the pipes to eliminate horizontal exchange, which mimics hydrologic conditions in the high marsh platform that we were simulating in this experiment (Jordan et al. 1983).

Mesocosms were filled with soils collected from specific areas and depths of Kirkpatrick Marsh. Most of Kirkpatrick Marsh is underlain by organic, sapric soils with C contents exceeding 40% and organic matter contents exceeding 80% to a depth of 5 m. These

soil characteristics hold regardless of whether the soils are presently dominated by C_3 or C_4 plant species. We collected soil from a C_4 grass (*Spartina patens*)-dominated area where the SOM carries a $\delta^{13}C$ signature significantly more enriched (-14.4‰) than the C_3 plant species (*Schoenoplectus americanus*) used in this study. Soil was collected from a depth of 50-100 cm to ensure the SOM was relatively old and recalcitrant compared to more surficial SOM. The soils were homogenized because our experimental design relied upon a uniform $\delta^{13}C$ signature in order to maximize our ability to separate soil and plant sources of CO_2 (see *Isotope and CO_2 flux calculations*). A hand-operated meat grinder was used to homogenize the soil, a process that may have increased the lability of organic matter by mechanically breaking apart macroscopic detritus and perhaps a few living roots. For this reason, we ran the experiment a second year in order to assess responses in the presence of less labile SOM compounds.

To test the second hypothesis that mesocosms with plants would support higher rates of SOM decomposition than mesocosms without plants, half of the mesocosms (n = 15) were planted with the C_3 sedge *Schoenoplectus americanus* (formerly *Scirpus olneyi*). This is a dominant macrophyte in large areas of the adjacent reference marsh platform where it can account for 75% of community biomass (Langley and Megonigal 2010, Langley et al. 2013). This widespread species is relatively flood tolerant (Kirwan and Guntenspergen 2012) and therefore served as the model species in our RSLR experiment. The other half of the mesocosms (n = 15) were left unplanted. *Schoenoplectus americanus* rhizome fragments were harvested from the adjacent marsh, cultivated in sand for three weeks, and transplanted to the mesocosms in mid-May 2012. Each planted mesocosm received four plants, simulating the natural stem density of approximately 500 stems m^{-2} (White et al. 2012, Langley et al. 2013). Mesocosms were deployed on rack with a stair step design resulting in 10 mesocosms per elevation, half of which were planted (n = 5) and half of which were unplanted treatments.

Soil freezing is normally limited to the upper few centimeters of the soil surface at this site; in order to prevent the mesocoms from unrealistically freezing to deeper depths over the winter, they were stored in a cold room at 4^oC from November 2012 (after plant senescence) through March 2013 (Langley et al. 2013). From March to May 2013, the mesocosms were stored in 120-L containers filled with tidal creek water to approximately 30 cm below soil surface. The mesocosms were redeployed to the marsh organ at their treatment elevations during the first week of June 2013 after shoots of approximately 5 cm height had emerged; this was a precaution to avoid flooding-stress at the lowest elevations that might otherwise have killed the plants due to continuous submergence.

Biomass sampling and plant $\delta^{13}C$ determination

Plant material was sampled for determination of plant tissue $\delta^{13}C$ required to distinguish between plant- and SOM-derived CO_2 flux (see *Isotope and CO_2 flux calculations*) and for the assessment of biomass parameters that we expected to scale with a potential priming effect on SOM decomposition. Plant material was sampled twice over the course of the experiment. Because we planned to redeploy the mesocosms after the first season, plant material for isotope analysis collected in September 2012 was sampled without destructively harvesting the mesocosms. For this initial assessment, one shoot from each mesocosm was cut at the soil surface and dried at 60°C to constant weight. The material was ground and a composite sample for each elevation was analyzed for $\delta^{13}C$. For isotope calculations, the 2012 results were compared and adjusted to those from a more thorough sample taken in 2013 (see *Isotope and CO_2 flux calculations*) when the mesocosms were destructively harvested for biomass assessment. Following gas sampling in the second season (August 2013), aboveground biomass was cut at the soil surface. Mesocosms were opened at the bottom by removing the cap, and the intact soil column was pushed out of the PVC pipe and cut in 10 cm increments. Belowground biomass (roots and rhizomes) was washed clean of soil with tap water, then given a final rinse with deionized water. Keeping each mesocosm separate, aboveground and belowground material was dried at 60°C to constant weight, ground and analyzed for $\delta^{13}C$. All tissue $\delta^{13}C$ analyses were performed on an element analyzer (HEKAtech, Wegberg, Germany) coupled to a Nu Horizon stable isotope-ratio mass spectrometer (Nu Instruments, Wrexham, UK) at the Applied Plant Ecology Stable Isotope Lab, University of Hamburg, Germany. Standing dead litter was excluded from isotope analysis, assuming that $\delta^{13}C$ was similar to living biomass and its relative contribution to CO_2 respiration was small (Kuehn and Suberkropp 1998, Kuehn et al. 2004).

Isotope and CO_2 flux calculations

The isotope calculations in this study followed Wolf et al. (2007), but were slightly modified to meet the requirements of the different experimental design. Briefly, the CO_2 emitted from planted mesocosms was a combination of plant- and SOM-derived sources characterized by different $\delta^{13}C$ signatures. We separated the relative contributions of plant- and SOM-derived flux using the equation

$$F_s = F_t (\delta_p - \delta_t) / (\delta_p - \delta_s) \quad (1)$$

where F_t is total CO_2 flux, F_s is SOM-derived CO_2 flux, δ_t is the $\delta^{13}C$ of the CO_2 produced by the whole system, δ_s is the $\delta^{13}C$ of the SOM-derived CO_2, and δ_p is the $\delta^{13}C$ of the plant-

derived CO_2. The elevation-specific averages of unplanted mesocosms were used for the δ_s end member terms, while the δ_p end member term was specific to each planted mesocosm. The δ_p end member was calculated as the mass-weighted average of the $\delta^{13}C$ of belowground and aboveground biomass. SOM-derived CO_2 flux was calculated for each mesocosm separately. Priming was calculated separately for each mesocosm as the difference between SOM-derived CO_2 flux from a planted mesocosm and mean CO_2 flux from unplanted mesocosms (n = 5) at the corresponding elevation.

Because the redeployment of the mesocosms after the first season did not allow a destructive harvest of the total biomass, a mass-weighted end member that included both belowground and aboveground biomass could not be used for isotope calculations in the first year of the experiment (2012). The option of using only the $\delta^{13}C$ of aboveground biomass would have led to a slight overestimation of SOM-derived vs. plant-derived CO_2 flux compared with the mass-weighted end member (Table 1). In order to avoid this potential bias, the $\delta^{13}C$ of 2012 belowground biomass was estimated from the $\delta^{13}C$ of 2012 aboveground biomass using a regression model developed for 2013 aboveground vs. belowground $\delta^{13}C$ values (belowground $\delta^{13}C = 10.51 + 1.37$ x aboveground $\delta^{13}C$, $r^2 = 0.921$, $p < 0.00001$).

We considered several assumptions embedded in our $\delta^{13}C$ end member assignments that may have affected our calculation of recalcitrant SOM decomposition rates. The assumption that aboveground and belowground tissues respire equally per unit biomass was addressed by using a range of potential δ_p end member terms in equation 1 (Tables 1 and 2). Specifically, we calculated F_s assuming that δ_p was the $\delta^{13}C$ of belowground biomass only (BEM), the $\delta^{13}C$ of aboveground biomass only (AEM), or the mass-weighted end member (MWEM). Because the $\delta^{13}C$ of plant tissue can differ from the $\delta^{13}C$ of CO_2 respired from that tissue, we sampled *S. americanus* from the adjacent marsh platform and compared the $\delta^{13}C$ of respired CO_2 to the tissue $\delta^{13}C$ itself (see *Supporting Information* for a detailed description of the methods). We used the range of divergence between the $\delta^{13}C$ of tissue and CO_2 respired by the tissue to develop two alternative mass-weighted end member terms that account for different scenarios of apparent ^{13}C enrichment or ^{13}C depletion during respiration. The alternative mass-weighted end member term 1 (aMWEM-1) accounts only for apparent ^{13}C enrichment during aboveground tissue respiration by +1‰, while aMWEM-2 accounts for both apparent ^{13}C enrichment during aboveground tissue respiration (+1‰) and ^{13}C depletion during belowground tissue respiration (-1‰), simulating the documented scenario of opposite ^{13}C discrimination (reviewed by Ghashghaie & Badeck 2014).

Carbon flux measurements

SOM decomposition was quantified by measuring SOM-derived CO_2 flux. Stable carbon isotopes (see *Isotope and CO_2 flux calculations*) were used to distinguish between recalcitrant SOM- and plant-derived CO_2. CO_2 flux measurements were conducted in September of the first growing season (2012) and in August of the second growing season (2013). At low tide, when the creek water level was 15 cm below the reference marsh soil surface (equivalent to the soil surface of the low-elevation mesocosms), mesocosms were carefully removed from the marsh organ and placed into 120-L containers. The containers were filled with tidal creek water to depths that corresponded to the water level their respective mesocosms were last exposed to in the creek. Algal growth was removed from the soil surface prior to sampling. Opaque PVC flux chambers of different lengths (depending on plant height) were placed onto each mesocosm and sealed. The headspace of each flux chamber was flushed with CO_2-free air for 4-8 min (depending on chamber volume) at a flow rate of 2 L min^{-1}. The resulting volume of CO_2-free air exceeded 3.5 times the headspace volume. We determined that this was sufficient to remove atmospheric CO_2 (Wunderlich and Borken 2012) by flushing sealed, empty chambers of different volumes and tracking [CO_2] with an LI-7000 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE, USA).

Chambers were left on the mesocosms for a period of 4 h in order to reach [CO_2] ≥ 1000 ppmv, at which point the headspace was sampled through a rubber septum using a 20-mL polyurethane syringe, and then transferred into a N_2-flushed and evacuated 12-mL Exetainer storage vial (Labco, High Wycombe, UK). Vials were checked for leaks during storage and transport by injecting a known volume of a CO_2 reference gas into 10 extra vials. All gas samples were analyzed for $\delta^{13}CO_2$ and [CO_2] at the Stable Isotope Facility of the University of California, Davis.

Our methods for quantifying SOM decomposition rate assume that CH_4 is a minor end product of microbial respiration in brackish systems (Poffenbarger et al. 2011) that can be ignored for the purposes of this study. We also assume that microbial oxidation of CH_4 to CO_2 had a negligible effect on $\delta^{13}CO_2$; otherwise isotopically depleted CO_2 derived from CH_4 would cause an underestimate of the contribution of SOM to total mesocosm (microbial+plant) CO_2 respiration. For these reasons, CH_4 flux and CH_4 oxidation were measured in September 2012 to coincide with the CO_2 flux measurements. A detailed description of CH_4 methods is provided in the *Supporting Information*.

Statistical analyses

Two-way ANOVAs were used to assess the effects of planting, elevation, and their interaction on SOM-derived CO_2 flux, CH_4 flux and soil redox potential. ANOVAs were applied separately for the two years. Tukey's HSD tests were used to make pairwise comparisons. One-way ANOVAs were used to test the effects of elevation on biomass production and plant tissue $\delta^{13}C$ and $\delta^{13}CO_2$. When Levene's test indicated heterogeneous variance, the data were log transformed. Linear regression was used to test for relationships between biomass production, priming, and SOM-derived CO_2 flux; elevation and redox potential; and the $\delta^{13}C$ of aboveground and belowground plant tissue. Analyses were performed using the statistical software STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA).

Results

Soil redox response to relative sea level

Soil redox potentials at 10 cm depth increased linearly with elevation in both planted mesocosms ($r^2 = 0.582$, $p < 0.05$ and $r^2 = 0.582$, $p < 0.01$, in 2012 and 2013, respectively) and unplanted mesocosms ($r^2 = 0.787$, $p < 0.01$ and $r^2 = 0.824$, $p < 0.001$, in 2012 and 2013, respectively). Redox potentials were higher in planted than unplanted mesocosms ($p < 0.001$ and $p < 0.0001$ in 2012 and 2013, respectively; Figure 1). In planted mesocosms, redox potentials were higher in 2013 than in 2012 ($p < 0.05$), while redox in the unplanted mesocosms did not differ between 2012 and 2013 ($p > 0.2$).

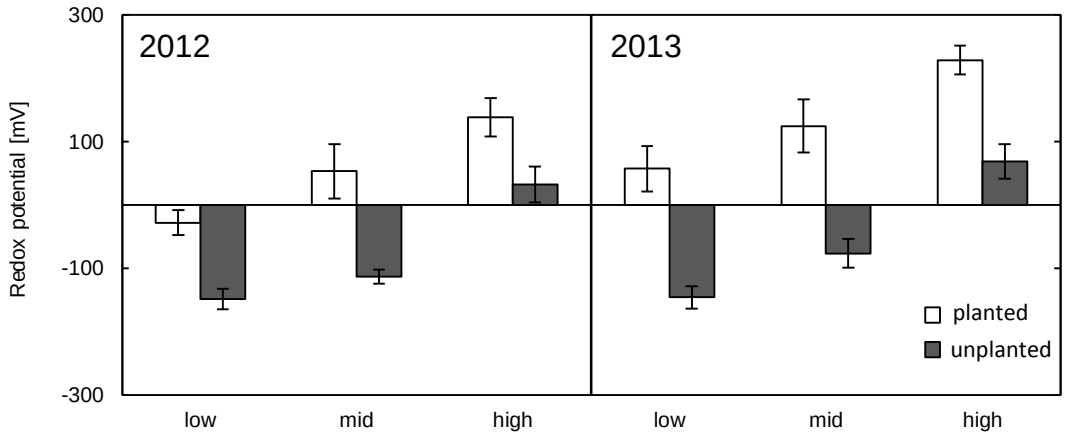

Figure 1 Soil redox potentials at 10 cm depth in unplanted and planted mesocosms measured at low tide at three different elevations relative to the reference marsh platform, low (-15 cm), mid (±0 cm), and high (+20 cm), in 2012 and 2013. Values are mean ± standard errors ($n = 3$)

Plant response to relative sea level

Aboveground, belowground and total biomass varied significantly with elevation ($p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively, Figure 2). The ratio of belowground-to-aboveground biomass also varied with elevation ($p < 0.001$), ranging from 3.7 at high elevation to 1.0 at low elevation. The main part of belowground biomass was allocated in the upper 10 cm of the soil profile at all three elevations, ranging from 84% of total belowground biomass at high elevation to 94% at low elevation. Single roots reached maximum depths of 45, 55, and 25 cm below surface in high, mid and low elevation mesocosms, respectively.

$\delta^{13}C$ of plant biomass and emitted CO_2

Above- and belowground plant biomass at the highest elevation was slightly ^{13}C enriched compared to the two lower elevations. At all elevations, belowground plant material was ^{13}C enriched compared to aboveground material (Table 2). The difference in $\delta^{13}C$ of *S. americanus* tissue and tissue-respired CO_2 (as determined on plant samples from the adjacent marsh) was <1‰ (tissue MWEM: -26.86 ± 0.20‰; respired CO_2: -26.13 ± 0.21‰).

$\delta^{13}CO_2$ was significantly different ($p < 0.001$) between planted and unplanted mesocosms (Table 2), reflecting the fact that CO_2 emitted by plant respiration of recent photosynthate was ^{13}C depleted compared to CO_2 emitted by microbial respiration of SOM. $\delta^{13}CO_2$ from the unplanted mesocosms did not vary with elevation in 2012 ($p > 0.1$), but in 2013 CO_2 at the lowest (wettest) elevation was ^{13}C depleted by 1.60-1.87‰ compared to the highest elevation ($p < 0.001$, Table 2).

Carbon fluxes and priming

There was a significant interaction between the effects of plant presence and elevation on SOM-derived CO_2 flux in both years ($p < 0.05$). Elevation had no effect on the rate of CO_2 flux from unplanted mesocosms ($p > 0.1$, Figure 3), all of which was considered SOM-derived due to the absence of plant respiration. In the planted mesocosms, SOM-derived CO_2 flux declined significantly from mid to high elevation ($p < 0.05$) in both years, but there were no significant differences between mid and low elevation ($p > 0.1$, Figure 3). The SOM-derived CO_2 flux was always higher in planted than in unplanted mesocosms, but the effect was more pronounced in 2012 than in 2013 (Figure 3). From 2012 to 2013, the CO_2 flux decreased by 27% from unplanted mesocosms ($p < 0.001$) and by 62% from planted mesocosms ($p < 0.001$, Figure 3).

Table 1 Left: r^2 values between biomass parameters (aboveground, belowground, and total biomass) and SOM-derived CO_2 flux. Right: Mean priming in percent of mean CO_2 flux from unplanted mesocosms of the three different elevations (high, mid, and low) relative to the reference marsh platform. All values are given under the assumption of different δ_p end member terms (see Isotope and CO_2 flux calculations). $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***); not significant (ns)

		r^2 values						*percentage priming*		
Year	assumption	Aboveground		belowground		total		high	mid	low
2012	*MWEM*	0.575	***	0.007	ns	0.154	ns	125	267	250
	AEM	0.528	**	0.046	ns	0.245	ns	148	292	256
	BEM	0.575	**	0.003	ns	0.134	ns	117	254	243
	aMWEM-1	0.570	**	0.004	ns	0.138	ns	120	263	243
	aMWEM-2	0.547	**	0.030	ns	0.216	ns	136	288	250
2013	*MWEM*	0.670	***	0.054	ns	0.303	*	17	125	63
	AEM	0.287	*	0.202	ns	0.389	*	77	154	80
	BEM	0.667	***	0.070	ns	0.334	*	-3	112	45
	aMWEM-1	0.521	**	0.133	ns	0.393	*	2	95	26
	aMWEM-2	0.388	*	0.307	*	0.567	**	51	161	61

MWEM: Mass-weighted end member

AEM: aboveground $\delta^{13}C$

BEM: belowground $\delta^{13}C$

aMWEM-1: alternative Mass-weighted end member 1

aMWEM-2: alternative Mass-weighted end member 2

Table 2 $\delta^{13}C$ of plant biomass and $\delta^{13}CO_2$ emitted from unplanted and planted mesocosms in 2012 and 2013. Values are mean ± standard error (n = 5)

	$\delta^{13}C$ aboveground biomass		$\delta^{13}C$ belowground biomass		$\delta^{13}CO_2$ planted mesocosms		$\delta^{13}CO_2$ unplanted mesocosms	
Elevation	*2012*	*2013*	*2012*	*2013*	*2012*	*2013*	*2012*	*2013*
High	-24.33	-25.56 ± 0.35	-22.74§	-24.23 ± 0.33	-19.14 ± 0.38	-22.97 ± 0.27	-16.80 ± 0.19	-15.22 ± 0.16
Mid	-25.68	-27.05 ± 0.03	-24.58§	-26.61 ± 0.07	-20.34 ± 0.35	-24.78 ± 0.06	-16.78 ± 0.08	-14.95 ± 0.34
Low	-25.69	-27.10 ± 0.03	-24.60§	-26.59 ± 0.03	-18.99 ± 0.23	-24.93 ± 0.11	-17.28 ± 0.31	-16.82 ± 0.24

§) Values are calculated from aboveground biomass $\delta^{13}C$ as described in *Isotope and CO_2 flux calculations*

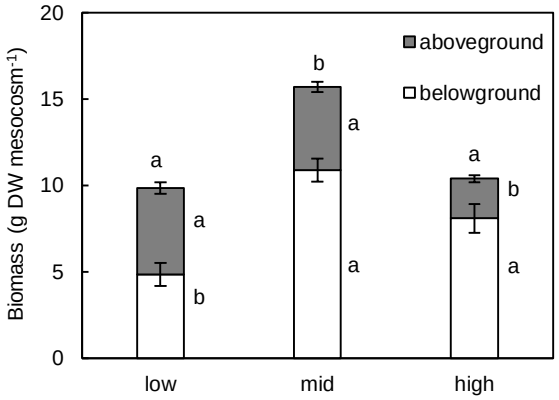

Figure 2 Belowground and aboveground biomass of *Schoenoplectus americanus* at three different elevations relative to the reference marsh platform, low (-15 cm), mid (±0 cm), and high (+20 cm), after two consecutive growing seasons in field-based mesocosms. Values are mean ± standard error (n = 5). Significant differences ($p < 0.05$) are indicated by different letters

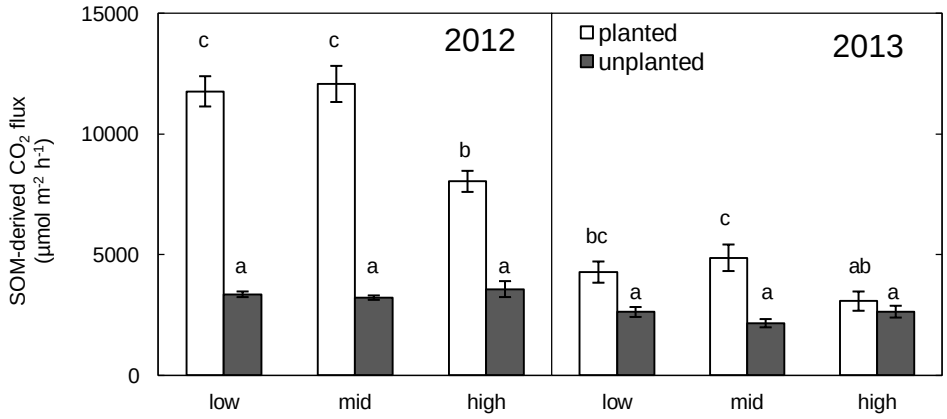

Figure 3 Soil organic matter derived CO_2 flux from unplanted and planted mesocosms at three different elevations relative to the reference marsh platform, low (-15 cm), mid (±0 cm), and high (+20 cm), in 2012 and 2013. Presented are mean values (n = 5) ± standard errors. SOM-derived CO_2 flux from planted mesocosms was calculated using the mass-weighted end member term in equation 1. Significant differences ($p < 0.05$) are indicated by different letters for 2012 and 2013 separately

Priming was greatest at mid elevation with no significant differences between mid and low elevation ($p > 0.1$) and was positively related to measures of plant biomass (Figure 4; Table 1). Both SOM-derived CO_2 flux and priming-driven increases in decomposition were positively related to aboveground biomass. This result held across the two years, and applied regardless of the δ_p end member assumption (Figure 4; Table 1). Furthermore, priming remained significantly and positively related to aboveground biomass when we excluded the driest, high elevation mesocosms from the 2013 regression analysis (Figure 4; $r^2 = 0.509$; $p < 0.05$), indicating that the relationship was not driven by the effects of desiccation on microbial activity. Priming also positively related to total biomass in both years, but these results were not robust under the assumption of different potential δ_p end member terms in 2012 (data not presented). SOM-derived CO_2 flux was related to total biomass in 2013 only (Table 1). Belowground biomass showed a weak but significant relationship with SOM-derived CO_2 flux and priming in 2013 only, but this result was not robust under the assumption of different δ_p end member terms (Table 1). The fact that the relationship between priming and biomass differed for aboveground vs. belowground biomass is consistent with the finding that no significant relationship was found between aboveground and belowground biomass ($r^2 = 0.002$, $p > 0.8$).

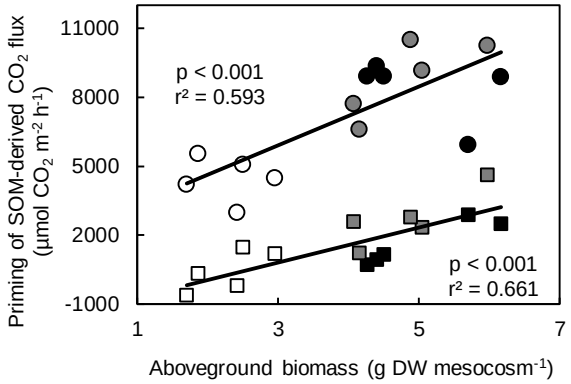

Figure 4 Priming of SOM-derived CO_2 flux as a function of aboveground biomass in 2012 (circles) and 2013 (squares). Data points are colored by elevation relative to the reference marsh platform: white = high (+20 cm), grey = mid (±0 cm), and black = low elevation (-15 cm). SOM-derived CO_2 flux was calculated using the mass-weighted end member term in equation 1

At all three elevations, CH_4 contributed <1% of total mesocosm C flux (CO_2 + CH_4) and <2% compared to SOM-derived CO_2 flux and was therefore negligible as a product of microbial respiration. Significant rates of CH_4 oxidation occurred only at the low elevation, where approximately 57% of CH_4 produced was oxidized to CO_2 (Table S1), an amount that accounts for <0.5% of the total mesocosm CO_2 flux at the corresponding elevation. Consequently, the likelihood that we underestimated SOM-derived CO_2 flux (and thus overestimated plant-derived flux) at the low elevation was assumed to be small.

Discussion

Contrary to our hypothesis, decomposition of aged, recalcitrant soil organic matter in a tidal wetland setting was largely insensitive to flooding depth and duration. By contrast, SOM decomposition rates were highly sensitive to the presence of plants (Table 1) and strongly positively related to plant biomass (Figure 4). Vascular plants can accelerate the decomposition of SOM in tidal marshes through at least two non-exclusive mechanisms. First, plants support microbial respiration by supplying terminal electron acceptors (TEAs) via root oxygen loss (ROL) to water-saturated soil environments. Oxygen via ROL directly supports aerobic respiration as a TEA (Wolf et al. 2007) and supports anaerobic respiration indirectly by regenerating oxidized forms of nitrogen, iron, or sulfur that function as alternative TEAs in the absence of O_2 (Frenzel et al. 1999, Neubauer et al. 2005, Laanbroek 2010, Sutton-Grier and Megonigal 2011). Second, plants deliver labile organic compounds to the rhizosphere, where they stimulate microbial activity and lead to accelerated mineralization of recalcitrant SOM, a phenomenon known as substrate-induced priming (Blagodatskaya and Kuzyakov 2008). Both substrate-induced and O_2-driven priming can be expected to scale positively with aboveground biomass, providing explanations for the relationships in Figure 4. Photosynthates are translocated to roots and available to support anaerobic respiration on a scale of hours (Megonigal et al. 1999), and anaerobic processes such as methanogenesis have been shown to vary directly in proportion to the photosynthetic capacity of wetland plants (Vann and Megonigal 2003).

By comparison, there is little known about how ROL rates scale with plant biomass because ROL is difficult to measure. The first step in ROL is for O_2 to enter aboveground biomass, making it possible that the process is limited by the surface area of shoots. Indeed, Tanaka et al. (2007) reported that ROL correlated to stem diameter in *Phragmites* stems. In addition, there is evidence that ROL is governed by the photosynthetic activity of a plant

(Caffrey and Kemp 1991, Frenzel et al. 1992, Connell et al. 1999). Although ROL rates might reasonably be expected to relate to belowground biomass, it is well established that only specific root tissues (e.g. tips and laterals) are permeable enough to allow for O_2 transfer (Colmer 2003, Koop-Jakobsen and Wenzhöfer 2015). Consequently, ROL does not necessarily correlate with belowground biomass (Wießner et al. 2002). In the present study, higher soil redox potentials in planted vs. unplanted mesocosms (Figure 1) support ROL as one mechanism responsible for accelerated SOM decomposition. However, the enhanced supply of TEAs cannot be the sole mechanism governing SOM decomposition because SOM-derived CO_2 flux and priming were not related to soil redox potentials. That is, redox increased linearly with elevation, while the relationship between elevation and SOM decomposition in the presence of plants did not (Figure 1 and 3).

Substrate-induced priming (SIP) was first invoked as an important control of organic decomposition in tidal marshes by Hemminga et al. (1988). However, while SIP has become an increasingly well understood process in terrestrial ecosystems, very little is known about SIP in wetlands generally and in tidal marshes specifically. SIP is driven by rhizodeposits and root exudates as carbon substrates for microbial metabolism (e.g. Kuzyakov 2002). Although comprehensive knowledge of both root exudate chemistry and *in situ* quantity does not exist (Jones et al. 2009, Haichar et al. 2014), it has been suggested that the release of root exudates to the rhizosphere is an inevitable result of plant productivity that accounts for 2-4% of net photosynthesis (Jones et al. 2004). However, root exudate-induced priming is not necessarily the result of an uncontrolled release of exudates. Under nutrient limitation, plants can benefit from active secretion of root exudates to prime the mineralization of organically bound nutrients such as nitrogen, which are mobilized through enhanced SOM decomposition (Jones et al. 2004, Blagodatskaya and Kuzyakov 2008, Phillips et al. 2011). Because plant growth at the present study site is nitrogen limited (Langley and Megonigal 2010, Langley et al. 2013), both uncontrolled and active release of exudates are plausible causes for the observed priming effect. The active release of exudates to enhance nutrient availability may be an overlooked process that structures plant communities and supports plant productivity in tidal marshes.

Regardless of the plant physiological mechanisms responsible for the observed priming effect, our results suggest that SOM decomposition in tidal marshes may be largely driven by aboveground plant productivity. A dependence of SOM decomposition on aboveground processes has previously been shown for several crop species such as maize, wheat, and sunflower (Kuzyakov and Cheng 2001, 2004, Dijkstra et al. 2006). Furthermore, there is a

growing body of literature illustrating a tight link between aboveground processes and soil microbial respiration, as well as the resulting consequences on carbon cycling in terrestrial ecosystems. In grasslands, the rate of photosynthesis drives both the autotrophic and heterotrophic components of soil respiration at seasonal timescales (Gomez-Casanovas et al. 2012), while in forests canopy processes influence soil respiration at seasonal and hourly timescales (Savage et al. 2013). Our results indicate that the coupling of these processes could be similarly relevant in tidal marshes and is likely to have important implications for the carbon balance of these ecosystems.

Plants increased SOM-derived CO_2 flux by 267% in 2012 and 125% in 2013. It is possible that the large drop in the magnitude of the SOM priming effect was the result of microbial respiration shifting from SOM in 2012 to belowground litter in 2013 when belowground litter from root turnover was presumably greater. Alternatively, the initial homogenization process may have artificially increased the lability of SOM in 2012, which then decreased in 2013 after the relatively labile compounds had been mineralized.

Effects of flooding on SOM decomposition in the absence of plants

Hydrology is a master variable that governs plant, microbial, and biogeochemical processes in wetlands, and the variable that our experiment was designed to capture with the most fidelity. Patterns of soil redox potential with elevation and the presence of plants were consistent with expectations based on tidal marsh biogeochemistry, decreasing with elevation loss (i.e. increasing flooding depth and duration) and increasing with plant presence (Figure 1). The increase in mean redox potential between the lowest and highest elevation mecososms (Δ = 198-235 mV, unplanted treatment; Δ = 90-155 mV, planted treatment) should have been large enough to stimulate microbial respiration rates based on controlled redox studies (Yu and Patrick 2003, Yu et al. 2007). Despite these expected responses, SOM decomposition in the absence of plants was not sensitive to flooding depth and duration (Figure 3). This counterintuitive result does not agree with our hypothesis, but it is consistent with a previous study performed in three Chesapeake Bay tidal marshes (including the present site) that used a membrane bag technique to quantify SOM decomposition rates (Kirwan et al. 2013).

Perhaps the most likely explanation for the insensitivity of SOM decomposition to flooding depth and duration is O_2 limitation. Redox potentials at 10 cm depth in the unplanted mesocosms were always <100 mV, suggesting that O_2 availability was limited in the absence of plants across all flooding treatments. It is possible that high water retention in the organic soils

used in our study limited O_2 diffusion even in the less frequently flooded mesocosms of the high elevation treatment (Rawls et al. 2003). Indeed, sapric organic soils (similar to the soil in this study) have been shown to have low water yield coefficients (i.e. the quantity of water that drains when the water level falls) and low hydraulic conductivity (Boelter 1969). A related point is that redox was measured only in the top 10 cm and did not represent the decomposition environment of most of the soil in our 70-cm-deep mesocosms; the influence of flooding depth and duration on redox is expected to decrease with increasing soil depth. Finally, it is not clear that SOM decomposition rates should have been sensitive to O_2 availability given the fact that SOM is recalcitrant by our operational definition.

Other mechanisms regulating SOM decomposition responses to sea level

SOM decomposition rates are controlled by a number of factors other than redox potential and the presence of plants that were not directly evaluated in the present study. For example, carbon quality can sometimes induce greater variation in litter decomposition rates than hydrology (Hemminga & Buth 1991). The SOM harvested from a depth of 50-100 cm for the present study was presumed to be quite recalcitrant under anaerobic conditions because of its age, but the large increase in decay rates in the presence of plants indicates that these aged SOM compounds were quite sensitive to biogeochemical phenomena in the rhizosphere.

Sulfate is the dominant alternative terminal electron acceptor in these iron-poor soils, and there is growing evidence that SO_4 availability may regulate SOM decomposition rates (Weston et al. 2006, Craft 2007, Sutton-Grier et al. 2011, Morrissey et al. 2014). It is possible that the negative effects of an increase in flooding on aerobic SOM decomposition were offset by the positive effects of an increase in SO_4 availability.

Although we did not measure soil moisture and porewater salinity, SOM decomposition may have declined at high elevation despite higher redox potentials as a consequence of suboptimum moisture levels and the accumulation of salts decreasing microbial activity. Specifically, phenol oxidase activity may have been inhibited in the relatively dry, high elevation treatments, causing phenolic compound concentrations to increase and microbial exoenzyme activity to decrease at the soil surface (Toberman et al. 2008). Finally, the relatively small priming response in the high elevation mesocosms may have been caused directly by the effects of dry soil conditions on microbial activity (Kuzyakov 2002), rather than by the effects of reduced plant biomass on the supply of labile carbon or O_2. However, we concluded that drying was not a major driver of the priming process because the plant biomass-priming

relationship held even when data from the high, drier elevation were excluded. The influence of these mechanisms on SOM decomposition responses to flooding requires additional research.

Implications for modeling tidal marsh elevation and stability against sea level rise

Widely used tidal marsh elevation models treat SOM decomposition differently and none incorporate feedbacks between flooding duration and depth and decomposition rates (Rybzcyk and Callaway 2009). The Callaway model (Callaway et al. 1996) and the modification named WARMER (Swanson et al. 2014) simulate burial by varying SOM decay rates as a function of age and soil depth, but there is no feedback between flood duration and decomposition rate. SOM decomposition rates respond only to temperature in the Kirwan and Mudd (2012) marsh elevation-carbon model. The Marsh Equilibrium Model (MEM) of Morris et al. (2002) adds a constant fraction of annual primary production to the SOM pool, which means that SOM storage (the inverse of decomposition) effectively varies only indirectly as a function of flooding on plant production. Our results suggest that SOM decomposition rates do vary with relative sea level, but the flooding-decomposition response curve may not be a simple monotonic decrease in rate with increasing flooding.

The present study was inspired by the fact that MEM is able to simulate an accurate soil carbon profile for our long-term study site (J. Morris, pers. comm.) based only on a plant production-flooding relationship. Here we interpret the positive linear relationship between aboveground plant biomass and SOM decomposition rate as evidence that plant production regulates microbial activity. An important implication of this relationship is that the flooding-SOM decomposition response curve should mirror the flooding-plant production curve. If so, MEM could be expected to generate an accurate soil carbon profile despite the lack of a flooding-SOM decomposition feedback, but only by overestimating carbon inputs through belowground production.

SOM decomposition in the presence of plants did not significantly differ between the mid-elevation mesocosms (mean soil elevation ± 0 cm relative to our reference marsh) and low-elevation mesocosms (mean soil elevation -15 cm) despite differences in flooding depth and duration. Based on this, we conclude that increased flooding due to RSLR will have a negligible impact on SOM decomposition, supporting the conclusions of Kirwan et al. (2013). However, we also demonstrate that SOM decomposition rates can be tightly coupled to the aboveground biomass-flooding response over a large range of elevations. Considering the sharp decrease in aboveground production with larger differences in relative elevation than we tested

here (Kirwan and Guntenspergen 2012, Langley et al. 2013), we propose that SOM decomposition will decline with plant production as marshes lose relative elevation. This may increase the fraction of plant production that is ultimately sequestered as SOM. Further research is required to determine the extent to which declining SOM decomposition rate will offset declining belowground production at higher rates of RSLR.

Acknowledgements

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Supporting information (Chapter 3)

CH_4 flux and CH_4 oxidation measurements

For CH_4 flux measurements, gas samples were taken every 30 min (total five per mesocosm) on four planted mesocosms per elevation (n = 4). Sampling followed the procedure for CO_2 flux measurements. Samples were analyzed within six hours using a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector (Kyoto, Japan). Emissions were calculated as change in methane concentration over time.

To investigate the extent to which methane oxidation contributed ^{13}C-depleted CH_4 to the $^{13}CO_2$ pool, we measured methane emissions in 2012 in the presence of a specific inhibitor of methane oxidation, methylfluoride (CH_3F). The method was adopted with slight modifications from Megonigal & Schlesinger (2002). Briefly, immediately following CO_2 and methane flux measurements (pre-treatment measurements), CH_3F was added to the headspace of closed flux chambers to a concentration of 1.5%, and allowed to diffuse through plants and soil for 12 hours. After venting the chambers for 1-2 hours, post-treatment measurements were conducted using the same procedure as described for the pre-treatment measurements. A second set of post-treatment measurements were conducted after 24 hours to observe the extent

to which methane oxidation activity returned once the methyl fluoride-driven inhibition of methanotrophy had been removed.

Table S1 Mean methane (CH_4) flux, total mesocosm C flux, and CH_4 oxidation as calculated from the difference between pre- and post-CH_3F treatment CH_4-flux measurements on planted mesocosms in 2012. P-values are for one-sided paired t-tests between pre- and post-treatment measurements

Elevation	CH_4 flux [µmol m^{-2} h^{-1}]	Total C flux [µmol m^{-2} h^{-1}]	CH_4 flux [% of total C flux]	CH_4 oxidation [% CH4 flux]	
high	91.1	13102.3	0.7	0*	p >0.1
mid	129.7	21801.7	0.4	37.6	p >0.5
low	59.5	15159.2	0.6	56.6	**p <0.5**

* Includes negative values

Comparision between tissue $\delta^{13}C$ and $\delta^{13}C$ of dark respired CO_2 in S. americanus

Schoenoplectus americanus was harvested in the adjacent marsh during the second half of June 2013. To prevent damage to the root system, soil of approximately 6 L containing roots and rhizomes was retrieved along with 5-8 shoots using a square shovel at each sampling spot. Plants were washed free of soil and kept in hydroculture for a period of 5-7 days, then roots and rhizomes were again thoroughly rinsed using deionized water prior to CO_2 flux measurements. Flux measurements followed the CO_2 flux procedure used on the marsh organ mesocosms. Plants were transferred to opaque PVC flux chambers (8-12 plants per flux chamber, n = 5). Flux chambers were capped on both ends and flushed with CO_2-free air. When CO_2 concentrations exceeded 1500 ppmv, gas samples were sampled through a rubber septum using a 20-mL polyurethane syringe, then transferred into N_2-flushed and evacuated 12-mL Exetainer storage vials (Labco, UK). Gas samples were analyzed for $\delta^{13}C$ in CO_2 at the Stable Isotope Facility of the University of California, Davis. Immediately after gas sampling, plants were separated into aboveground and belowground biomass and dried at 60°C to constant weight. Aboveground and belowground material was then ground and analyzed for $\delta^{13}C$ at the Smithsonian Stable Isotope Mass Spectrometry Facility, Suitland, MD.

4 Man-made blue carbon: short- and long-term carbon sequestration in artificial salt marshes of the European Wadden Sea

Under Review for Ecological Applications

Peter Mueller, Nils Ladiges, Alexander Jack, Gerhard Schmiedl, Lars Kutzbach, Kai Jensen, and Stefanie Nolte

Abstract

Salt marshes and other "blue carbon" ecosystems have been increasingly recognized for their carbon-sink function. Yet, an improved assessment of both organic carbon (OC) stocks and C sequestration rates is still required to include blue C in C-crediting programs. Particularly the variability in long-term rate and permanence of sequestration across systems and regions, and the potential of artificial ecosystems for C sequestration, such as constructed and restored tidal wetlands, requires an improved understanding. This study reports on C sequestration in the salt marshes of the UNESCO world-heritage site of the European Wadden Sea and provides first data on short (years), mid (decades), and long-term (centuries) C sequestration in man-made blue C systems. We studied C sequestration in three mainland marshes conducting deep (1.3 m) sampling and regular surface accretion measurements in combination with available historic accretion data based on radionuclide dating. Different ecosystem stages were identified by the down-core distribution of foraminifera and C stable-isotope analyses. Although large amounts of OC are lost down-core in the well-aerated marsh soils, long-term and mid-term C sequestration rates (1.12-1.49 Mg C ha^{-2} yr^{-1}, respectively) are in range with rates reported from other blue C systems. Our study emphasizes the importance of deep sampling in combination with long-term accretion records and knowledge on ecosystem development to avoid vast overestimation of C sequestration and OC stocks in temperate marsh systems with decomposition maintained deep in the profile. Despite large differences in top soil OC contents, elevation, and other factors, we demonstrate remarkably similar mean OC densities across sites after few decades of ecosystem development. If this finding is applicable to other tidal wetland systems within a given regional context (i.e. within one estuary), it has important implications for modeling long-term C sequestration in young artificial sites such as constructed or restored tidal wetlands.

Introduction

Salt marshes and other vegetated coastal ecosystems, such as mangroves and seagrass beds, have been increasingly recognized for their carbon-sink function in recent years. In these "blue C" ecosystems, rates of C sequestration exceed those of most other ecosystem types, because slow rates of organic matter decomposition co-occur with high rates of organic matter input, through both autochthonous primary production and import of allochthonous organic matter (Duarte et al. 2005, Bridgham et al. 2006, Mcleod et al. 2011). Furthermore, regular sulfate inputs through seawater effectively suppress methane production and consequently emissions, thereby improving the greenhouse-gas (GHG) balance of coastal ecosystems (Poffenbarger et al. 2011). Therefore, protection, restoration, and construction of blue C ecosystems as GHG-offset activities are of high interest for policy makers in the context of C crediting and climate-change policy (Nellemann et al. 2009, Callaway et al. 2012). Yet, such measures should be based on a thorough understanding of processes in these ecosystems.

The understanding of factors controlling C sequestration in coastal ecosystems has greatly improved over recent years. For instance, efforts have been devoted to explore the effects of land use, disturbance, and management on C sequestration (Yu and Chmura 2009, Macreadie et al. 2013, 2017, Kauffman et al. 2014, Elschot et al. 2015). Further research was focused on the effects of invasive species (Martin and Moseman-Valtierra 2015, Mueller et al. 2016a, Bernal et al. 2017) and global change factors such as sea level rise (Kirwan et al. 2013, Mueller et al. 2016b), elevated concentration of atmospheric CO_2 (Langley et al. 2009, 2013), or rising temperatures (Kirwan and Mudd 2012) on primary production, soil organic matter decomposition, and GHG emissions to estimate the extent to which rates of C sequestration may change in future. However, in order to include blue C in C-crediting programs, an improved assessment of both current OC stocks and sequestration rates is still required (Mcleod et al. 2011), particularly with respect to the areal extent of blue C ecosystems and variability in long-term rate and permanence of sequestration across ecosystem types and regions (Nellemann et al. 2009, Mcleod et al. 2011, Saintilan et al. 2013, Ewers Lewis et al. 2017).

Carbon sequestration in blue C systems is typically described by the product of OC density of the soil or sediment (g OC cm^{-3}) and accretion rate (cm yr^{-1}) of the system (Chmura et al. 2003). Unlike most terrestrial soils, which become OC-saturated over time (Schlesinger and Lichter, 2001), soils or sediments of blue C systems build up vertically with rising sea level. As a consequence, these ecosystems can keep up C sequestration over millennia, leading to rates of long-term C sequestration per area that exceed those of terrestrial climax systems by

orders of magnitude (Mcleod et al. 2011). Differences in C-sequestration rate between blue C and terrestrial systems consequently depend on the time period considered, with increasingly higher rates in blue C vs. terrestrial systems over time. Therefore, it is important to always relate rates of C sequestration to the time period considered. For instance, credits for blue C are likely to apply to OC that is removed permanently or over centuries, but not to OC that is dissimilated and returned to the atmosphere over shorter time periods (Crooks et al. 2010, Sifleet and Murray 2011, Callaway et al. 2012, Murray 2012).

The distinction between deep long-term (centuries-millennia) and surface short-term OC accumulation (months-years) is necessary, however, surprisingly often left unconsidered. For instance, a considerable amount of data on which estimates of global blue-C sequestration rates are based stems from studies using feldspar-horizon techniques (e.g. Chmura et al. 2003 and references therein), thereby only assessing the OC density of the top (usually <20cm) soil/sediment. For systems in which OC density and content are relatively stable with soil depth, such shallow assessments are usually sufficient because long-term sequestration and surface OC accumulation should not substantially differ. If larger amounts of OC are continuously lost in deeper soil horizons (Cahoon et al. 1996, Connor et al. 2001, Wang et al. 2011, Livesley and Andrusiak 2012), however, deeper sampling is required and distinctions between short-term and long-term C sequestration over different time periods are necessary in order to avoid overestimation (Saintilan et al. 2013, Kelleway et al. 2016).

The present study provides first data on short- and long-term C sequestration for the salt marshes of the UNESCO world-heritage region of the European Wadden Sea (WS). The salt marshes of the WS region account for approx. 20% of the European salt-marsh area (Doody 2008). Among these, the salt marshes on the mainland coast are mostly artificial systems which have been constructed for means of land reclamation in the late 19^{th} and early 20^{th} century (Hofstede 2003, Esselink et al. 2009). To our knowledge, there are no reports on long-term C sequestration for artificial systems such as constructed or restored tidal wetlands available yet, because most of these systems are simply too young for long-term assessments (Craft et al. 1999, 2003, Callaway et al. 2012, Ballantine and Schneider 2014). The WS salt marshes therefore provide a unique system to study man-made blue C.

Yet, for these artificial WS salt marsh systems, the separation of surficial OC accumulation and long-term C sequestration may be particularly relevant. Firstly, OC content and density are not expected to be stable with depth. Surface accretion in these minerogenic systems depends on inputs of mineral sediments as opposed to persistence of organic matter in

organic marshes, therefore declines in organic-matter content with depth are less relevant for marsh stability against sea level rise in these systems (Allen 2000). Indeed, a large fraction of the organic matter feeding the OC pool is of allochthonous origin and may be rapidly decomposed upon deposition. Therefore, it is possible that a considerable amount of allochthonous material in the top soil is lost with soil depth (Saintilan et al. 2013). Furthermore, WS marshes are situated relatively high in the tidal frame, which leads to oxidizing conditions in the top soil and substantive rates of decomposition (Allen 2000, Mueller et al. 2017).

The determination of long-term C sequestration rates is additionally challenging because these young and rapidly developing systems have undergone several vegetation shifts in the course of ecosystem succession over the last 80-120 years. However, predictions of long-term C sequestration rates should be based on a stable/mature ecosystem stage. C sequestered during early developmental stages (i.e. the pioneer zone) may be relevant for stock estimation, yet reveals little information for the actual rate of long-term sequestration. The identification of these different successional stages down-core is crucial to interpret possible changes in OC density and content. Furthermore, accretion rates may have varied considerably during different developmental ecosystem stages (van Wijnen and Bakker 2001). Thus, knowledge on accretion rates during early marsh development and their relation to accretion rates of the mature/stable ecosystem stage is equally important as knowledge on changes in OC density for a precise assessment of C sequestration in young systems.

The aim of this study is to test the predictability of both OC density down-core and accretion over time, and consequently short- and long-term C sequestration rates in young, artificial salt-marsh systems. We hypothesize first (i), that OC contents and thus also OC density will decrease down-core and stabilize at a certain depth at which decomposition is suppressed by low redox potentials or increasing recalcitrance of the remaining organic material. Consequently, rates of short-term C sequestrations that are based on OC density of the top soil will be substantially higher than rates based on deeper assessments (i.e. mid- and long-term C sequestration), if stable rates of accretion are assumed. We hypothesize second (ii), that accretion during early ecosystem development was higher (van Wijnen and Bakker 2001, Butzeck et al. 2014) than current rates, thereby partially counterbalancing the effect of decreases in OC density down-core on C sequestration.

Methods

Study sites and accretion record

The study was conducted in three artificial mainland salt marshes along the German WS coast in the Wadden Sea National Park Schleswig-Holstein. All marsh sites have traditionally been used for livestock grazing. Since the foundation of the National Park in 1985, grazing has been abandoned in some parts of the sites, yet, has been sustained as a nature-management practice in others (Esselink et al. 2009).

The Dieksanderkoog marsh (DSK) is situated at the outer mouth of the Elbe estuary. The adjacent sea wall was built in 1935 for land reclamation, and construction of brushwood groins and ditching in the mudflat led to high sedimentation rates and a rapid growth of the salt marsh that today extends approx. 2 km from the seawall to the mud flats. The marsh elevation ranges from 1.2 to 2.8 m NHN. Tidal range in the area is 3.0 m with a mean high tide at 1.62 m (BSH, 2011, Müller et al. 2013).

The Westerhever marsh (WH) is situated on the peninsula of Eiderstedt. It is part of a larger marsh complex in the Tümlau Bay. In 1897, a small patch of naturally developed salt marsh of approx. 3 ha, separated from the mainland by a tidal creek, was accessed through dam construction. Damming and ditching activities led to a rapid increase in salt-marsh area to at least 161 ha until 1909. Today the marsh extends approx. 1 km from the seawall to the mud flats and covers an area of approx. 250 ha. The marsh elevation ranges from 1.5 to 2.4 m NHN at a tidal range of approx. 3 m with mean high tide at 1.45 m NHN (Peiter 2002, BSH 2011).

The Sönke-Nissen-Koog marsh (SNK) is situated in the North of the National Park. The adjacent sea wall was built in 1924, and the salt marsh began to develop. Today, the marsh extends approx. 1 km from the seawall to the mud flats and has an elevation between 0.9 and 2.6 m NHN at a tidal range of 3.4 m with mean high tide at 1.59 m NHN (BSH 2011, Müller et al. 2013).

In all three marsh sites, annual measurements of surface elevation are conducted using Sedimentation Erosion Bars (SEBs; Nolte et al. 2013a) since 2006 at DSK and SNK and since 2008 at WH, allowing calculation of current accretion rates. For WH, additional accretion data obtained from regular leveling campaigns since 1998 is available (Stock 2012). For the assessment of historic accretion rates at DSK and SNK, ^{137}Cs dating was conducted in deep soil cores that were taken directly next to the SEB positions in 2006 (Nolte et al. 2013b). The available historic accretion data for WH is based on ^{137}Cs dating of a soil core taken in an adjacent marsh patch of the Tümlau Bay in 2013 (Müller-Navarra et al. unpublished) (Figure

S1). In all soil cores, ^{137}Cs peaks of two historic events could be identified: an upper peak caused by the Chernobyl disaster of 1986 and a deeper peak caused by nuclear bomb tests during the 1960s that had their maximum in 1963 (Kirchner and Ehlers 1998).

Sampling and elemental analyses

Duplicate soil cores of 130 cm depth were taken within grazed areas of each site in order to sample a profile that has developed under the same land use or management over time. Cores were taken in April 2016 within 2 m of the SEB positions. We used an Eijkelkamp peat sampler with a 50-cm long, 500-cm^3 shuttle head (equipment that is usually used for sampling of peat soils, e.g. bogs or organic marshes) for our mineral-dominated marsh soils. As the peat sampler cuts side-wise into the soil profile, the technique allowed for uncompacted and undisturbed sampling (Jowsey 1966). Soil cores were sliced into eight increments of 5 cm (top 40 cm), 5x10 cm (40-90 cm), and 2x20 cm (90-130 cm).

Bulk density and OC content were determined to calculate OC density. Samples were dried at 105°C for 72 h and weighed to determine bulk density. OC contents were determined using a total carbon analyzer (liquiTOC II, Elementar, Hanau, Germany).

In order to assess origin of the accumulated organic matter (autochthonous vs. allochthonous sources), we determined OC/N ratios and δ^{13}C (Khan et al., 2011). Total nitrogen (N) for calculation of OC/N ratios was analyzed using an element analyzer (VARIO Max Cube, Elementar, Hanau, Germany). δ^{13}C was analyzed using an isotope-ratio mass spectrometer (New Horizon, HEKAtech, Wegberg, Germany). Results are given in δ-notation vs. VPDB and measurements were calibrated using the international standard caffeine. δ^{13}C was assessed in one core per site.

Down-core identification of successional ecosystem stages

The identification of different successional stages down-core is crucial to interpret changes in OC density and content. In the present study, we utilized the down-core distribution of foraminifera because their abundance and species composition exhibits a distinct vertical zonation in salt marshes relative to the tidal frame (Scott and Medioli 1978, Edwards et al. 2004). Between 3.0 and 3.5 g dry sediment/soil was washed over a 63-µm sieve and the residue >63 µm was dried and inspected for benthic foraminifera under a stereo microscope. Drying of samples may result in the destruction of fragile agglutinated tests (Scott and Medioli 1980);

however, the frequent occurrence of fragile tests of *Jadammina macrescens* in the studied salt-marsh samples indicated a reasonable representation of agglutinating taxa.

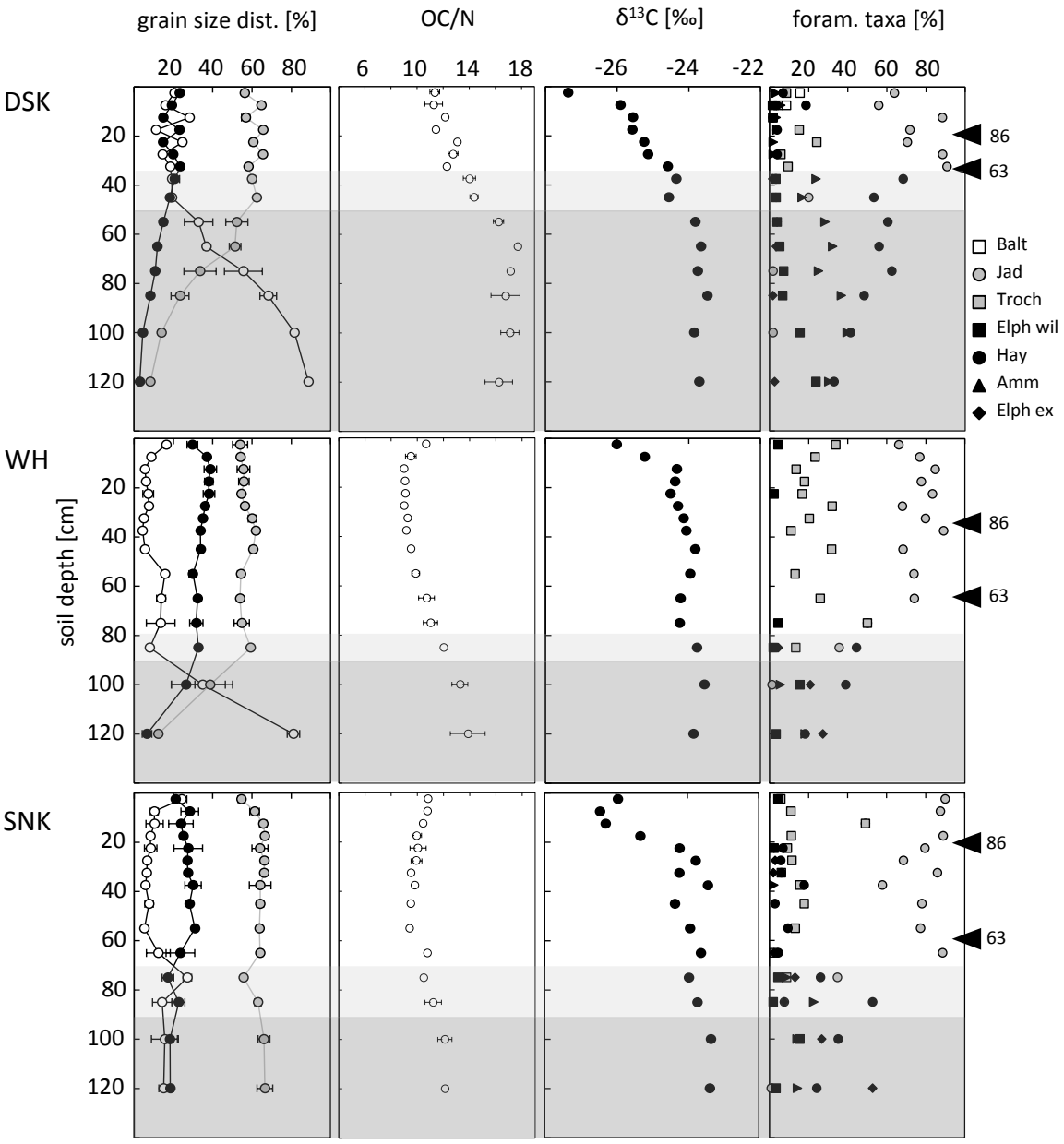

Figure 1 Grain-size distribution (white = sand; gray = silt; black = clay), organic C/N ratio, $\delta^{13}C$ of the organic material, and relative abundance of different foraminiferal taxa (black = indicators for mud flat; gray = indicators for salt marsh; white = indicators for high marsh) with soil depth in three artificial Wadden Sea salt-marsh sites Dieksanderkoog (DSK), Westehever (WH), and Sönke-Nissen-Koog (SNK). Triangles on the right indicate depth of 1986 and 1963 ^{137}Cs peaks. The darker gray area of each panel indicates the depth zone of the former mud flat, the lighter gray area indicates the transition zone between mud flat and salt marsh (i.e. pioneer zone), and the white area indicates the salt-marsh zone of the profile. Shown are mean values ± SD of duplicate cores for grain-size distribution and OC/N ratio. $\delta^{13}C$ and foraminiferal data are based on single cores. The species names of foraminifera are: Balt = *Balticammina pseudomacrescens*, Jad = *Jadammina macrescens*, Troch = *Trochammina inflata*, Elph wil = *Elphidium williamsoni*, Hay = *Haynesina germanica*, Amm = *Ammonia batava*, Elph ex = *Elphidium excavatum*.

If present, between 50 and 100 individuals were counted, which provides a confident estimate of environmental changes based on the overall low diversity of tidal-flat and salt-marsh foraminiferal assemblages (Fatela and Taborda 2002). The species designation was mainly based on Gehrels and Newman (2004) and Müller-Navarra et al. (2016). The down-core distribution of foraminifera was assessed in one core per site.

High percentages of the hyaline species *Ammonia batava, Elphidium williamsoni, Haynesina germanica,* and *Elphidium excavatum* indicate the depth zone of the former mud flat in all three marsh profiles (Müller-Navarra et al. 2016; Figure 1). In turn, high percentages of the agglutinating species *Jadammina macrescens* and *Trochammina inflata* are indicators for the vegetated salt-marsh system (Gehrels and Newman, 2004, Müller-Navarra et al. 2016; Figure 1). Between these clearly identifiable zones, a zone of 10-20 cm with intermediate abundances of both groups indicate the transition between mud flat and salt marsh, which is considered as pioneer zone here (Figure 1). Only at DSK, *Balticammina pseudomacrescens* is moderately abundant in the top soil (10 cm), indicating the zone of the less frequently flooded high marsh (Alve and Murray 1999, Gehrels and Newman 2004). The absence of this species at SNK and WH is in accordance with the current low-marsh vegetation in these sites, dominated by *Puccinellia maritima*. Contrary, *Festuca rubra*, a high-marsh plant species, is dominant at DSK.

Grain-size distribution was anticipated to be an additional indicator for ecosystem stage, as the fraction of larger particles (i.e. sands) is often greater in the lower elevated, early developmental ecosystem stages (i.e. pioneer zone) than in the higher elevated, late stages (i.e. high marsh) (Allen 2000). Grain-size fractions <63µm (silt fractions and clay) were determined on an automated sedimentation analyzer system (SEDIMAT 4-12, UGT, Müncheberg, Germany). Sand fractions were determined on a sieve shaker (Retsch, Haan, Germany).

Data Analysis

Rates of C sequestration were calculated by multiplying OC density [g OC cm^{-3}] (product of OC content [%] and bulk density [g cm^{-3}]) with accretion rate [cm yr^{-1}]. We base our calculations on three quantification periods: 1.) Short-term C sequestration is based on the OC density of the top soil (5 cm) and current (SEB based) accretion rates. OC density for the calculation of C sequestration over (2.) 30- and (3.) 53-year quantification periods, here classified as mid-term, are based on the mean OC density above the ^{137}Cs peaks for the years

1986 and 1963, respectively, calculated dividing the total amount of OC [g] by the volume of the core [cm^3] above the respective peak. The 30-year and 53-year accretion rates are calculated by dividing peak depth [cm] by time [years].

In order to test if OC density, accretion rate, and C sequestration depend on the quantification period, we compared mean OC density, mean annual rates of accretion, and C sequestration of the three defined time periods (short-term, 30 years, and 53 years*)* using Friedman's ANOVA. Additionally, stable regions of OC density down-core within the marsh zone of the profiles were assessed visually. This stable minimum OC density describes the value that mean OC densities integrating over long-term periods (e.g. centuries-millennia) approach and it is therefore used to estimate minimum or long-term C sequestration.

In order to identify important predictors other than time/depth on OC density, we conducted linear and non-linear regression analyses to test for relations between grain-size distribution, $\delta^{13}C$, OC/N, OC content, bulk density, and OC density. Analyses were conducted in Microsoft Excel 2010 (Microsoft, Redmond, WA, US) and STATISTICA 10 (StatSoft, Tulsa, OK, US).

Results

Identification of ecosystem stages and OC sources

Different ecosystem stages down-core could be identified quantifying the relative abundance of foraminiferal taxa (Figure 1). Additionally, at DSK and WH, sand contents are considerably higher and clay contents lower in the mud-flat zone of the profile compared to the salt-marsh zone. Differences in grain-size distribution between salt-marsh and mud-flat zone are not distinct at SNK.

Down-core changes in the stable OC-isotope composition and OC/N ratio, as potential indicators of organic-matter source, show few consistent patterns across sites. At all three sites, OC/N ratios are higher in the mud flat zone (10-18) than in the top soil (8-12). While the net change in OC/N from top soil to mud flat is >6 at DSK, net changes are <4 and <2 in the profiles of WH and SNK, respectively. However, OC/N ratios down-core reveal little information on the ecosystem succession.

At DSK, abrupt changes in $\delta^{13}C$ down-core coincide with the transitions from mud flat to pioneer zone and from pioneer zone to low marsh as based on the foraminiferal records. In the profiles of WH and SNK, relations between the foraminiferal record and $\delta^{13}C$ are less pronounced. Generally, OC of the top soil is more ^{13}C depleted (-26 to -28‰) compared to the

zone of the mud flat (≥-24‰; Figure 1). While ^{13}C enrichment occurs in the top 15 cm of the DSK and WH profiles, little change in $\delta^{13}C$ is detectable in the top 15 cm of the SNK profile (Figure 1). Overall, organic matter that accumulated since 1963 in the DSK profile is more ^{13}C depleted than that in the WH and SNK profiles (Figure 1). Organic matter that accumulated before 1986 in the SNK profile is less ^{13}C depleted than the organic matter that accumulated after 1986. In comparison, the $\delta^{13}C$ of the organic matter in the WH profile remains relatively stable for >10 cm above the 1986 mark (Figure 1).

Factors controlling OC content, bulk density, and OC density down-core

Organic C density is the product of OC content [%] and bulk density [g cm^{-3}]. In order to identify factors controlling OC density down-core, it is therefore necessary to identify the factors affecting OC content and bulk density.

Bulk density is predominantly controlled by OC content, with OC content explaining >90% of the variability in bulk density (Table 1). OC content considerably deceases with soil depth in all three marshes from 6-8% in the top soil down to 0.1-1.5% in the mud flat zone, whereas bulk density increases from 0.5-0.7 g cm^{-3} to 1.2-1.9 g cm^{-3} (Figure 2).

The strongest predictor for OC density is OC content, explaining >95% of the variability in OC density (Table 1). Similar to OC content, OC density decreases considerably with depth (Figure 2). In the profiles of WH and SNK, regions of both stable OC content and OC density within the salt-marsh zone of the profile are clearly discernible. In the WH profile, OC content and density are stable between 40 and 80 cm depth (mean ± SD: 1.51 ± 0.12%; 0.018 ± 0.002 g cm^{-3}). In the SNK profile, OC content and density are stable between 25 and 60 cm depth (2.12 ± 0.17%; 0.022 ± 0.001 g cm^{-3}). The salt-marsh zone of the DSK profile is shallow compared to WH and SNK. Still, within this shallow marsh zone in the profile, OC content and density tend to stabilize at 20 cm down to 35 cm (mean ± SD: 1.08 ± 0.09%; 0.015 ± 0.001 g cm^{-3}).

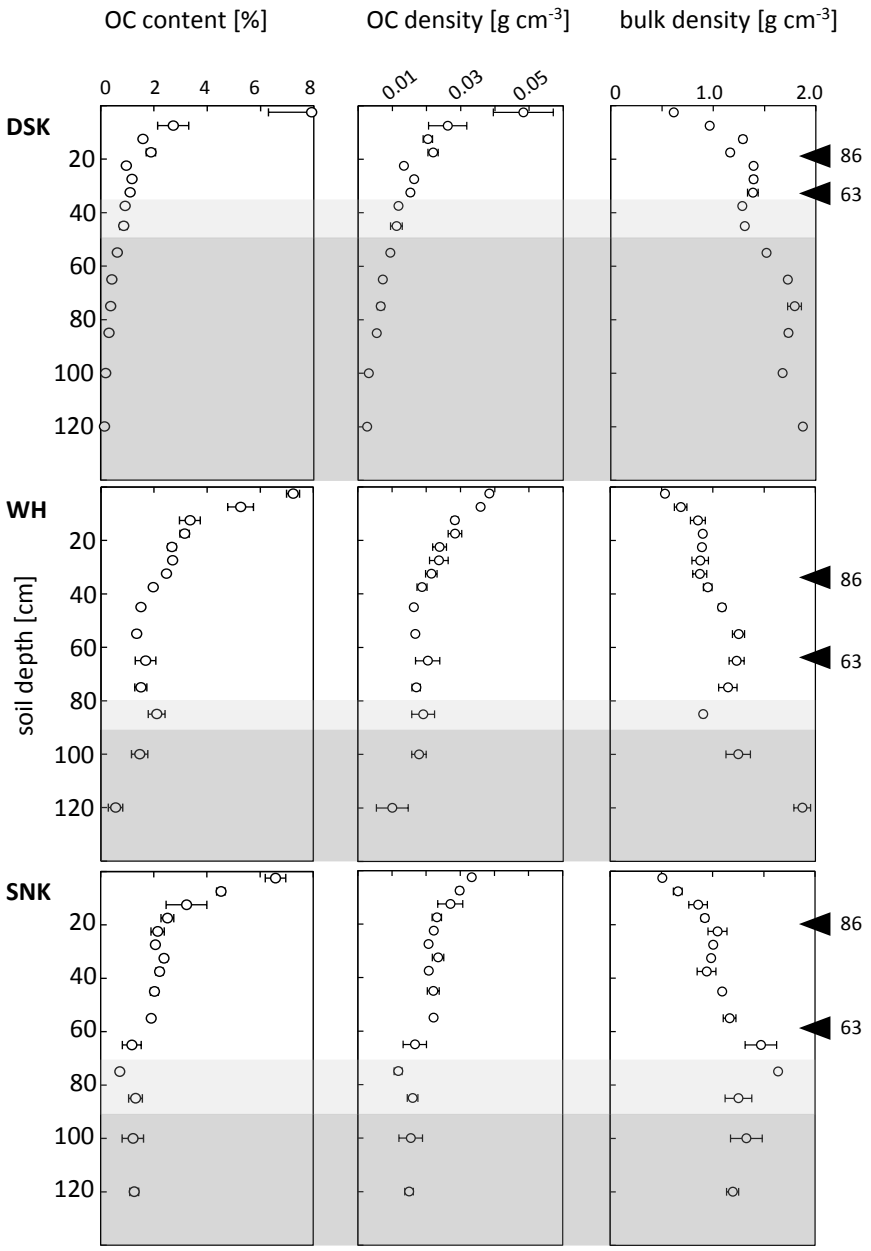

Figure 2 Organic C content [%], OC density [g cm^{-3}], and bulk density [g cm^{-3}] with soil depth in three artificial Wadden Sea salt-marsh sites Dieksanderkoog (DSK), Westehever (WH), and Sönke-Nissen-Koog (SNK). Triangles on the right indicate depth of 1986 and 1963 ^{137}Cs peaks. The darker gray area of each panel indicates the depth zone of the former mud flat, the lighter gray area indicates the transition zone between mud flat and salt marsh (i.e. pioneer zone), and the white area indicates the salt-marsh zone of the profile. Shown are mean values ± SD of duplicate cores.

Table 1 Linear and non-linear regression matrix: Lower left half presents R^2 values for relationships of best fit (based on linear, logarithmic, exponential, and power functions) between the parameters (from: left to right): OC density (OCD) soil depth; OC content (OCC), bulk density, sand, silt, and clay contents, $\delta^{13}C$ of the organic material, and OC/N ratio. Upper right half indicates the direction (+/-) of significant relationships. ns = not significant at $p > 0.05$; log = logarithmic function ; pow = power function; lin = linear function; exp = exponential function. Strong relationships with $R^2 > 0.5$ are bold typed

	OCD	depth	OCC	BD	sand	silt	clay	13C	OC/N
OCD		-	+	-	-	+	+	-	-
depth	**0.712 log**		-	+	+	-	-	+	+
OCC	**0.961 pow**	**0.773 log**		-	-	+	+	-	-
BD	**0.833 log**	**0.669 pow**	**0.904 log**		+	-	-	+	+
sand	**0.654 exp**	0.357 lin	**0.590 log**	**0.529 lin**		-	-	ns	+
silt	**0.526 pow**	0.336 exp	0.443 pow	0.349 log	**0.874 lin**		+	ns	-
clay	**0.714 pow**	0.317 exp	**0.658 pow**	**0.580 log**	**0.879 exp**	**0.679 pow**		ns	-
13C	**0.506 lin**	**0.767 log**	**0.536 lin**	0.392 exp	0.007 log	0.067 lin	0.035 log		ns
OC/N	**0.630 pow**	0.273 lin	0.629 log	**0.556 lin**	**0.767 pow**	0.410 lin	**0.743 exp**	0.054 lin	

Also bulk density shows stable regions down-core. However, stable regions of bulk density are not necessarily parallel to the stable regions of OC density. In the WH profile, bulk density is stable between 15 and 35 cm (0.885 ± 0.012 g cm^{-3}), which is located higher in the profile than the stable region of OC content and density. In the SNK profile, bulk density is stable between 20 and 40 cm (0.994 ± 0.038 g cm^{-3}), partly overlapping with the stable region of OC content and density (Figure 2). Only in the DSK profile, bulk density stabilizes with OC content and density at 20 cm down to 35 cm (1.390 ± 0.003 g cm^{-3}).

The high degree of inter-correlation between parameters (Table 1) makes it difficult to construct models that include multiple predictor variables (e.g. multiple regression) to explain OC density. Besides depth and OC content, bulk density is also strongly correlated (based on r^2 >0.5) with sand and clay contents and the OC/N ratio (Table 1). OC contents are also strongly correlated with $\delta^{13}C$, OC/N, as well as clay and sand contents (Table 1). However, while sand and clay contents seem to be important predictors for the OC content over the whole profile, there are no significant correlations between the two parameters and OC content when considering the top 40 cm only, where >90% of the change in OC content occurs (Figure S2).

Comparison of OC density, accretion, and C sequestration over time

Mean OC density decreases significantly with the quantification period and shows consistent decline patterns across sites (Figure 3). Surface OC density, a value required for the assessment of short-term C sequestration, is 0.040 ± 0.008 g cm^{-3} and shows high variability across sites (Figure 3). In comparison to that, mean OC density integrated over 30 years is reduced by 28% to 0.029 ± <0.001 g cm^{-3} and shows considerably less variability. Considering a time period of 53 years, this value decreases again by 17% to 0.024 ± <0.001 g cm^{-3} and also shows surprisingly little variability across sites (Figure 3). Stable OC density, the value required for the assessment of long-term C sequestration, is 0.018 ± 0.003 g cm^{-3} and thus 45% (SNK) to >400% (DSK) higher than the stable OC density of the mud flat (Figure 2). In contrast to the changes in OC density over time, which are similar across sites, mean accretion rates for the three time periods considered show large variation (Figure 3).

Thus, the constant decrease in OC density and the consistent pattern across sites seems to be independent of accretion rate. While the DSK experienced a slight, but constant increase in accretion rate from 0.64 cm yr^{-1} over a 53-years period, 0.65 cm yr^{-1} over 30 years, to currently 0.85 cm yr^{-1}, the opposite is true for the WH site. Here, accretion over 53 years

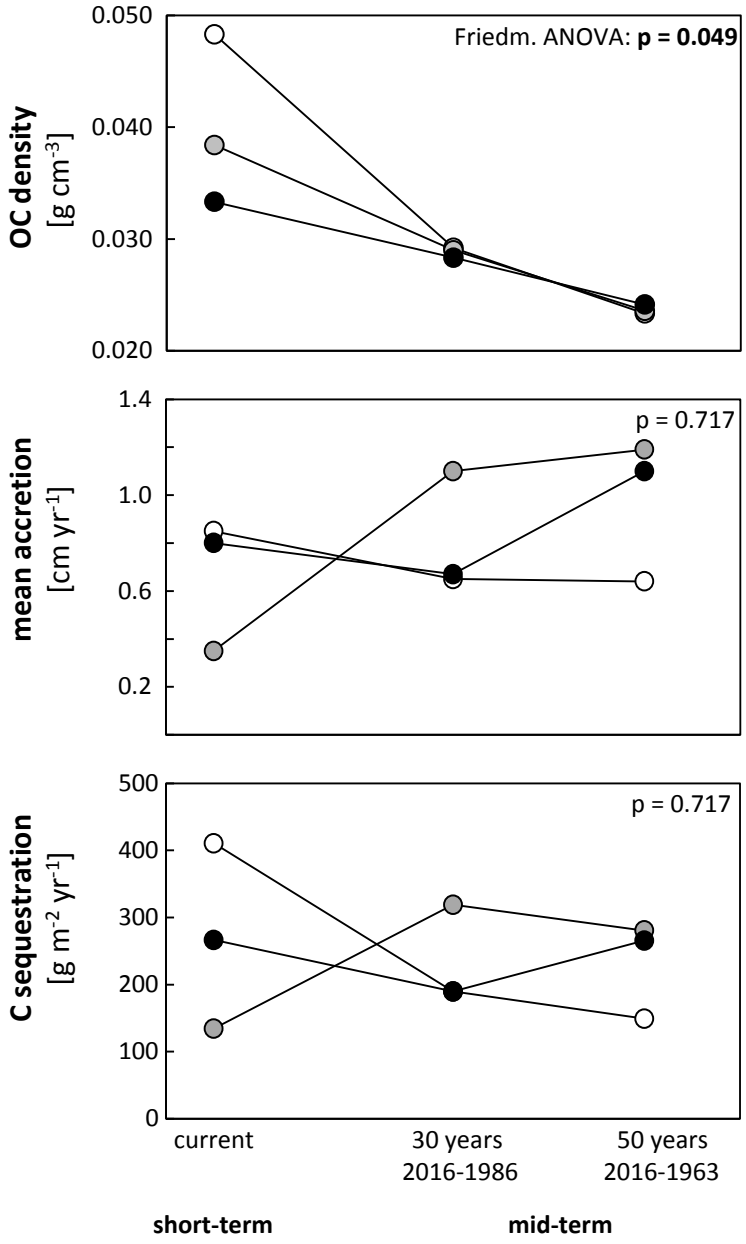

Figure 3 Mean OC density [g cm^{-3}], accretion [mm yr^{-1}], and the resulting rate of C sequestration [g C m^{-2} yr^{-1}] over the three quantification periods short-term (based on OC density of the top soil (5cm) and current (SEB based) accretion rates), mid-term 30 years (soil depth based on ^{137}Cs dating back to 1986), and mid-term 53 years (soil depth based on ^{137}Cs dating back to 1963). Data points are colored by site: white = Dieksanderkoog (DSK); gray = Westerhever (WH); black = Sönke-Nissen-Koog (SNK)

is 1.2 cm yr^{-1}; however, the rate dramatically slowed down during the last two decades to only 0.4 cm yr^{-1} as the current rate (compare Figure S1 for details on surface-elevation developement of the WH site). The SNK also shows highest mean accretion of 1.1 cm yr^{-1} over 53 years, whereas accretion over 30 years and current accretion is comparable to rates at DSK (Figure 3).

Carbon sequestration results as the product of OC density and accretion rate. Because accretion shows large variability across sites and no consistent pattern over time, also C sequestration is not significantly affected by the time period considered (Figure 3). In each site, considerable changes in C sequestration rate occur over the time periods considered. Thus, short-term C sequestration ranges from 134-411 g C m^{-2} yr^{-1} and C sequestration over 53 years ranges from 149-281 g C m^{-2} yr^{-1} (Figure 3).

Discussion

We show considerable declines in OC density down-core within the salt-marsh zone of the profiles (Figure 2). Yet, stable or minimum OC density in the salt-marsh zone is at least 45% (SNK) to >400% (DSK) increased compared to the mud-flat zone (Figure 2). Given that accretion of the marsh system is at least equal to that of the mud flat, these values illustrate the greater capacity of the vegetated ecosystem to sequester OC.

In accordance with our first hypothesis, OC content decreased significantly with depth in all three sites, and consequently both mean OC content and density decline over time. This decline in OC density is independent from changes in accretion rate over time (Figure 3). Instead, it is due to the loss of organic matter down-core through unsuppressed decomposition (compare: Kelleway et al. 2016, Schile et al. 2017), particularly in the top 15 cm of the profiles, where >50% of the OC is lost (Figure 2). Continuous records of soil redox conditions in the low-marsh zone of DSK support this notion. Soil redox is often oxic down to 15 cm and occasional oxic events down to 30 cm occur during periods of low precipitation and low water levels (Figure S3), thereby enabling aerobic microbial metabolism deep in the soil profile. Increasing organic matter input in the course of ecosystem development could have also contributed to the observed decline in OC content down-core. Indeed, data from a natural zonation stretching from pioneer zone to high marsh at DSK show that top soil (5 cm) organic matter contents increase along this gradient (4.7% in pioneer zone; 7.8% in low marsh; 11.6% in high marsh; P. Mueller, unpublished data; also compare Elschot et al. (2015) for

successional changes in top-soil OC contents). However, this increase is less steep than the decline in OC content down-core as reported here. Furthermore, based on the foraminiferal record and the present plant-species composition, our sampling positions at WH and SNK have undergone only two major shifts in their ecosystem development: from mud flat to pioneer zone and from pioneer zone to low marsh. More than the top 70 cm of the profiles consequently represent low-marsh environments. We therefore suggest that changes in organic matter input are only of secondary importance for the OC distribution down-core.

In contrast to our second hypothesis, which states that accretion rates are consistently higher during early ecosystem development and decrease with ecosystem age (van Wijnen and Bakker 2001, Butzeck et al. 2014), the observed changes of accretion rate over time were highly variable between sites. While accretion at WH indeed slowed down in the course of ecosystem development (Figures 3 and S1), the opposite seems to be true for the DSK site (Figure 3). Still, high mean rates of accretion exceeding 1.0 cm yr^{-1} were only observed for quantification periods >30 years, indicating that such rapid rates may indeed be more typical for early marsh development (van Wijnen and Bakker 2001). We therefore demonstrate that high rates of accretion during early ecosystem development can counterbalance the effect of low OC densities on C sequestration (Figure 3); however, this is not necessarily the case. As a consequence, a priori estimates of long-term C sequestration for young developing or artificial blue C systems are challenging and require precise regional-scale accretion models.

Regional scale applicability of OC density

Mid-term OC densities are remarkably similar after few decades of ecosystem development only, despite large differences in top-soil OC contents, quality of the organic material, grain-size distribution (Figures 1 and 2), site elevation, and plant-species composition (Table 2; Figure 3). We suppose that this effect is either caused by the overriding effect of decomposition on OC density in this system or by counterbalancing effects between primary production (or OC input), accretion, and decomposition. That is, OC input seems to be highest at DSK and lowest at SNK as evident by OC contents and densities of the top soil (Figure 2). At the same time, the decline in OC content and density is steepest in the DSK profile and most modest in the SNK profile: The OC content of the top 5 cm at DSK (7.9%) is 20% higher than at SNK (6.6%); however, it decreases sharply by ~70% to 2.7% within the top 10 cm at DSK compared to only ~30% decrease to 4.5% at SNK (Figure 2). Thus, the positive effect of high primary production on OC content and density at DSK seems to be counterbalanced with depth by higher rates of

decomposition. This co-occurrence of high primary production and high decomposition is potentially driven by high levels of soil aeration favoring both production and decay in temperate European marshes (Cooper 1982, Hemminga et al. 1991). Also, biomass-dependent rhizosphere priming effects could have contributed to the effect (Mueller et al. 2016b).

Variability in stable or long-term OC density is slightly higher than in mid-term OC densities, but still small compared to variance in top-soil OC density (Table 2). It is determined by the depth at which decomposition stops due to recalcitrance of the material and/or availability of terminal electron acceptors to support microbial metabolism (Megonigal et al. 2004). Because oxidizing conditions reach deep into the profile (Figure S3) only a small fraction of primary production and allochthonous organic matter input can remain with depth (Figure 2), which leads to similar values of stable OC content and density. Indeed, stable OC density (converted from organic matter density following Craft et al. (1991)) in the grazed mainland salt marsh site of Noord Friesland Buitendijks, NL (Nolte et al. 2013b) is with 0.017 g OC cm^{-3} not different to the values reported here, although the marsh is even younger than the sites sampled in the present study, and large differences in mean bulk density and grain-size distribution exist compared to our three sites (Nolte et al. 2013b).

We therefore suggest that our data on both mid-term (decades) and long-term (stable/centuries) OC density are applicable to a large part of the Wadden Sea salt-marsh area, potentially excluding very sandy systems or island marshes, and yields important implications for modeling and upscaling C-sequestration rates in the region.

Estimates of C sequestration in Wadden Sea salt marshes

Driven by the large variability in accretion rates over time, total amounts of sequestered C vary considerably over short- and mid-term periods across our three sites (Figure 3). However, we also demonstrate almost negligible variability in mid-term (Figure 3) and small variability in stable (Figure 2) OC densities. This in combination with a representative mean accretion value for the total mainland salt-marsh area would allow us to propose a realistic range for rates of mid- and long-term C sequestration in the region.

Suchrow et al. (2012) determined accretion rates over 20 years in over 400 plots between the Elbe Estuary and the Danish border (~200 km shoreline) across early and late successional stages and report a mean accretion for the WS foreland mainland salt marshes of 6.2 mm yr^{-1}. We use this value to estimate C sequestration for the type of mainland foreland marshes that comprise with 20,560 ha approximately 50% of the total WS salt-marsh area and

exclude island marshes, barrier-connected marshes, and polders (Esselink et al. 2009). Based on our stable OC density of 0.018 g cm^{-3} and an accretion of 6.2 mm yr^{-1}, long-term (century scale) C sequestration is 1.12 tons C ha^{-1} yr^{-1}, resulting in a total annual rate of 22,950 tons C yr^{-1} or 84,150 tons CO_2 equivalents yearly in the foreland marshes of the WS alone (Table 2). Decennial scale C sequestration over a 53-year period results in 30,600 tons C yearly. In comparison, short-term C sequestration based on the same mean accretion rate and the OC density of the top soil results in 2.49 tons C ha^{-1} yr^{-1} and in a total annual rate of 51,000 tons yr^{-1}, and thus to an overestimation of >100% compared to the actual long-term rate of C sequestration (Table 2).

Table 2 Carbon sequestration in the foreland mainland marshes of the Wadden Sea region. Values are based on a mean accretion rate of 6.2 mm yr^{-1} (Suchrow et al. 2012) and short-, mid-, and long-term assessments of OC density. Time scales (Scale) refer to the potential permanence of the C sequestartion

Assessment	Scale	OC density [g cm^{-3}]		C sequestration [tons per year] per hectare		mainland marsh area		
		mean	SD	mean	SD	mean	min	max
Short-term	years	0.040	0.008	2.49	0.50	51194	42644	61807
Mid-term	decades							
30 years		0.029	0.001	2.11	0.03	43382	42612	44216
53 years		0.024	0.000	1.49	0.02	30634	30183	31224
Long-term	centuries	0.018	0.003	1.12	0.19	23027	19189	28144

Organic carbon sources

The distinction between allochthonous and autochthonous OC to the total OC pool is potentially relevant for C crediting approaches, as the alternative fate of the allochthonous OC, if it had not accumulated in the wetland soil, is unclear. Although quantification of the contributions of different OC sources to the total soil OC pool is commonly conducted by means of ^{13}C natural abundance and OC/N ratios (Bouillon et al. 2003, Khan et al. 2015), these approaches are only insightful if large differences in both $\delta^{13}C$ and OC/N exist between the two sources (Khan et al. 2015). With the present data, we are not able to adequately quantify the relative amounts of allochthonous versus autochthonous OC because considerable differences in both $\delta^{13}C$ and OC/N exist within the autochthonous source between aboveground and

belowground biomass (delta $\delta^{13}C$ >2‰; delta OC/N >12) and deviations of the allochthonous from the mean autochthonous $\delta^{13}C$ end member are only moderate (approx. 4‰) (Mueller et al. 2017).

Considerable ^{13}C enrichment with depth as reported in the present study, but also elsewhere (Wang et al. 2011, Saintilan et al. 2013), could point to at least three non-exclusive processes: 1.) higher contributions of belowground vs. aboveground organic matter, because belowground biomass is generally less ^{13}C depleted compared to aboveground biomass (Ghashghaie and Badeck 2014). 2.) Higher contributions of less ^{13}C depleted allochthonous organic matter (Middelburg et al. 1997). 3.) ^{13}C enrichment during aging and repeated cycling of the organic material as generally found in terrestrial soil profiles (Boström et al. 2007, Kohl et al. 2015).

In the present study, changes in $\delta^{13}C$ down-core are not strictly continuous, but also show stable regions. We therefore argue that aging and repeated cycling of the organic matter is not the sole mechanism inducing ^{13}C enrichment with depth. Furthermore, stable and less ^{13}C depleted regions in the profiles of WH and SNK could be related to the high historic accretion and allochthonous-input rates in these systems. For instance, organic matter that accumulated since 1963 in the DSK profile is more ^{13}C depleted than that in the WH and SNK profiles (Figure 1), potentially indicating a larger contribution of autochthonous organic matter to the total organic matter pool in the overall slower accreting system of the DSK. Likewise, organic matter that accumulated before 1986 in the SNK profile is less ^{13}C depleted than the organic matter that accumulated after 1986, potentially indicating larger contributions of allochthonous organic matter before 1986 when also accretion rates were considerably higher (Figures 1 and 3). Additionally, $\delta^{13}C$ of the organic matter in the WH profile remains relatively stable for >10 cm above the 1986 mark, while also accretion rates remained higher for a longer period compared to SNK (Figures 1 and 3). We therefore suggest that greater contributions of allochthonous vs. autochthonous organic matter down-core are an important factor determining the observed shifts in $\delta^{13}C$, and large relative differences in the organic matter source both across sites and over ecosystem development exist that are likely attributable to differences and changes in sedimentation and accretion rates.

Anthropogenic effects on carbon sources and accretion rates

The development of most WS mainland salt marshes relied on artificial drainage and accretion enhancement techniques in the past (Hofstede 2003). While the frequent maintenance of the

drainage-ditch system, which is characterized by the deposition of sediment from the ditches onto the marsh (Hofstede 2003), does not seem to directly affect vertical accretion rates (Esselink et al. 2009), effects on the input of allochthonous OC to the marsh surface cannot be excluded. Patterns of grain-size distribution and OC/N ratio down-core are regular and show no signs of large disturbances caused by potentially infrequent and excessive artificial deposition events. Also, the $\delta^{13}C$ signature down-core shows little noise at DSK and WH. At SNK, in contrast, relatively large variability in $\delta^{13}C$ between 20 and 40 cm could indeed point to effects of infrequent artificial deposition events. The foraminiferal fauna of this interval contains a considerable proportion of hyaline taxa suggesting either re-working of mud-flat sediments during storm surges or re-deposition of material during maintenance of ditches. However, these possible effects did not translate to changes in OC density or content in this section of the profile. In fact, the consistent decline patterns of OC content and density with depth in all three sites suggest that either the development of the profiles was predominantly regular (potentially due to frequent maintenance of the ditch system), or that effects of only potentially strong and infrequent events of additional sediment deposition are overridden by high rates of organic matter decomposition.

Conclusion and Implications

The mainland salt marshes of the WS region are mainly artificial systems that could only develop in their exposed, high-energy environments through accretion enhancement techniques, like construction of brushwood groynes, and ditching which have been conducted since the middle of the 19th century (Hofstede 2003). The original purpose for artificial salt-marsh development was land reclamation for agricultural use, whereas the important role of salt marshes for coastal protection and biodiversity support was recognized later on (Hofstede 2003). In the present study we demonstrate the large C-sequestration potential of these man-made blue C systems. Although large amounts of OC are lost down-core in the well-aerated marsh soils of the WS region, mid- and long-term C sequestration rates are well in range with rates reported from other blue C systems (Mcleod et al. 2011) and clearly higher than those of the surrounding un-vegetated inter-tidal system.

At the same time, our study emphasizes the importance of deep sampling in combination with long-term accretion records and knowledge on ecosystem development (as based on our foraminiferal record) in order to accurately assess and avoid vast overestimation of C sequestration and OC stocks in blue C ecosystems. Finally, despite large differences in top

soil OC contents, surface elevation, and other factors (Figures 1 and 2), we demonstrate remarkably similar OC densities after few decades of ecosystem development in our three sites. If this finding is applicable to other tidal wetland systems within a given regional context (i.e. within one estuary), it has important implications for modeling long-term C sequestration in young artificial sites such as constructed or restored tidal wetlands (Craft et al. 1999, 2003, Callaway et al. 2012, Osland et al. 2012, Ballantine and Schneider 2014).

The WS salt marshes represent a powerful man-made C sink with an estimated OC stock of 24.7 Mio. tons in the top 0.5 m of the foreland mainland marshes alone, if we base our estimate on mid-term (53 years quantification period) OC density and the total foreland salt-marsh area (Esselink et al. 2009). In the context of climate change mitigation, this important ecosystem service should find consideration in future habitat-management directives, specifically with regard to maintaining and increasing current expansion rates of the salt-marsh area. Currently, the WS salt-marsh area is still expanding at rates exceeding 200 ha yr^{-1} (Esselink et al. 2009), thereby yearly increasing the capacity for long-term C sequestration by 224 tons C yr^{-1}, corresponding to 821 tons CO_2 yr^{-1}. Maintaining the expansion of salt-marsh area therefore represents a promising measure for the reduction of net CO_2 emissions, particularly with regard to the broadly announced society goal of “net zero” CO_2 emissions since the *Paris 2015 agreement* (Rogelj et al. 2016).

Future research, in turn, should aim at quantifying the net impact of land-use cessation (livestock grazing) on C sequestration (Schuman et al. 1999, Elschot et al. 2015). Although livestock grazing considerably reduces the rate of organic matter decay (Mueller et al. 2017), greater primary production in ungrazed systems (Morris and Jensen 1998) may counterbalance this effect. However, since the abandonment of livestock grazing in most areas was initiated only <30 years ago, long-term changes in C sequestration are difficult to assess. Further work in island marshes and restored/de-embanked sites is needed to improve our estimates on C sequestration for the entire salt-marsh area of the WS region.

Acknowledgements

K. Müller-Navarra is thanked for discussion and providing information on accretion rates in the Westerhever salt marsh. Many thanks go to Birgit Grabellus for providing support in the laboratory of the Institute of Soil Science. N. Ladiges and L. Kutzbach were supported through the Cluster of Excellence "CliSAP" (EXC177), University of Hamburg, funded by the German Research Foundation (DFG). P. Mueller and S. Nolte were supported by the Bauer-Hollmann Stiftung and the Rudolf und Helene Glaser Stiftung in the framework of the research project INTERFACE (Interaction of fish, plants, carbon & sediment: management and ecosystem functions of Wadden Sea salt marshes). Finally, we thank Martin Stock and all project partners of the Wadden Sea National Park Schleswig-Holstein for their cooperation

Supporting information (Chapter 4)

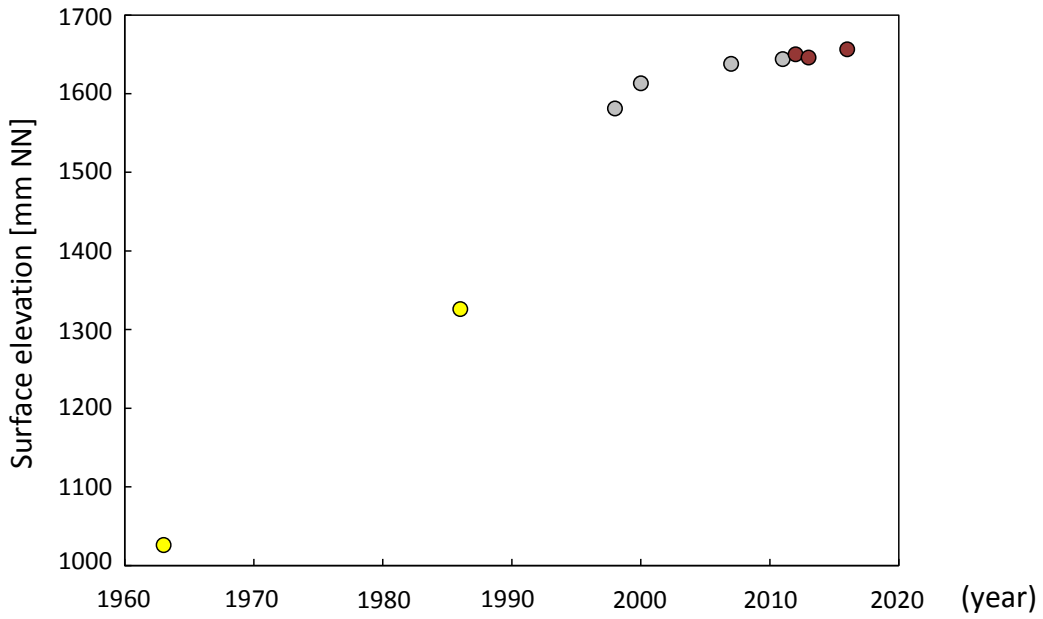

Figure S1 Development of surface elevation [mm NN] of the sampling position at Westerhever marsh (WH). Yellow: data points based on ^{137}Cs dating of a neighboring marsh patch; not corrected for compaction processes (Müller-Navarra et al. unpublished). Gray: leveling-derived data points. Red: data points based on regular SEB measurements (Stock 2012, TMAP database)

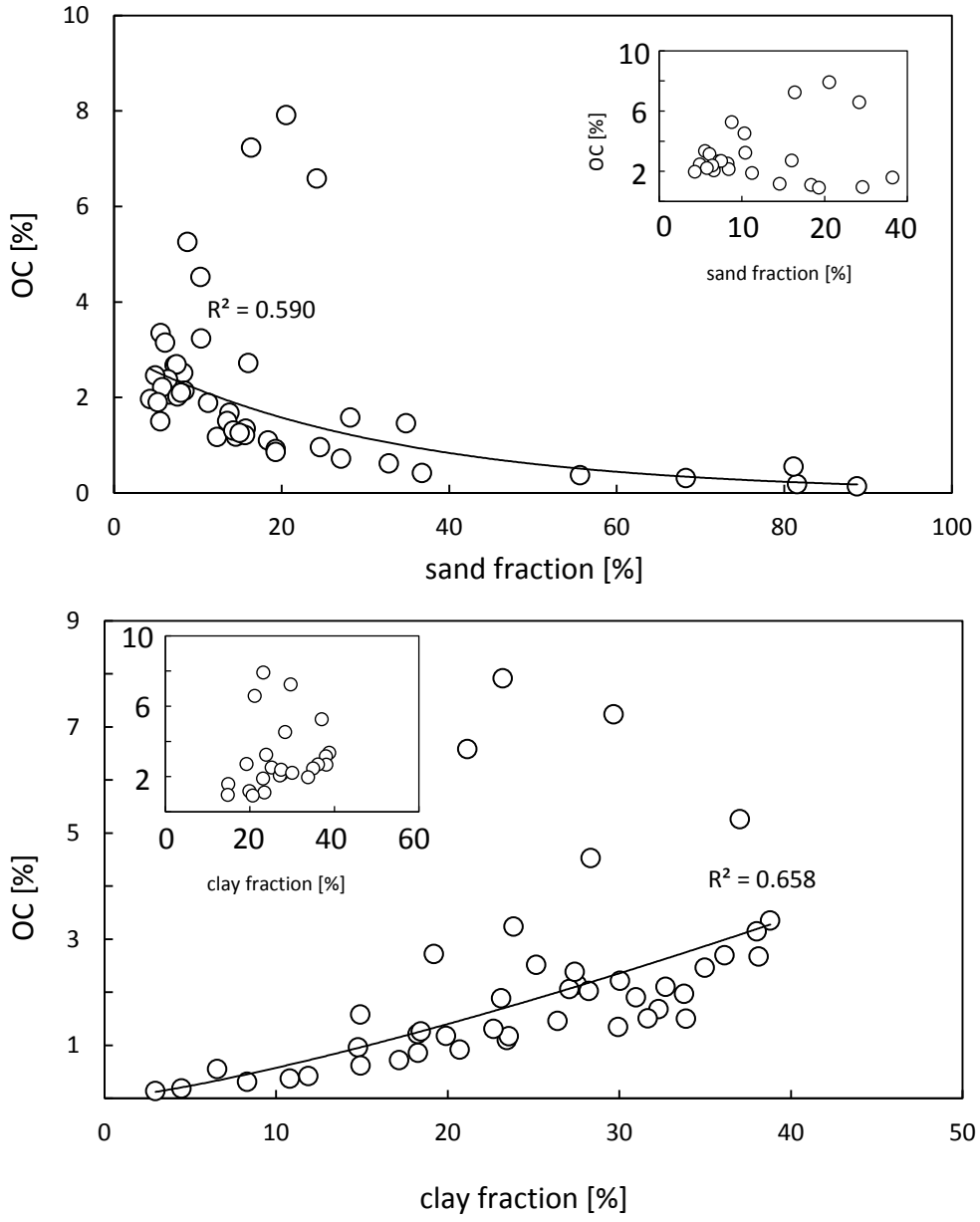

Figure S2 Relationship between sand- and organic-carbon content (top panel) and clay- and organic-carbon content (bottom panel) in 130 cm soil profiles of three artificial Wadden Sea salt-marsh sites. Inlets show relationship within the top 40 cm of the soil profiles.

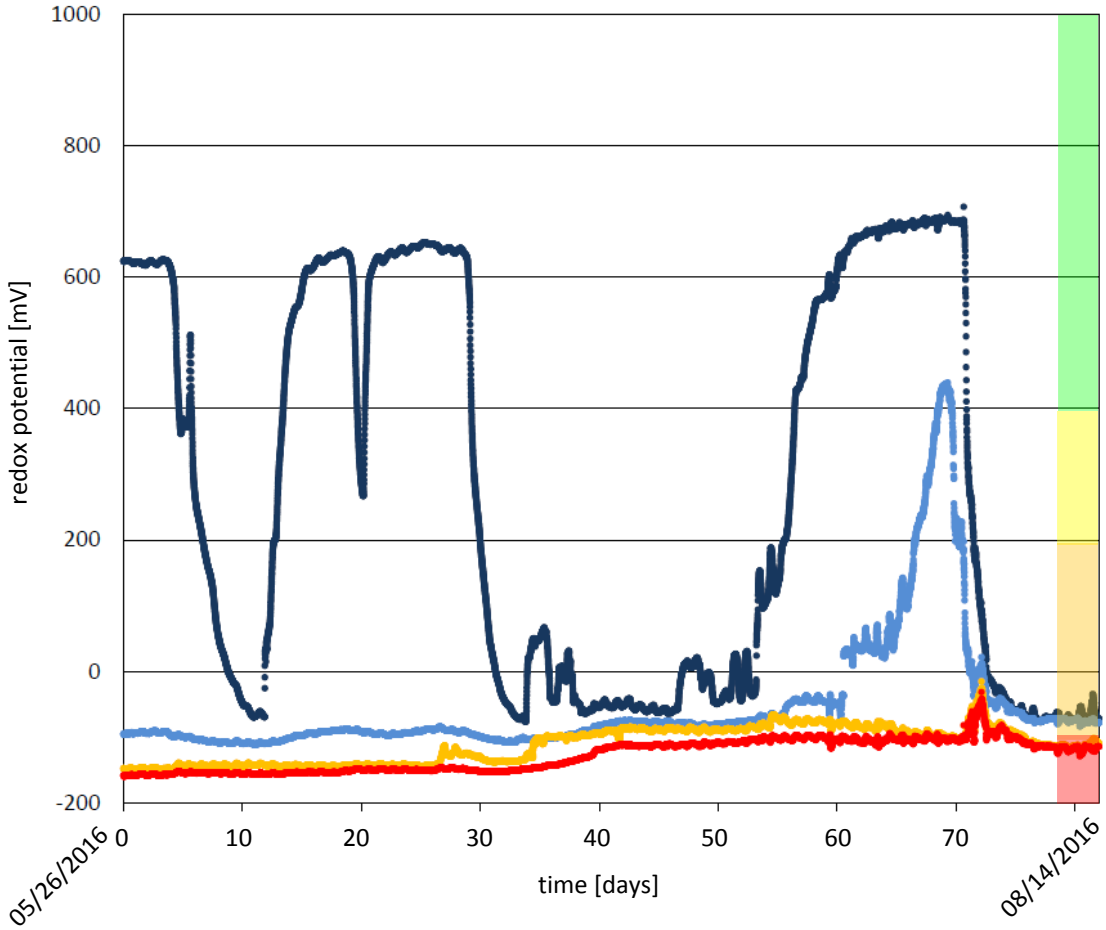

Figure S3 Soil redox conditions in a low-marsh profile of the DSK site continuously measured between 26 June - 14 Aug in **15 cm (dark blue)**, **30 cm (light blue)**, **45 cm (orange)**, and **60 cm (red)** soil depth. Color bar on the right illustrates redox classes after Zhi-Guang (1985): Green = oxidizing (>400 mV); yellow = weakly reducing (200 to 400 mV); orange = moderately reducing (-100 to 200 mV); red = strongly reducing (<-100 mV)

Methods summary: Pt-tipped redox probes were deployed in four depths. Values presented are means of 4 measuirng positions on each probe. Redox was referenced against an Ag/AgCl electrode in 3M KCl solution (214 mV vs. standard hydrogen electrode) and corrected for soil pH.

BOX A

Indicator of Reduction in Soils (IRIS) sticks: pilot study in a Wadden Sea salt marsh

The IRIS technique has recently been developed to detect reducing soil conditions and quantify redox conditions in a time- and cost-efficient manner (Jenkinson 2002, Castenson and Rabenhorst 2006). Due to rapidly changing hydrological conditions, soil redox conditions are highly variable in tidal wetlands. Therefore, installation of permanently measuring redox probes (i.e. Pt-tipped measuring electrode plus reference electrode) is required to accurately assess redox conditions. If it is anticipated to assess redox in multiple positions and depths within the wetland system, however, this approach is relatively time and cost consuming. As a potential alternative, I tested if the IRIS technique is able to capture relative differnces in redox conditions in a Wadden Sea salt marsh:

Application and theory

- iron-oxide painted PVC is inserted in soils to characterize redox conditions with depths over several weeks
- As water saturated soils become progressively reduced, microbes oxidizing organic matter will reduce solid phase Fe(III) (iron oxides) to soluble Fe(II). This will dissolve and consequently remove the iron oxide paint applied to the PVC surface, indicating reducing soil conditions (Rabenhorst 2008).
- Castensen and Rabenhorst (2006) demonstrated that the quantity of removed Fe(III) and the indication of reducing conditions is in well agreement with soil redox data.

Protocol, application, and evaluation

- In the present case study, I used finely sanded PVC pieces of 5x40 cm instead of PVC pipe as in the original protocol (Rabenhorst 2008).
- The laboratory procedure for paint preparation followed the original protocol (Rabenhorst 2006, 2008)
- IRIS sticks were inserted between Oct and Nov 2016 for a duration of six weeks in five zones (n = 3) along an intertidal flat to salt-marsh ecotone at Dieksanderkoog, German Wadden Sea.
- Upon retrieval, IRIS sticks were gently cleaned, and IMG-file format images of the sticks were obtained using an image scanner. Paint removal was evaluated using the open source software *Fiji* (ImageJ, GNU).

Results and implications

- Reducing conditions based on Fe(III) removal could be detected within the top 5 and 20 cm in all five zones except the *Elymus athericus* dominated high marsh (Figure 1 and image, right).
- Reduction within the top 5 cm was similar and highly variable across the four zones from intertidal flat to *Puccinellia martima* dominated low marsh (Figure 1, top panel).
- Integrating over the top 20 cm, reduction was surprisingly low in the *Salicornia* dominated pioneer zone (Figure 1, bottom panel).
- My initial results suggest that a clear gradient in reduction from intertidal flat to high marsh is not present in the top 20 cm, however, is likely to establish in deeper assessments (image)
- The obtained results agree well with those obtained in parallel redox campaigns. Particularly, both approaches suggest well-aerated conditions in the *Salicornia* and *Elymus* zones of the ecotone (Chapter 7; Figure 3A).
- The approach may allow for the detection of small-scale patterns in oxygen availability, i.e. as caused by bioturbation in anaerobic environments (image, tidal flat stick).
- This IRIS-stick approach has proven useful in capturing the relative redox conditions within a site and in identifying reduction. Further work is required to calibrate IRIS against redox data.

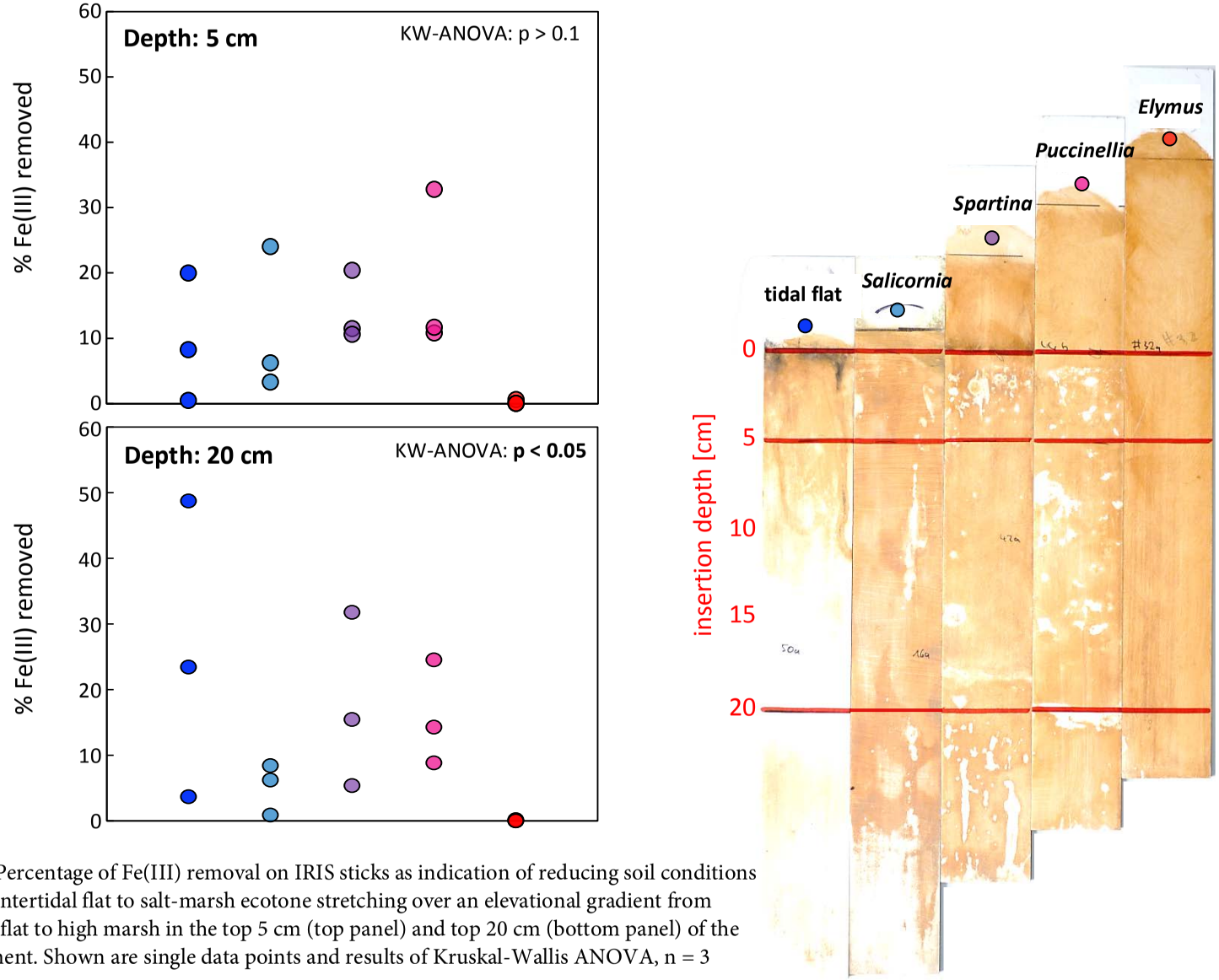

Figure 1 Percentage of Fe(III) removal on IRIS sticks as indication of reducing soil conditions along an intertidal flat to salt-marsh ecotone stretching over an elevational gradient from intertidal flat to high marsh in the top 5 cm (top panel) and top 20 cm (bottom panel) of the soil/sediment. Shown are single data points and results of Kruskal-Wallis ANOVA, n = 3

5 Top-down control of carbon sequestration: grazing affects microbial structure and function in salt marsh soils

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Peter Mueller, Dirk Granse, Stefanie Nolte, Hai Thi Do, Magdalena Weingartner, Stefan Hoth, and Kai Jensen

Abstract

Tidal wetlands have been increasingly recognized as long-term carbon sinks in recent years. Work on carbon sequestration and decomposition processes in tidal wetlands focused so far mainly on effects of global change factors such as sea level rise and increasing temperatures. However, little is known about effects of land use, such as livestock grazing, on organic matter decomposition and ultimately carbon sequestration. The present work aims at understanding the mechanisms by which large herbivores can affect organic matter decomposition in tidal wetlands. This was achieved by studying both direct animal-microbe interactions and indirect animal-plant-microbe interactions in grazed and ungrazed areas of two long-term experimental field sites at the German North Sea coast. We assessed bacterial and fungal gene abundance using quantitative PCR as well as the activity of microbial exo-enzymes by conducting fluorometric assays. We demonstrate that grazing can have a profound impact on the microbial community structure of tidal wetland soils, by consistently increasing the fungi-to-bacteria ratio by 38-42%, and therefore potentially exerts important control over carbon turnover and sequestration. The observed shift in the microbial community was primarily driven by organic matter source, with higher contributions of recalcitrant autochthonous (terrestrial) vs. easily degradable allochthonous (marine) sources in grazed areas favoring relative fungal over bacterial abundance. We propose a novel and indirect form of animal-plant-microbe interaction: top-down control of aboveground vegetation structure determines the capacity of allochthonous organic matter trapping during flooding and thus the structure of the microbial community. Furthermore, our data provide the first evidence that grazing slows down microbial exo-enzyme activity and thus decomposition through changes in soil redox chemistry. Activities of enzymes involved in C cycling were reduced by 28-40%, while activities of enzymes involved in N cycling were not consistently affected by grazing. It remains unclear if this is a trampling driven direct grazing effect, as hypothesized in earlier studies, or if the effect on redox chemistry is plant mediated and thus indirect. This study improves our process-level understanding of how grazing can affect the microbial ecology and biogeochemistry of semi-terrestrial ecosystems that may help explain and predict differences in C turnover and sequestration rates between grazed and ungrazed systems.

Introduction

In recent years, salt marshes and other tidal wetlands have been increasingly recognized as long-term carbon sinks, sequestering organic carbon (OC) at rates exceeding those of most other ecosystem types (Duarte et al. 2005, Mcleod et al. 2011). Carbon sequestration in tidal wetlands depends on the rates of organic matter (OM) input (from both autochthonous and allochthonous sources) and microbial OM decomposition, which is often assumed to be inhibited under the prevailing reducing soil conditions (Kirwan and Megonigal 2013).

Recent work on carbon sequestration in tidal wetlands focused on effects of global change factors, such as sea level rise (Kirwan et al. 2013), rising temperatures (Kirwan and Mudd 2012), and salt-water intrusion (Morrissey et al. 2014). Land use and habitat management of tidal wetlands, such as livestock grazing as conducted in large parts of the European salt marsh area (Esselink et al. 2000, Bakker et al. 2002) and elsewhere (Yang et al. 2008, Gedan et al. 2009, Di Bella et al. 2014) have not intensively been studied. They could, however, greatly influence the capacity of these systems to sequester carbon (Kauffman et al. 2014, Bhomia et al. 2016), thus yielding important implications for management policies.

Indeed, previous studies suggested an impact of livestock grazing on the net C balance of salt marshes. Morris and Jensen (1998) show lower rates of C sequestration in grazed versus ungrazed parts of a mainland North Sea salt marsh, attributing this effect to the removal of aboveground biomass and the resulting lower litter supply. Contrary to these findings, Elschot et al. (2015) could demonstrate higher rates of C sequestration in cattle grazed versus ungrazed parts of a back-barrier North Sea island marsh, giving two possible explanations for the observed effect. On the one hand, higher belowground biomass allocation under grazing could have increased OC inputs. On the other hand, lower soil redox conditions in more compacted grazed soils could have decreased OM decomposition. In order to understand the mechanisms causing such equivocal results, it is necessary to separate the two factors influencing C sequestration, namely OM input through primary production and microbial OM decomposition (Hackney 1987). Yet, in comparison to effects of grazing on salt-marsh primary production and community composition (Reimold et al. 1975, Morris and Jensen 1998, Di Bella et al. 2014), very few studies investigated mechanisms by which grazing could affect microbial processes leading to changes in OM decomposition (but see Olsen et al. 2011, Ford et al. 2013). Livestock grazing can potentially affect OM decomposition through both direct animal-microbe interactions and indirect animal-plant-microbe interactions. Direct effects of grazing on microbial activity and consequently decomposition rate could be induced by changes in soil aeration and

redox conditions through trampling-driven soil compaction (e.g. Kauffman et al. 2004). Indeed, grazing effects on soil redox conditions have been demonstrated for clay-rich salt marsh soils (Schrama et al. 2013); however, an influence of grazing-induced changes in soil redox on OM decomposition has so far only been proposed (Elschot et al. 2015) but not demonstrated. Furthermore, soil redox is not necessarily related to OM decomposition rate in tidal wetland soils (Kirwan et al. 2013, Mueller et al. 2016b). Direct grazing effects on decomposition could additionally be induced by the return of highly decomposable excretory products that increase labile C and N pools (Frank et al. 2000). However, the input of excretory products is both spatially patchy and seasonally variable (Bakker et al. 2004). Furthermore, direct effects of excretory products on both microbial activity and structure could often not be confirmed (Bardgett et al. 1998a, de Vries et al. 2006), making it difficult to develop hypotheses about effects on decomposition processes.

Indirect grazing effects could play a crucial role in regulating various belowground processes and ultimately OM decomposition (Bardgett et al. 1998b). Such plant mediated processes affecting microbial activity or structure (animal-plant-microbe interactions) could be particularly relevant in wetland systems. Here, plant-biomass parameters and certain physiological and morphological plant traits have been identified as important biotic drivers of decomposition in tidal wetlands (Neubauer et al. 2005, Wolf et al. 2007, Mozdzer and Megonigal 2013), and, in fact, plant biomass-dependent rhizosphere priming effects are a major control of decomposition rate irrespective of hydrology and redox conditions (Guenet et al. 2010, Mueller et al. 2016b). Furthermore, shifts in plant-species composition, biomass quality, and changes in litter accumulation can induce substrate control over both microbial activity and community composition (Jones et al. 2004, Strickland and Rousk 2010). Given this overriding role of plant effects on decomposition in tidal wetland soils, also top-down or consumer controls of plant community composition, biomass, and biomass allocation (Schuman et al. 1999, Silliman and Zieman 2001, He and Silliman 2016) could exert indirect but important control over decomposition processes. First insight into these kinds of animal-plant-microbe interactions in wetlands came from studies on effects of herbivory on methane production and emissions in freshwater lake systems (Bodelier et al. 2006, Dingemans et al. 2011); however, little is known about such indirect effects on OM decomposition or microbial activity per se.

The present study investigates the effect of livestock grazing on OM decomposition by assessing microbial abundance, and microbial exo-enzyme activity (EEA) as the rate limiting step of OM decomposition (Sinsabaugh et al. 1991). Although the investigation of

EEAs allows one to distinguish between the decomposition of different OM types (e.g. as opposed to C-flux measurements), these initial steps of decomposition are still poorly understood in tidal wetland soils (Morrissey et al. 2013, Kirwan and Megonigal 2013). We assayed enzymes involved in both C and N cycling in order to relate EEAs to potential differences in substrate sources and quality in grazed versus ungrazed marshes. Considering functional differences between fungi and bacteria, as the two major decomposer groups, with fungi having a higher C-use efficiency, lower N demand, and greater ability to decompose recalcitrant OM (van der Heijden et al. 2008, Strickland and Rousk 2010), we quantified the abundance of both groups separately by assaying specific gene abundance using quantitative polymerase chain reaction (qPCR). Quantifying the decomposition of different OM types as well as major decomposer groups separately could be particularly relevant to understand decomposition processes in coastal ecosystems. Here, autochthonous and allochthonous sources of OM with large differences in substrate quality (Khan et al. 2015) contribute to different amounts to the total soil- or sediment-OM pool, thus shaping the microbial community structure (Fagervold et al. 2014).

We hypothesized first that grazed marshes would support a lower fungal vs. bacterial abundance than ungrazed marshes through both direct and indirect grazing effects. As a direct effect, we hypothesized that grazing induced soil compaction lowers soil oxygen availability and leads to more frequent events of hypoxia and anoxia, thereby decreasing the abundance of fungi, which are assumed to be less adapted to anaerobic conditions than bacteria (Bossio and Scow 1998, Chambers et al. 2016). Grazing often reduces aboveground biomass and litter accumulation (Bakker et al. 1993, Olsen et al. 2011, Ford et al. 2013) as a recalcitrant substrate source (high C/N, rich in structural components compared to allochthonous marine organic matter (Baldock et al. 2004, Khan et al. 2015)) that is assumed to be more effectively degraded by fungal than bacterial communities. As an indirect (plant mediated) effect, we therefore hypothesized that grazing decreases the contribution of recalcitrant autochthonous OM to the soil OM pool, thereby leading to a lower relative fungal abundance. Irrespective of potential differences in OM quality and microbial structure between treatments, we hypothesized second that grazing induced reductions in soil-oxygen availability would have an overriding influence on decomposition rate by decreasing microbial EEA.

Material and methods

Study sites and field-study design

The study was conducted from May to November 2015 in the high marsh zones of two long-term experimental field sites. Both sites are minerogenic, meso-tidal North Sea mainland salt marshes of the Schleswig-Holstein Wadden Sea National Park, Germany. A rectangular network of creeks and ditches still reveals the artificial origin of the marsh sites that were created for land reclamation in the early 20th century (Müller et al. 2013). The Dieksanderkoog marsh (DSK) is situated in the south of the National Park at the outer mouth of the Elbe Estuary. The Sönke-Nissen-Koog marsh (SNK) is situated in the northern part of the National Park, approx. 30 km south of the border to Denmark. Both marsh sites were entirely grazed by sheep until 1988 and 1992 (SNK and DSK, respectively), when the long-term field experimental sites were established and grazing was abandoned in distinct areas of the marsh sites (Stock 2005). The high marsh of both sites is divided by a straight central creek of 2-3 m width, separating a grazed from an ungrazed area. In each marsh, two sampling locations separated by approximately 100 m and with different distance to the seawall (landward/seaward) were defined on each treatment side of the central creek to account for possible variability in land-sea direction. At each sampling location, n = 5 sampling positions were defined in the grazed treatment and were mirrored on the creek line to the ungrazed treatment, resulting in 10 sampling positions per treatment per marsh site and a total of N = 40 sampling positions (Figure S1). Sampling positions were in a distance of 2-36 m from the central creek. As micro-topographical site parameters, surface elevation of sampling positions (using a laser level (Trimble, Sunnyvale, CA, US)) and distance to creek were determined. Independent of sampling location, the ungrazed treatment of both sites are homogeneously covered by the tall growing grass *Elymus athericus*, while the grazed treatment of DSK and SNK are characterized by short and homogeneous lawns of *Festuca rubra* and *Puccinellia maritima*, respectively (Table S1). Compare Müller et al. (2013) for a more detailed description of the study area, aerial pictures, and maps of study sites.

Soil sampling and processing

Sampling took place from 27 May - 5 June 2015 before livestock had access to the sites (approx. Jun-Nov). From each sampling position, one soil sample was taken as a core from the top 5 cm using a volumetric steel ring (100 cm^3). Aboveground biomass and litter were removed prior sampling. Until further analysis, intact soil cores were kept frozen at -20°C

within 8 h after sampling. A subsample of each core was homogenized by passing it through a 2.5-mm sieve. This also removed coarser materials such as rhizomes. 20 g of the homogenate was suspended in 20 mL deionized water and then mixed on an overhead shaker. The resulting slurry was stored at -20°C and used as base material for further analysis (DNA extraction, element and isotope analyses, EEA assays). The residual sample was air dried at 65°C for 48 h and used to determine dry weight, bulk density, OM content, and soil pH.

Soil redox chemistry

The effect of grazing on the oxidation-reduction state (redox) of the soil was assessed *in situ* and used as a proxy for O_2 availability. Redox was measured at 5 cm depth within 50 cm of each sampling position using a Pt-tipped redox electrode and an Ag/AgCl reference electrode (ecoTech, Bonn, Germany) connected to a portable, high impedance redox- and pH-meter (ecoTech, Bonn, Germany). Readings were corrected to the redox potential of the standard hydrogen electrode (+207 mV) but not for pH, as a linear relationship between pH and redox in soils is questionable (Mansfeldt 2003). Redox values were interpreted after Zhi-Guang (1985) as oxidizing (>400 mV), weakly reducing (200-400 mV), moderately reducing (-100 – 200 mV), and strongly reducing (<-100 mV). Soil redox is highly variable, e.g. with changing hydrological conditions. In order to asses this variability, we measured redox not only when soil samples were taken, but also on three additional occasions between June and November 2015. Along with redox measurements, soil moisture was measured in the same spots with a portable ThetaProbe TDR-probe connected to an HH2 readout system (Delta-T Devices, Cambridge, UK).

In order to interpret redox readings and assess the redox chemistry of the bulk soil in more detail, soil pH was determined in $CaCl_2$ solution using an Eutech Instruments pH probe (Thermo Fisher Scientific Inc., Waltham, MA, US) connected to an 18.82 multimeter (Eijkelkamp, Giesbeek, NL). 10 g of air dried soil was incubated in 25 mL 10 mM $CaCl_2$ solution for 1 h under continuous shaking. The measurement was taken after all soil particles had precipitated and the solution was slightly turbid but transparent.

Plant-species composition and biomass parameters

Prior soil sampling, species composition and cover using Londo scale were assessed in 400-cm^2 plots around each sampling point. As measures for the autochthonous production and litter pool, aboveground biomass was assessed as after-season standing biomass. Clip plots of 60 cm^2 (grazed) and 315 cm^2 (ungrazed) were sampled in each sampling position.

Belowground biomass of the top 5 cm was assessed sampling three volumetric rings (100 cm^3) of soil within 50 cm of each sampling point. Belowground biomass was washed free of soil over a 200-µm sieve. Above and belowground biomass was dried at 60°C and weighed. The thickness of the litter layer was assessed prior soil sampling using a metric ruler.

Table 1 Redox chemistry and plant biomass parameters in grazed and ungrazed treatments of the two marsh sites DSK and SNK

Site	Dieksanderkoog (DSK)				Sönke-Nissen-Koog (SNK)			
Treatment	grazed		ungrazed		grazed		ungrazed	
	mean	SE	mean	SE	mean	SE	mean	SE
soil redox [mV]								
average	404	8	450	2	390	7	433	6
low	297	19	438	3	313	10	418	15
high	460	5	463	5	437	6	448	2
pH	7.54	0.03	7.77	0.04	7.47	0.04	7.60	0.04
plant biomass [g m^{-2}]								
aboveground	460	41	1080	62	437	65	1387	108
belowground	994	50	1755	126	1360	82	1273	147
litter layer [cm]	0.4	0.1	2.4	0.3	0.0	0.0	2.8	0.4

Notes: Values presented are mean values ± standard errors (n = 10). Redox values are reported as average of four measuring campaigns (average) and for both the campaign characterized by lowest (low) and highest (high) soil redox

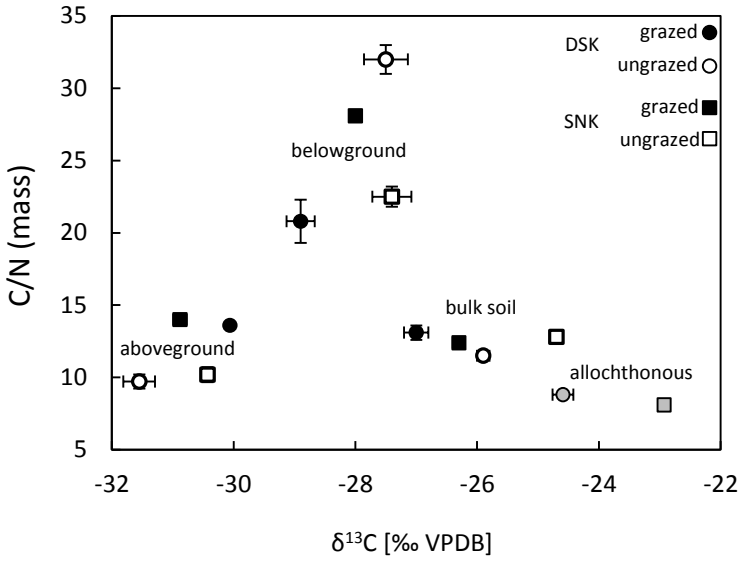

Figure 1 $\delta^{13}C$ and C/N of plant biomass (aboveground and belowground tissues, n = 4) and bulk-soil OM (n = 10) in grazed and ungrazed treatments, and deposited allochthonous OM (n = 6; independent of treatment, gray symbols) at DSK and SNK site. Presented are mean values ± standard errors

Substrate quality of soil organic matter and plant biomass

Soil OM content was determined by loss on ignition (LOI). Approx. 15 g of pre-dried soil (105°C to constant weight) was ignited at 550°C for 2.5 h and cooled in a desiccator. LOI was calculated as the difference (% gDW) between pre- and post-ignition sample weight.

C and N contents as well as $\delta^{13}C$ of bulk-soil OM, fresh plant biomass (green leaf tissue and fresh root material), and allochthonous OM (organic fraction of deposited sediment) were determined to assess microbial substrate quality. $\delta^{13}C$ of OM was used to distinguish between allochthonous (marine) and autochthonous (terrestrial) OM sources that greatly differ with respect to substrate quality and degradability (Khan et al. 2015). Allochthonous OM was collected during flooding events using sediment traps (compare Müller et al. 2013). Traps were positioned on the grazed creek bank with a distance of 0.5-2 m to the central creek. This was done to avoid trapping of autochthonous OM mixed within in the deposited material (e.g. with increasing distance to the sediment source (creek) or within the dense ungrazed vegetation. Sampling was conducted when no livestock was present in the sites during winter 2014/15. C, N, and $\delta^{13}C$ were determined on an element analyzer (HEKAtech, Wegberg, Germany) coupled to a Nu Horizon isotope-ratio mass spectrometer (Nu Instruments, Wrexham, UK). $\delta^{13}C$ of soil OM was determined on acidified (10% HCl) samples to remove carbonates.

DNA extraction and quantitative PCR

Microbial abundance was quantified by assaying specific gene abundance of fungi and bacteria using qPCR(Smith and Osborn 2009). Soil DNA was extracted using the PowerSoil DNA extraction kit (MoBio, Carlsbad, USA) following the manufacturer's protocol with slight modifications. Soil slurry was transferred into the reaction vial (PowerSoil bead tube) using a disposable 3-mL pipette with wide opening (2 mm). First, the slurry was pumped several times in and out of the pipette to avoid obstruction of the pipette's opening before dropping 8 to 12 droplets of slurry into the bead tube. This volume corresponded to the equivalent of approximately 0.25 g air dried soil. The PowerSoil DNA extraction method was reported to be variable in DNA yield (Feinstein et al. 2009). Therefore, four consecutive DNA extraction loops were conducted for each sample to gain higher accuracy in determination of microbial gene copies as recommended by Feinstein et al. (2009). The DNA concentration and extraction quality parameters (260/280 nm and 260/230 nm ratio) of each extraction were determined using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, US).

For qPCR, SYBR Green assays were performed on two technical replicates per sample using a Qiagen Rotor-Gene Q (Qiagen, Venlo, NL). Quantitative PCR runs were conducted with 5 µL template DNA (pooled from extraction loops 1 to 4). Cycling conditions (40 cycles) were: denaturation at 95°C for 10 s, annealing at 55°C for 15 s, extension at 72°C for 20 s, and a 10-min hold at 72°C before the final melt analysis. Data were analyzed using Rotor-Gene Q software (version 2.0.3 build 2; Model 5-Plex). Cycle threshold was automatically determined by the software and number of gene copies was calculated from the reported cycles by means of plasmid standard curves (Smith and Osborn 2009).

To estimate bacterial abundance, the primer pair B341F/B805R was used to target the prokaryotic 16S rRNA gene region (Herlemann et al. 2011). The commonly used primer pair Eub338/Eub518 (Fierer et al. 2005) was not used because it interacts with eukaryotic DNA (Klindworth et al. 2013). In preliminary investigations for this study, the Eub338/Eub518 primer pair showed signals in qPCR runs with fungal DNA from *Saccharomyces cerevisiae*, *Fusarium oxysporum*, and *Fusarium graminearum,* and its use was therefore discarded. Fungal abundance was estimated using the primer pair FR1/FF390 (Chemidlin Prévost-Bouré et al. 2011) targeting the fungi-specific eukaryotic 18S rRNA gene region. Specificity and coverage of both primer pairs were tested *in silico*.

For plasmid standard curves, DNA from pure cultures of *Agrobacterium tumefaciens* and *Fusarium oxysporum* was extracted and used in PCRs with bacteria and fungi primer pairs, respectively. PCR products were cloned into a plasmid vector and the resulting plasmid DNA was purified. Quality of the purified plasmid DNA was examined using agarose gel electrophoresis (1.5%) and ethidium bromide staining. Efficiency of standard curves was 0.78-0.84 and 1.02-1.03 with r^2 >0.99 for fungi and bacteria, respectively. Values are presented as number of gene copies per unit OM (Morrissey et al. 2013).

Exo-enzyme assays

Potential exo-enzyme activity (EEA) of cellobiosidase, ß-glucosidase, leucine-aminopeptidase, and chitinase was determined in fluorometric assays following German et al. (2011). Substrates and fluorophore standards were obtained from Sigma-Aldrich Germany (Munich, Germany). The list of enzymes, substrates, and degradable compounds is given in Table S2. Potential enzyme activity at pH 8 was measured in 50 mM bicarbonate buffer (Sinsabaugh et al. 2003). 2 g of soil slurry were mixed with 20 mL buffer. 200 µL bicarbonate buffer or 200 µL buffered soil slurry (continuously stirred) were added to 50 µL

of one of the following solutions pre-pipetted into black 96-well microplates: i) 50 mM bicarbonate buffer (for blank or control, respectively) ii) 100 µM fluorophore standard (for reference or quench, respectively), and iii) 1.6 mM substrate solution (for substrate control or enzyme assay at substrate saturation, respectively). The microplates were incubated in the dark at 20°C for 16 h and read on a PerkinElmer LS 55 plate reader (PerkinElmer Inc.,Waltham, MA, US) set at 460 nm emission and 365 nm excitation wavelength. Values are presented as enzyme activity per unit OM to obtain a measure for OM decomposition rate (Morrissey et al. 2013).

Statistical analyses

Three-way ANOVAs were conducted to test for effects of treatment (grazed vs. ungrazed), land-sea location (landward vs. seaward), and site (DSK vs. SNK) on plant and soil properties, elevation, microbial abundance and structure (specific gene abundance of fungi and bacteria and the fungi-to-bacteria-ratio), and EEA (ß-glucosidase, cellobiosidase, leucine-aminopeptidase, chitinase). When significant interaction effects between treatment, site, and land-sea location existed, subsequent two-way ANOVAs were conducted within each site or location to better understand potential grazing effects on microbial abundance and EEA. Effects on soil redox and moisture were tested with repeated measures ANOVAs. Tukey's HSD tests were conducted for pairwise comparisons. Normal distribution of residuals (as checked visually) and equal sample sizes across groups assured robustness for parametric testing (McGuinness 2002). Correlation analysis was used to further explore relations between environmental parameters (elevation, soil, and plant properties), EEA, and microbial abundance and structure. Exponential regression was used to better illustrate the significant relationship between $\delta^{13}C$ of bulk-soil OM and the fungi-to-bacteria ratio. Effects of plant-species composition on environmental parameters, EEA, and microbial abundance and structure were not independently considered in our analyses because plant-species composition could not be separated from grazing treatment in either site (Table S1). Analyses were conducted in STATISTICA 10 (StatSoft Inc., Tulsa, OK, US).

Results

Site elevation, soil parameters, and organic-matter sources

Grazed plots were on average 15 cm lower than ungrazed plots in DSK (210 ± 4 vs. 225 ± 2 cm NHN; $p < 0.05$). In contrast, elevation did not differ between grazed and ungrazed plots in SNK (215 ± 2 vs. 214 ± 1 cm NHN; $p > 0.4$). Land-sea location had no effect on elevation ($p > 0.1$; Figure S2).

Grazing increased bulk density in both marshes ($p < 0.001$). However, this treatment effect was only significant at the landward locations (*treatment x location*: $p < 0.01$). Here, grazing increased bulk density by 22% and 28% in DSK and SNK, respectively (Figure S2).

OM contents were lower under grazing ($p < 0.01$). Along with the differences in bulk density between treatments, differences in OM were only significant when comparing the landward locations (Figure S2). Bulk density and OM were negatively correlated ($r^2 = 0.779$; $p < 0.0001$).

Bulk-soil C/N (mass basis) was 12.3 ± 0.4 in DSK and 12.6 ± 0.2 in SNK ($p > 0.3$). Differences between treatments were only present in DSK with higher C/N under grazing (13.1 ± 0.5 vs. 11.5 ± 0.3; $p < 0.05$). Land-sea location had no effect on bulk-soil C/N ($p > 0.4$). $\delta^{13}C$ of bulk-soil OM differed significantly by site ($p < 0.001$) with more depleted signatures in DSK (-26.4 ± 0.2‰) vs. SNK (-25.5 ± 0.2‰). At both sites, bulk-soil OM was consistently more depleted in ^{13}C under grazing by >1‰ ($p < 0.001$; Figure 1). Land-sea location had no effect on $\delta^{13}C$ of bulk-soil OM ($p > 0.2$).

Autochthonous OM was highly variable in $\delta^{13}C$ across sites, treatments, and tissue types: belowground material from grazed samples was slightly more depleted in ^{13}C than from ungrazed samples. In contrast, aboveground material was most depleted in ungrazed samples (Figure 1). Allochthonous OM contributions are considerably ^{13}C enriched compared to autochthonous OM contributions: During winter 2014/15, $\delta^{13}C$ of sediment OM was -24.6 ± 0.2 in DSK and -22.9 ± 0.1 in SNK (Figure 1). Because relatively large differences in $\delta^{13}C$ between aboveground and belowground biomass exist (Figure 1), precise determinations of autochthonous vs. allochthonous contributions to the total OC pool are not possible based on ^{13}C natural abundance only.

Soil redox chemistry

Mean soil redox conditions were mostly oxic (>400 mV) and occasionally weakly reducing (200-400 mV). Weakly reducing redox potentials were almost exclusively measured in grazed plots (Table 1). Here, increasing soil moisture negatively affected redox ($r^2 = 0.436$; $p < 0.0001$; moisture range: 31.7-48.2%), whereas there was no moisture effect on redox in ungrazed plots ($r^2 = 0.003$; $p > 0.5$; moisture range: 10.7-48.5%). Overall, redox was significantly lower (RM-ANOVA, $p < 0.001$; Table 1) in grazed vs. ungrazed plots of both sites, and slightly higher at DSK compared to SNK (RM-ANOVA, $p < 0.05$). Grazing also decreased soil pH by 0.23 and 0.13 pH units in grazed vs. ungrazed plots in DSK and SNK, respectively (Table 1; $p < 0.001$). An effect of land-sea direction on soil pH ($p > 0.5$) or

redox (RM-ANOVA, $p > 0.5$) could not be detected.

Plant biomass parameters

After season aboveground biomass was >100% greater in ungrazed vs. grazed treatments of both sites with highest values in SNK (Table 1; $p < 0.001$). Differences in belowground biomass only existed in DSK with greater belowground biomass in the ungrazed treatment (Table 1, $p < 0.001$). However, belowground biomass was 18.5 ± 4% greater in landward vs. seaward sampling areas ($p < 0.01$).

Microbial abundance and structure

Bacterial gene abundance was strongly affected by land-sea location, particularly at DSK (three-way ANOVA: *location:* $p < 0.01$; *site x location:* $p < 0.01$; Figure 2). Grazing did not affect bacterial gene abundance at either location (two-way ANOVA: $p > 0.4$ and > 0.9 at landward and seaward locations, respectively). Similarly to bacterial abundance, fungal abundance was also affected by land-sea location at DSK (three-way ANOVA: *site x location:* $p < 0.01$). In contrast, fungal abundance was significantly increased under grazing at landward locations of both marshes (two-way ANOVA: $p < 0.01$), but was unaffected at seaward locations (two-way ANOVA: $p > 0.4$; Figure 2).

Relative fungal abundance (fungi-to-bacteria ratio; F/B) was significantly affected by grazing and varied between sites (Figure 3). In contrast to microbial abundances, F/B was unaffected by land-sea location (three-way ANOVA: *location:* $p > 0.9$, no significant interactions). Grazing reduced F/B consistently at both sites, leading to a higher relative fungal abundance in grazed vs. ungrazed treatments ($p < 0.05$). Relative fungal abundance was higher in both grazed and ungrazed plots of DSK compared to SNK ($p < 0.01$). Variability in both bacterial and fungal abundance is best but only weakly explained by bulk density and bulk-soil OM contents (Table 2). In contrast, variability in F/B is strongly determined by the $\delta^{13}C$ of the bulk-soil OM (Figure 4, Table 2), with relative fungal abundance increasing toward more depleted $\delta^{13}C$ signatures of the organic source material (terrestrial source ^{13}C end member; Figure 1). Irrespective of the study site, the negative relationship between $\delta^{13}C$ of bulk-soil OM and F/B holds true within each treatment ($p < 0.001$; Figure 4).

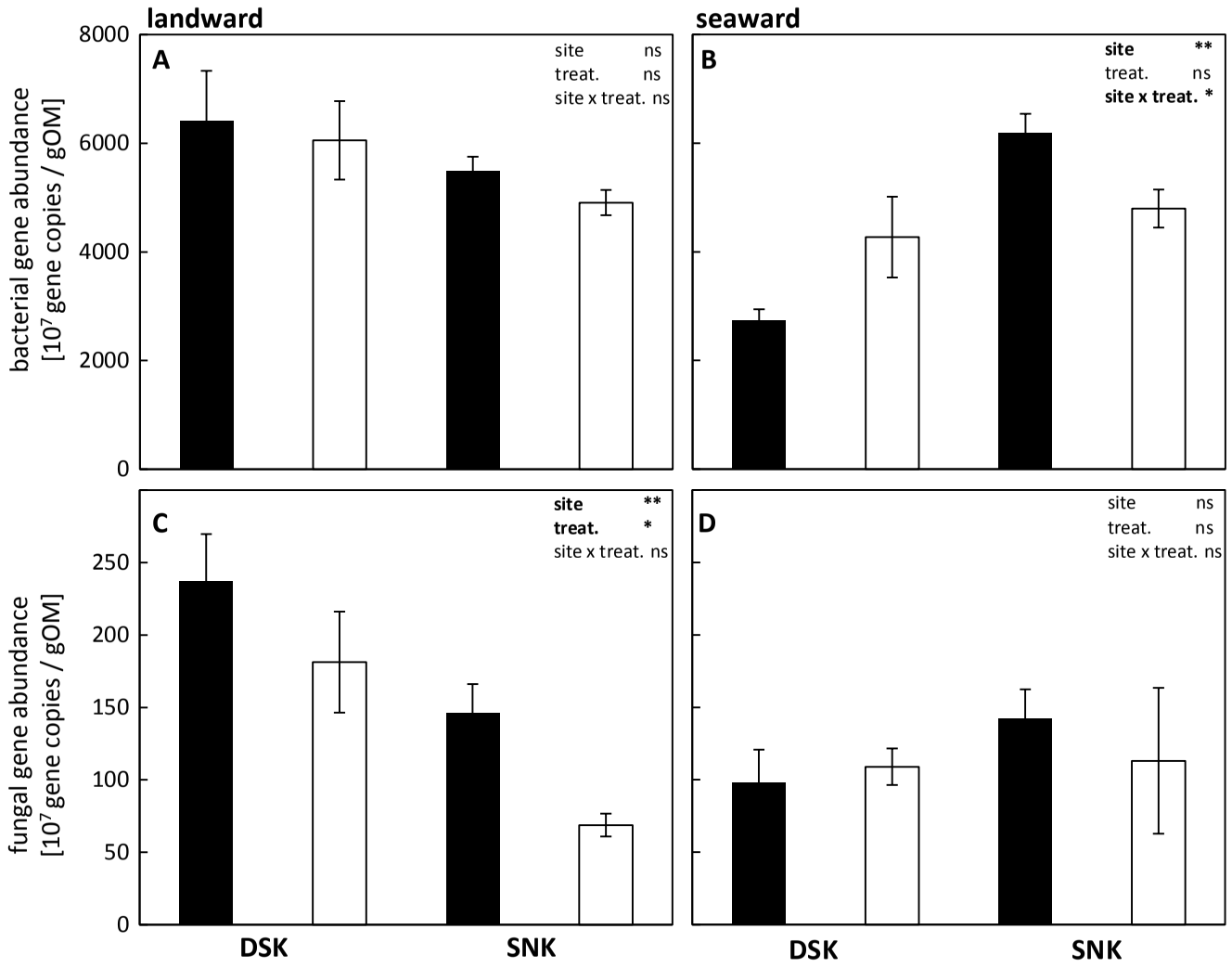

Figure 2 (A and B) Bacterial and (C and D) fungal gene abundance in grazed (filled bars) and ungrazed (open bars) treatments of the two marsh sites DSK and SNK. Panels A and C show results for landward sampling locations, Panels B and D for seaward locations. Presented are mean values ± standard errors (n = 5) and results of two-way ANOVAs for effects of site and treatment (ns, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$)

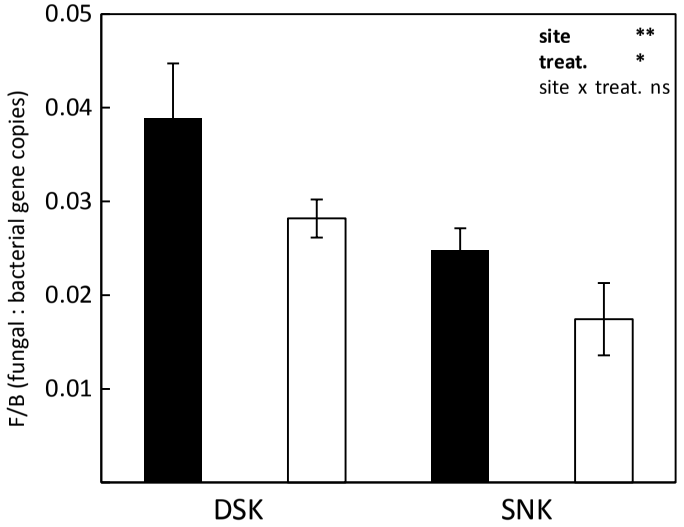

Figure 3 Fungi-to-bacteria ratio in grazed (filled bars) and ungrazed (open bars) treatments of the two marsh sites DSK and SNK. Presented are mean values ± standard errors (n = 10) and results of three-way ANOVA (ns, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$). Sampling location (landward vs. seaward) had no effect ($p > 0.9$; all interactions $p > 0.4$)

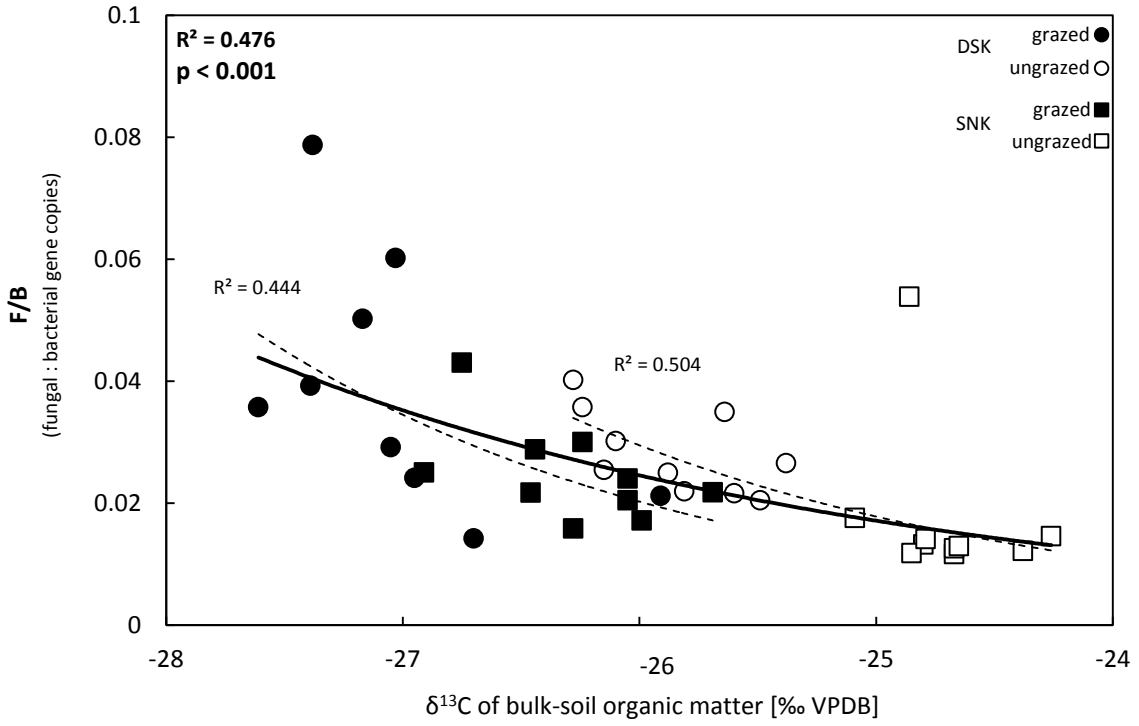

Figure 4 Fungi-to-bacteria ratio as a function of $\delta^{13}C$ in bulk-soil OM across and within grazed (filled symbols) and ungrazed (open symbols) treatments of the two marsh sites DSK (circles) and SNK (squares)

Exo-enzyme activity

Grazing consistently reduced activity of the two cellulolytic enzymes ß-glucosidase and cellobiosidase by 28-40% at both sites (Figure 5). Activity of the exo-enzymes involved in N cycling (aminopeptidase and chitinase) was not consistently affected by grazing (Figure 5). While grazing decreased aminopeptidase activity at DSK (two-way ANOVA; $p < 0.01$), there was no grazing effect at SNK (two-way ANOVA; $p > 0.2$). Chitinase activity was not affected by grazing at either site; however, activity varied significantly between sites and was higher at DSK (Figure 5). Land-sea location had no effect on the activity of any exo-enzymes (three-way ANOVA; all $p > 0.4$, no significant interactions).

Variability in the grazing-affected EEAs of ß-glucosidase and cellobiosidase is best explained by the inter-correlated parameters of redox chemistry (soil redox and pH) and aboveground biomass (Table 2). In contrast, redox chemistry and biomass parameters showed no strong correlations with EEAs of aminopeptidase or chitinase (Table 2).

Discussion

Grazing effects on microbial abundance and structure

Livestock grazing had no effect on bacterial abundance, but increased fungal abundance in the landward locations of our two marsh sites (Figure 2). Here, grazing pressure is higher, as evident by higher bulk densities (Figure S2), and consequently differences between

grazed and ungrazed plots are expected to be more pronounced than in seaward plots.

Grazing affected the soil microbial structure opposite as hypothesized by consistently increasing relative fungal abundance. In contrast to other studies that suggested negative effects of flooding or lower soil redox on fungal abundance (Bossio and Scow 1998, Chambers et al. 2016), differences in soil redox (that were profound between treatments; Table 1) did neither negatively affect absolute nor relative fungal abundance in the present study (Table 2). Our data therefore do not provide evidence of a direct grazing effect on relative and absolute fungal abundance.

We further hypothesized that grazing also indirectly affects the relative fungal abundance through decreased autochthonous production and litter accumulation, representing a recalcitrant substrate source that is assumed to be more effectively degraded by fungal than bacterial communities (van der Heijden et al. 2008, Strickland and Rousk 2010). Therefore, relative fungal abundance was expected to increase by higher rates of autochthonous production and litter accumulation in the ungrazed treatments and to decrease by higher relative contributions of high quality and easily degradable allochthonous input from marine sources (e.g. Khan et al. 2015) in the grazed treatments. Indeed, our data support OM source as an important factor shaping the microbial community in tidal wetland systems, as relative fungal abundance increased with more depleted $\delta^{13}C$ signatures within and across treatments (Figure 4). This indicates that fungi are indeed favored by higher contributions of autochthonous vs. allochthonous OM. However, contrary to our hypothesis, the contribution of autochthonous vs. allochthonous OM could be larger in the grazed treatments (Figure 1) and soil C/N was not lower under grazing; in fact, the opposite was true in one of our sites (DSK). We propose that differences in the (allochthonous) sediment-trapping capacity between grazed and ungrazed treatments are responsible for these unexpected results. Aboveground vegetation structure has a large influence on sediment trapping during inundation events (Morris et al. 2002, Temmerman et al. 2005). Both aboveground biomass and canopy height can be several fold decreased under grazing (Table 1). Consequently, also the input of high quality, marine allochthonous OM to the top soil might be lower under grazing. In support of this concept, aboveground biomass is positively correlated with the $\delta^{13}C$ of bulk-soil OM (^{13}C enrichment of bulk-soil OM with increasing aboveground biomass; Table 2). We therefore propose a novel and indirect form of animal-plant-microbe interaction: grazing affects aboveground vegetation structure, which in turn determines allochthonous OM trapping and thus microbial structure.

Models and concepts on terrestrial ecosystem functioning and stability include relative fungal abundance as a key factor (e.g. Wardle et al. 2004, Neutel et al. 2007). Although experimental support for links between microbial structure and ecosystem functions are often scarce (Rousk and Frey 2015), fungal dominance of the microbial community is often assumed to be indicative for slow carbon- and nutrient-cycling ecosystems (Moore et al. 2004, Wardle et al. 2004, de Vries et al. 2012). Therefore, higher relative fungal abundance under grazing, as found in the present study, could have important consequences for the C-turnover rate and ultimately the C-sequestration capacity of salt-marsh ecosystems. While the present study did not explicitly assess C turnover, recent data from a similar system demonstrate that both ecosystem C turnover and turnover of the microbial C pool is indeed slowed down under grazing (Olsen et al. 2011).

Although relative fungal abundance was small in the present study (<5% across treatments and sites), we propose that grazing induced shifts towards a higher relative fungal abundance can have a strong influence on tidal-wetland biogeochemistry. We therefore offer an alternative explanation for differences in rates of C turnover and sequestration found between grazed and ungrazed areas of salt marsh systems (Morris and Jensen 1998, Olsen et al. 2011, Elschot et al. 2015) that is not based on direct grazing effects on soil redox conditions or primary production per se, but on origin and quality of OM exerting control over the microbial community structure. However, we acknowledge that links between microbial structure, particularly with regard to relative fungal vs. bacterial dominance, and tidal-wetland biogeochemistry are not well understood, yet, and should therefore be addressed, particularly with regard to tidal-wetland stability and C sequestration.

Grazing effects on microbial exo-enzyme activity

Livestock grazing considerably reduced the activity of the two major cellolytic enzymes cellobiosidase and ß-glucosidase consistently by over 28-40% in both marsh sites (Figure 5), thus slowing down the initial steps of cellulose decomposition and ultimately C turnover. In accordance with our findings, Olsen et al. (2011) also suggest slower C turnover under grazing; however, their data suggest slower turnover of the microbial C pool as opposed to slowed down substrate decomposition in our study. In accordance with our second hypothesis, we demonstrate that the reduction in cellulolytic activity is induced by grazing effects on soil redox (Table 2). Grazing considerably reduced soil redox, lowering the redox state of the top soils from always oxic to frequently weakly to moderately reducing at high soil moisture levels.

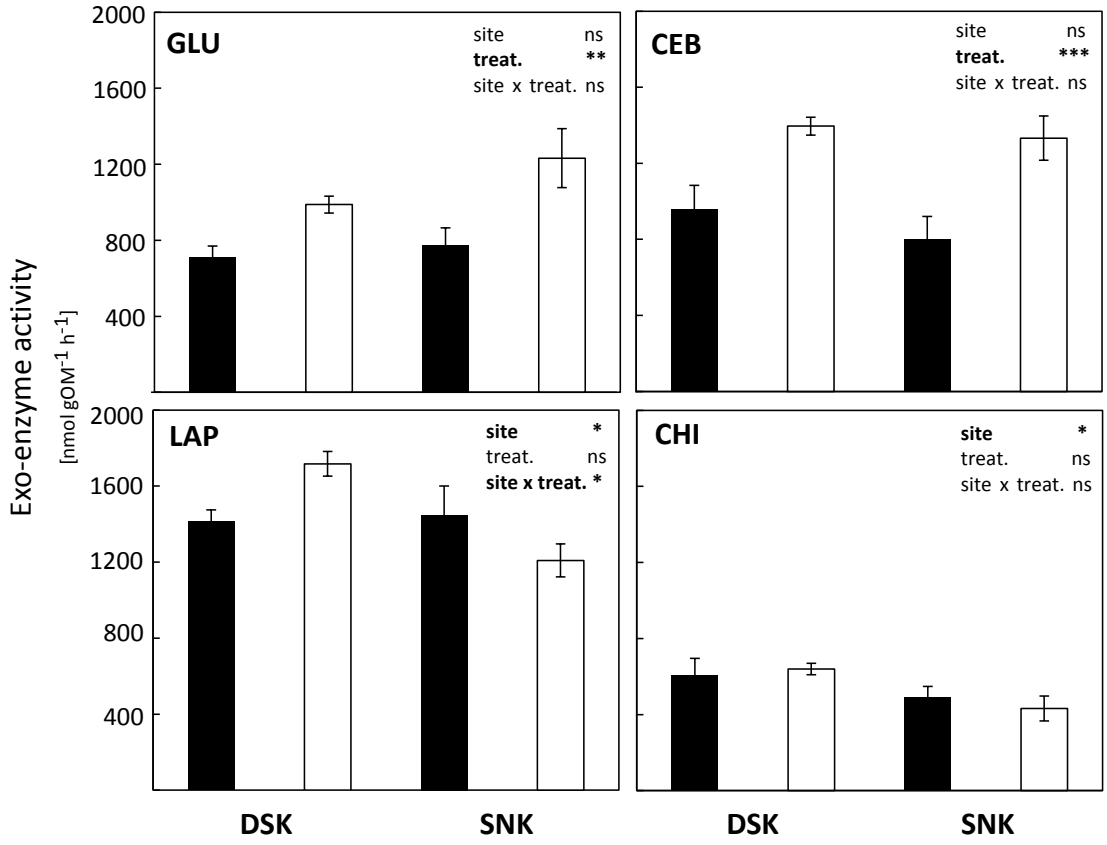

Figure 5 Activity of the exo-enzymes ß-1,4 glucosidase (GLU), cellobiosidase (CEB), leucine-aminopeptidase (LAP), and chitinase (CHI) in grazed (filled bars) and ungrazed (open bars) treatments of the two marsh sites DSK and SNK. Presented are mean values ± standard errors (n = 10) and results of three-way ANOVAs are shown (ns, $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Sampling location (landward vs. seaward) had no effect (all $p > 0.4$; all interactions $p > 0.1$)

A reduction of soil redox under grazing has previously been demonstrated by Schrama et al. (2013) and Elschot et al. (2015). However, although soil redox conditions are not necessarily related to decomposition rate in tidal wetland soils (Kirwan et al. 2013, Mueller et al. 2016b), implications on decomposition rate have been proposed (Elschot et al. 2015), but not demonstrated.

This study provides first evidence that grazing induced changes in redox chemistry (redox and pH) slow down OM decomposition (Table 2), whereas it remains unclear if these changes are caused by direct or indirect grazing effects. Previous studies explained lower soil redox under grazing being a direct grazing effect induced through trampling, which leads to lower soil porosity and higher bulk density (Nolte et al. 2013b), slowing down drainage and lowering oxygen supply to the soil (Schrama et al. 2013, Elschot et al.

2015). Our data also suggest a strong impact of grazing on bulk density, particularly in the landward locations (Figure S2) that are generally more frequently used by livestock (Bakker et al. 2002, Stock 2011, Suchrow et al. 2012). Furthermore, our data demonstrate a narrower soil moisture range in grazed vs. ungrazed plots, indicative of slower drainage in grazed soils, and a strong relationship between soil moisture and redox within the grazed treatment, but not in the ungrazed treatment. Both features are likely attributable to direct grazing or trampling effects on soil porosity and drainage. However, in the present study soil redox is not significantly related to bulk density (Table 2). In that regard, our data do not support lower redox conditions under grazing as the result of a direct grazing effect. The missing relationship between bulk density and soil redox may in part be due to limitations in the available redox data (e.g. four point measurements for relative comparisons of redox between treatments (Davy et al. 2011) as opposed to permanent measurements).

However, also indirect grazing effects on redox chemistry that are mediated through plant processes may exert control over both soil redox and pH. Indeed, redox and also pH are weakly, but closer related to biomass parameters and elevation than to bulk density in the present study (Table 2). Thus, it seems possible that greater aboveground biomass and shoot density increase oxygen supply to the rhizosphere and thus increase soil redox (Mueller et al., 2016 and references within). Additionally, a higher sediment-trapping capacity of taller vegetation (Morris et al. 2002) in the ungrazed treatment could increase soil pH by trapping carbonate-rich sediments. Eventually, greater belowground and fine root biomass are likely to affect soil pH through protonation of the rhizosphere (Yang et al. 2012). Therefore, also indirect grazing effects mediated through plant processes could have caused the observed differences in redox chemistry ultimately affecting decomposition rate.

The activity of enzymes involved in N-acquisition was unresponsive to changes in redox chemistry and thus decoupled from the grazing response of the cellulolytic enzymes. Grazing effects on the activity of enzymes degrading N-rich polymers were either not consistent between sites (aminopeptidase activity) or not present (chitinase activity). The activity of aminopeptidase and chitinase in soils has often been interpreted in the context of microbial N acquisition or microbial N demand (Sinsabaugh et al. 2008). In the present study, chitinase activity was unaffected by grazing and aminopeptidase activity was reduced under grazing at DSK, but remained unaffected at SNK (Figure 5). In contrast to the two cellulolytic enzymes assayed in this study, aminopeptidase and chitinase activity were not affected by changes in redox chemistry, but were instead linked to microbial structure and abundance and to micro-topographical parameters (distance to creek and elevation). For instance, we demonstrate weak but positive relationships between aminopeptidase activity

and both fungal abundance and F/B, which could result from a higher bacterial N demand under increasing competition for nutrients with fungi or higher production of enzymes in fungi (Table 2). However, linking microbial structure to functions such as activity of certain exo-enzymes is difficult due to the high degree of functional redundancies across microbial groups and taxa (Nannipieri et al. 2003, Morrissey et al. 2013, Chambers et al. 2016). Additionally, the primers used in our qPCR assays do not allow a precise separation of saprotrophic from symbiotic groups (e.g. arbuscular mycorrhizae) within fungi, making it difficult to interpret links between microbial structure and function. Still, as our data demonstrate microbial structure to be strongly influenced by OM source, also N acquisition activities could be indirectly linked to changes in allochthonous OM contributions. It has been demonstrated that stoichiometries of exo-enzyme activities involved in the acquisition of different nutrients relate to OM quality and can reveal important insights into microbial nutrient demand (Sinsabaugh et al. 2008, 2014, 2016). In accordance with this, our data suggest a strong influence of OM source ($\delta^{13}C$) on the microbial C- vs. N-acquisition activity (ratio of cellulolytic activities to N-acquisition activities) with decreasing relative investment in N-acquisition with more enriched signatures indicative of allochthonous, high quality marine sources ($r^2 = 0.467$, $p < 0.001$; Figure S3).

Site effects on microbial structure and function

It must be noted here that site effects on the microbial structure were even stronger than treatment effects. The higher elevated DSK site showed higher relative fungal abundance in both grazed and ungrazed treatments than the SNK site (Figure 4). Likewise, chitinase and aminopeptidase activity responded stronger to site than treatment effects (Figure 5). The lower elevated SNK site experiences >3 times the sediment deposition than the DSK site (Müller et al. 2013) and thus a considerably larger amount of high quality, allochthonous material (Figure 1). This is in accordance with our conclusion that the rate of allochthonous OM input is an important driver of the microbial structure and possibly function, causing a greater bacterial abundance and lower N-acquisition activity in SNK compared to DSK.

Table 2 Pearson's correlation matrix. Presented are r-values (top right half) and p-values (bottom left half) for correlations between parameters of micro-topography (elevation, distance to creek), plant biomass (aboveground and belowground biomass), soil (bulk density, organic matter content, $\delta^{13}C$ of organic matter, C/N, mean redox, pH), microbial abundance/structure (fungal and bacterial gene abundance, F/B ratio), and microbial exo-enzyme activity (ß-glucosidase, cellobiosidase, leucine-aminopeptidase, chitinase). r- and p-values are bold-typed at $p < 0.05$

	micro-topography and plant biomass					soil characteristics				microbial abundance				exo-enzyme activity			
	elev	dist	AB	BB	BD	OM	13C	C/N	redox	pH	fungi	bacteria	F/B	GLU	CEB	LAP	CHI
elev		-0.139	**0.347**	**0.480**	0.038	-0.130	-0.011	**-0.483**	**0.516**	**0.405**	**0.338**	0.106	**0.346**	0.239	**0.356**	**0.490**	0.206
dist	0.393		0.105	-0.060	**-0.487**	**0.422**	0.279	-0.056	-0.309	-0.282	**-0.340**	-0.094	**-0.368**	-0.160	-0.270	**-0.392**	-0.172
AB	**0.028**	0.519		0.260	**-0.537**	**0.375**	**0.667**	-0.091	**0.658**	**0.449**	-0.274	-0.179	-0.264	**0.576**	**0.568**	-0.036	-0.135
BB	**0.002**	0.714	0.105		-0.069	-0.053	0.250	**-0.389**	0.304	**0.478**	-0.032	0.189	-0.159	0.201	0.239	**0.434**	0.183
BD	0.818	**0.001**	**0.000**	0.670		**-0.883**	**-0.422**	0.156	-0.209	-0.152	**0.531**	**0.416**	**0.371**	-0.175	-0.189	**0.411**	0.001
OM	0.424	**0.007**	**0.017**	0.744	**0.000**		0.201	-0.169	0.160	0.125	**-0.489**	**-0.549**	-0.181	0.129	0.119	**-0.432**	0.095
13C	0.945	0.082	**0.000**	0.119	**0.007**	0.215		0.000	**0.437**	0.185	**-0.349**	0.147	**-0.601**	**0.458**	**0.380**	-0.158	-0.308
C/N	**0.002**	0.733	0.577	**0.013**	0.337	0.298	0.999		-0.266	**-0.436**	0.027	0.042	0.040	-0.121	**-0.326**	-0.239	-0.221
redox	**0.001**	0.053	**0.000**	0.057	0.195	0.325	**0.005**	0.097		**0.507**	0.053	0.005	0.066	**0.474**	**0.719**	0.301	-0.030
pH	**0.010**	0.078	**0.004**	**0.002**	0.348	0.443	0.253	**0.005**	**0.001**		-0.027	-0.154	0.046	**0.439**	**0.563**	**0.382**	0.278
fungi	**0.033**	**0.032**	0.087	0.846	**0.000**	**0.001**	**0.027**	0.869	0.744	0.870		**0.561**	**0.713**	0.098	0.137	**0.413**	0.128
bacteria	0.514	0.563	0.269	0.243	**0.008**	**0.000**	0.364	0.795	0.974	0.344	**0.000**		-0.108	0.073	0.033	0.263	-0.149
F/B	**0.029**	**0.019**	0.099	0.326	**0.018**	0.263	**0.000**	0.806	0.685	0.776	**0.000**	0.505		0.006	0.071	**0.322**	**0.356**
GLU	0.137	0.324	**0.000**	0.215	0.281	0.429	**0.003**	0.457	**0.002**	**0.005**	0.546	0.656	0.970		**0.772**	0.245	0.153
CEB	**0.024**	0.092	**0.000**	0.137	0.242	0.464	**0.016**	**0.040**	**0.000**	**0.000**	0.399	0.839	0.665	**0.000**		**0.371**	0.261
LAP	**0.001**	**0.012**	0.826	**0.005**	**0.008**	**0.005**	0.329	0.137	0.059	**0.015**	**0.008**	0.101	**0.043**	0.128	**0.018**		**0.356**
CHI	0.203	0.290	0.405	0.259	0.997	0.558	0.053	0.170	0.856	0.082	0.432	0.360	**0.024**	0.345	0.103	**0.024**	

Müller et al. (2013) reported considerable rates of sediment deposition to the high marsh zones of both SNK and DSK with 3.6 ± 0.9 and 1.0 ± 0.3 kg m^{-2} $year^{-1}$, respectively. Assuming a mean OM content of approx. 10% in the deposited material (Butzeck et al. 2014), these values correspond to 9.3-21.8% and 26.0-82.4% of the standing aboveground biomass in DSK and SNK, respectively, thus representing a major microbial substrate source.

We therefore emphasize the important role of the mostly overlooked influence of allochthonous OM on tidal wetland biogeochemistry. Even though the importance of allochthonous OM as microbial C source has long been demonstrated (Boschker et al. 1999), effects of allochthonous OM inputs on rhizosphere biogeochemical processes and the microbial ecology of tidal wetland systems remain poorly understood (Bouillon et al. 2004). Particularly with regard to global warming, sea level rise, changes in inundation frequency, sediment load, and allochthonous and autochthonous OM production (Boyce et al. 2010, Kirwan and Megonigal 2013, Baldwin et al. 2014), future research should address potential impacts of changes in OM source on tidal-wetland biogeochemistry.

Alternative considerations for C/N and $\delta^{13}C$ *dynamics*

In this study, we did not find the expected relationship between C/N and $\delta^{13}C$ of bulk-soil OM as demonstrated elsewhere (Bouillon et al. 2004). However, events of sediment deposition and therefore allochthonous OM inputs to our high marsh sites are episodic, and predominantly occur during storm surges between November and March (Müller et al. 2013, Butzeck et al. 2014). Depending on sampling time, additional N from high quality marine sources may have left the soil pool through mineralization, rapid plant uptake, and allocation to N-rich aboveground biomass. We therefore acknowledge that it will be necessary to investigate the seasonal dynamics in soil C/N, $\delta^{13}C$, and microbial structure in order to understand their interactions. However, the effects of marine allochthonous inputs on the microbial structure of salt marsh soils may not solely be due to the low C/N of the material. Also structural, more recalcitrant components like cellulose or lignin are less abundant or not present in marine OM (Baldock et al. 2004), thus potentially affecting competition between bacterial and fungal decomposers.

Besides input from allochthonous sources, also other processes can cause ^{13}C enrichment in bulk-soil relative to autochthonous OM. For instance, OM becomes enriched in ^{13}C with age and depth in terrestrial soil profiles. This process has often been attributed to the

gradual enrichment of OC when being repeatedly incorporated and cycled in the microbial biomass pool over time (Cifuentes and Salata 2001), thereby gradually decreasing in quality (Churchland et al. 2013, Rousk and Frey 2015). Alternatively, Kohl et al. (2015) suggest ^{13}C depletion in fungal relative to bacterial biomass, concluding that changes in $\delta^{13}C$ with age or depth could be explained by changes in the F/B alone.

However, in the present study the divergence of $\delta^{13}C$ in bulk-soil OM from the $\delta^{13}C$ of the fresh biomass (Figure 1) is too large to be driven by aging of the material only within the top 5 cm. Furthermore, in the present study the range of relative fungal abundance (1.5-7.5%) is too small to explain variation in $\delta^{13}C$ of bulk-soil OM of >3‰. First findings from terrestrial systems demonstrate order of magnitude differences in relative fungal abundance (1-55%) to impact the $\delta^{13}C$ of bulk-soil OM in a range of only <3‰ (Kohl et al. 2015). Irrespective of the reason for the gradual enrichment of bulk-soil OM with aging, ^{13}C enrichment in the range as demonstrated in the presented study require cycling of OM through several soil horizons (e.g. Arrouays et al. 1995, van Kessel et al. 2006, Yoneyama et al. 2006, Kohl et al. 2015). Data of deeper soil samples from one of our sites (SNK) show that $\delta^{13}C$ of bulk-soil OM does not significantly change downcore even within the first 20 cm of the soil profile in neither grazed nor ungrazed areas (H. T. Do, unpublished data), indicating that considerable enrichment of the organic C downcore also requires longer time periods in these fast accreting systems (Suchrow et al. 2012, Nolte et al. 2013b).

Lastly, digestive processes of herbivores have been reported to induce slight ^{13}C fractionation in the digested material (Sponheimer et al. 2003). Therefore, it is possible that the return of excretory products to the soil surface in our grazed treatment could have affected the $\delta^{13}C$ of bulk-soil OM. However, Codron et al. (2012) could demonstrate that the $\delta^{13}C$ of grass diet is not affected by digestive processes along the gastrointestinal tract of ruminants, making it unlikely that excretory products caused the large difference in $\delta^{13}C$ of bulk-soil OM observed between our treatments.

Limitations of qPCR-based quantification of microbial abundance

The present study assessed microbial abundance by quantifying fungi-or bacteria-specific gene regions using qPCR. It needs to be noted here that qPCR results may not directly translate to the relative abundances of these groups on a per biomass basis, as fungi and bacteria differ physiologically (Strickland and Rousk 2010). It is neither recommended to confer the number of cells from the number of gene copies, because the number of rRNA gene regions per cell can

be variable within both fungi and bacteria (Smith and Osborne 2009). Therefore, qPCR-detected shifts in both relative and absolute abundance of the two groups can be influenced by profound shifts within the bacterial or fungal community. However, previous work assessing fungal and bacterial abundance using different methods demonstrate that qPCR results are often highly correlated with results based on other methods (e.g. PLFA techniques or microscopy) and that conclusions based on different methods are in well agreement (e.g. Boyle et al. 2008, Bachar et al. 2010, Buckeridge et al. 2013).Therefore, we are confident that the qPCR-based results presented here are suitable to capture relative shifts in both microbial abundance and structure between sites and treatments.

Implications and Synthesis

Our data on microbial structure and function suggest reduced C turnover under grazing. In the context of blue C, livestock grazing therefore yields the potential to significantly increase the C value of salt-marsh area. Livestock grazing is conducted in salt marshes worldwide (e.g. Yang et al. 2008, Gedan et al. 2009, Di Bella et al. 2014). Particularly in Europe, large parts of the salt-marsh area are used for livestock grazing. Here, the proportion of grazed vs. ungrazed area as well as the grazing intensity is regulated through habitat-management directives (e.g. Esselink et al. 2009). However, the need for livestock grazing as habitat management was mainly discussed in the context of biodiversity support in the past (Bakker et al. 2002, Klink et al. 2016), while effects on ecosystem services such as C sequestration were left unconsidered. At the current stage, we cannot give management recommendations with respect to grazing effects on C sequestration because our understanding is based on few case studies with equivocal results only. Specifically, we need to understand under which conditions frequent biomass removal and lower production under grazing (Morris and Jensen 1999) is counterbalanced by reduced decomposition, and how this translates to changes in C sequestration in order to predict and ultimately to optimize the C sink function of salt marsh systems.

In the present study, we demonstrate that livestock grazing can have a strong effect on the microbial structure of tidal wetland soils, increasing the relative fungal abundance and therefore potentially exerts important control over C turnover and sequestration rates. Our data suggest that this shift in the microbial structure is primarily driven by OM source, with higher contributions of recalcitrant autochthonous vs. easily degradable allochthonous sources in

grazed areas favoring relative fungal over bacterial abundance. We therefore propose a novel and indirect form of animal-plant-microbe interaction, by which livestock grazing affects microbial structure via changes in vegetation structure and allochthonous OM trapping. We further provide first evidence that grazing indeed slows down OM decomposition through changes in redox chemistry, while it remains unclear if this is a direct grazing effect, and trampling driven as hypothesized in earlier studies, or if the grazing effect on redox chemistry is plant mediated and thus indirect.

In conclusion, our study improves the process-level understanding of how livestock grazing can affect the microbial ecology and biogeochemistry of salt marsh ecosystems that helps to explain and predict differences in C turnover and sequestration rates between grazed and ungrazed systems.

Acknowledgements

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Supporting information (Chapter 5)

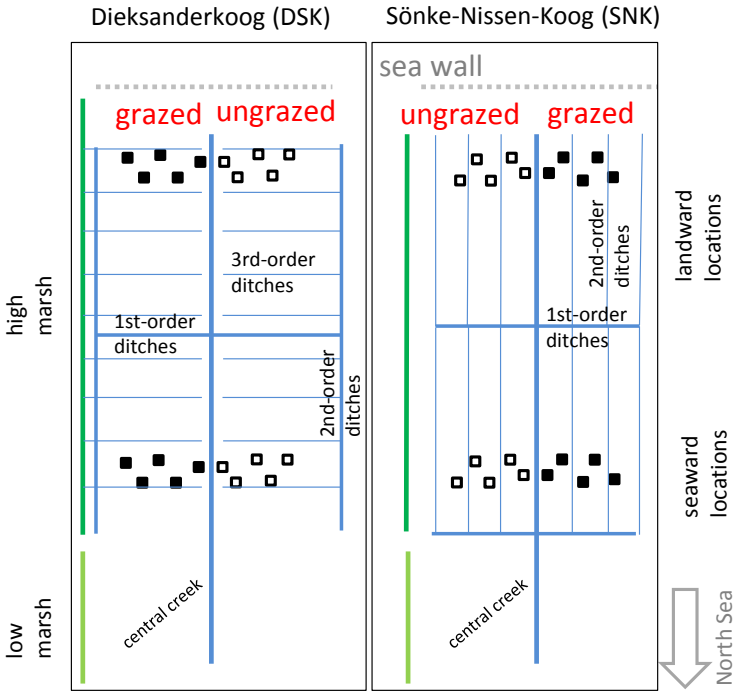

Figure S1 Experimental design for DSK and SNK marsh. Diagrams are not to scale

Table S1 Mean species cover (Londo scale; n = 5) and frequency (f; number of sampling positions with presence of species; 1-5) of the three dominant grasses *Festuca rubra*, *Puccinellia maritima*, *Elymus athericus*, and forbs around soil sampling locations

Site	treatment	location	*Festuca*[1]		*Puccinellia*		*Elymus*		forbs[2]	
			cover	f	cover	f	cover	f	cover	f
DSK	grazed	land	96	5	0	0	0.4	1	7	5
		sea	77	4	17	1	1.6	4	5	4
	ungrazed	land	0	0	0	0	100	0	0	0
		sea	0	0	0	0	100	0	0	0
SNK	grazed	land	36	4	54	4	0	0	9	5
		sea	39	3	46	5	1.2	1	12	5
	ungrazed	land	0	0	0	0	100	0	0	0
		sea	0	0	0	0	100	0	0	0

1) contains contributions of *Agrostis stolonifera*
2) sum of forbs, including *Salicornia* spec., *Spergularia media*, *Triglochin maritima*, *Artemisia maritima*, *Aster tripolium*, and *Trifolium* spec.

Table S2 List of enzymes, substrates, and degradable compounds

enzyme	artificial substrate	compounds
ß-Glucosidase	4-MUF-ß-D-glucopyranoside	cellulose, cellobiose
Cellobiosidase	4-MUF-ß-D-cellobioside	cellulose
Leucine-aminopeptidase	L-Leucine-7-AMC	peptides
Chitinase	4-MUF-N-acetyl-ß-D-glucosaminide	chitin

4-MUF = 4-methylumbelliferone; 7-AMC = 7-amido-4-methylcoumarin hydrochloride

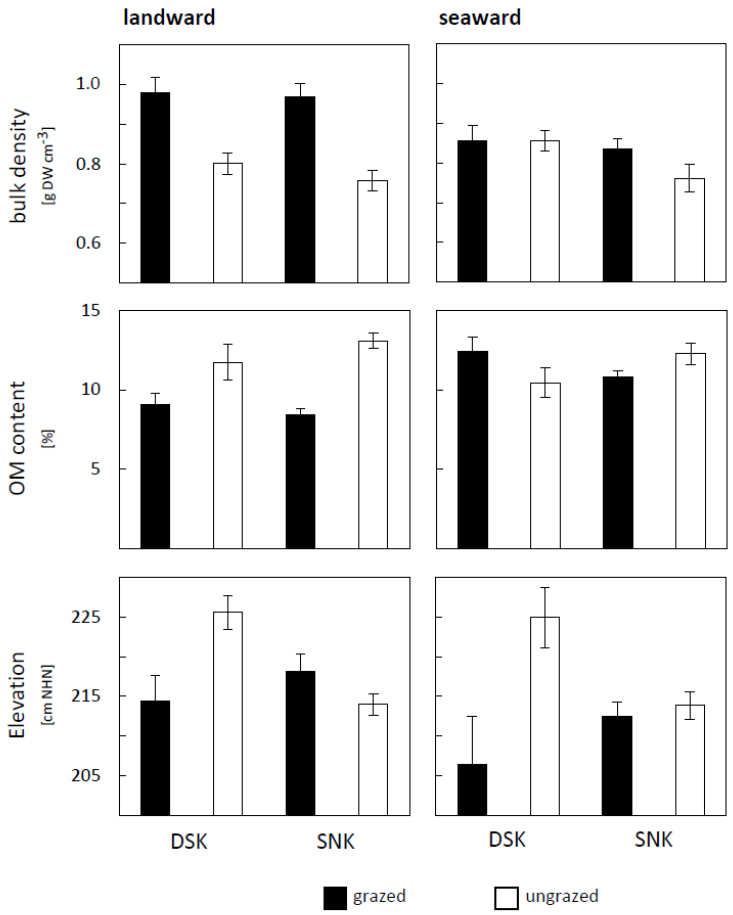

Figure S2 Bulk density (top-row panels), OM contents (mid-row panels), and surface elevation (bottom-row panels) in grazed and ungrazed treatments of the two marsh sites DSK and SNK. Left panels show results for landward sampling locations, right panels for seaward locations. Presented are mean values ± standard errors (n = 5)

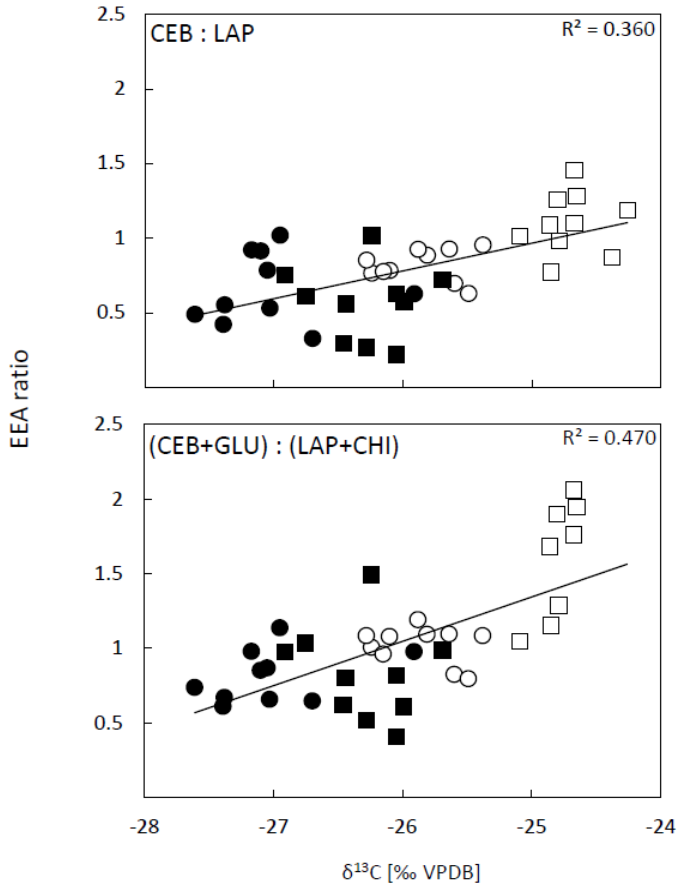

Figure S3 Stoichiometry of microbial C vs. N acquisition in relation to substrate source. Ratio of cellulolytic EEA vs. N-acquisition EEA as a function of $\delta^{13}C$ in bulk-soil OM across grazed (filled symbols) and ungrazed (open symbols) treatments of the two marsh sites DSK (circles) and SNK (squares).

Top: cellobiosidase/aminopeptidase
Bottom: (cellobiosidase + ß-glucosidase)/ (aminopeptidase+chitinase)

6 Wetland carbon sequestration potential is reduced by rising temperatures and sea level rise

In preparation as Letter to Nature Climate Change

Peter Mueller*, Lisa Schile*, Thomas Mozdzer, Thomas Dinter, Yakov Kuzyakov, Alma de Groot, Peter Esselink, Chris Smit, Andrea D'Alpaos, Carles Ibáñez, Magdalena Lazarus, Urs Neumeier, Beverly Johnson, Andrew Baldwin, Stephanie Yarwood, Diana Montemayor, Jihua Wu, Zaichao Yang, Kai Jensen, and Stefanie Nolte

*The first two authors contributed equally to this manuscript

Tidal wetlands, such as tidal marshes and mangroves, are global hotspots for carbon (C) sequestration (Duarte et al. 2013). The preservation of organic matter (OM) is a critical process by which tidal wetlands exert their strong influence over the global C cycle and at the same time gain elevation to keep pace with sea level rise (SLR) (Chmura et al. 2003, Duarte et al. 2013, Kirwan and Megonigal 2013). The present study provides the first global-scale field-based experimental evidence of temperature and relative sea level effects on OM decomposition and stabilization in tidal wetlands. This study, assessing the Tea-Bag Index (TBI) (Keuskamp et al. 2013), was conducted in 25 marsh and mangrove sites across four continents, utilizing commercially available standardized OM. While effects on decomposition rate per se were minor, OM stabilization was strongly and negatively affected by temperature (~5% reduction per 1°C increase) and reduced by 27% in low vs. high elevation zones. These unanticipated combined negative effects of temperature and relative sea level on OM stabilization yield the potential to strongly reduce the fraction of net primary production and other OM sources that are transformed to stable soil organic matter (SOM), thus reducing the C sequestration potential and ecosystem stability against accelerated SLR of tidal wetlands.

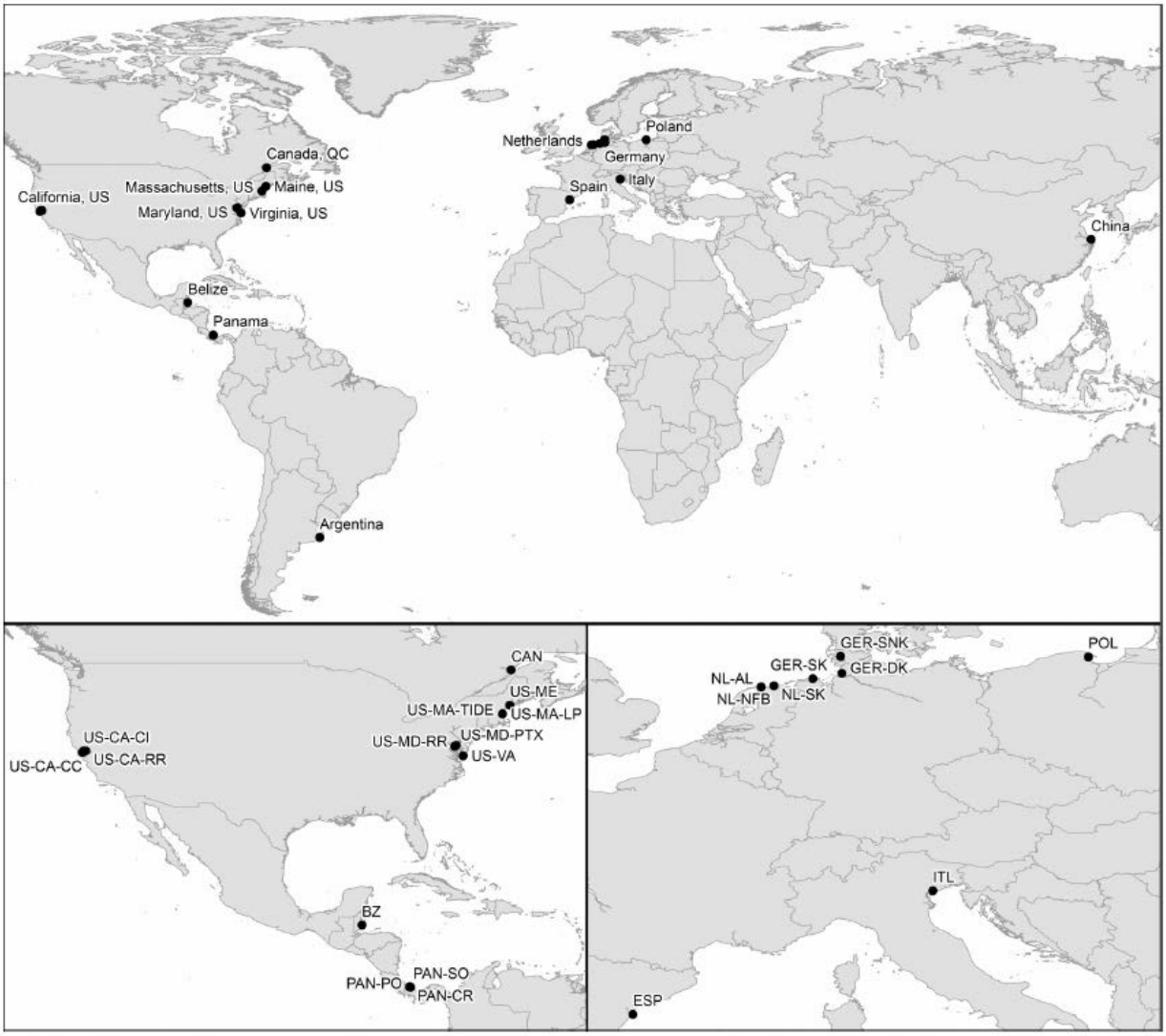

Figure 1 Overview map of study sites. See Methods Table 1 for site details

Tidal wetlands provide numerous ecosystem services, making them some of the most economically important ecosystems on Earth, and are valued at approximately US$ 10,000 per hectare (Barbier et al. 2011, Kirwan and Megonigal 2013). Yet, they are among the most threatened ecosystems, experiencing dramatic loss due to direct anthropogenic impacts and accelerated SLR (Mcleod et al. 2011, Kirwan and Megonigal 2013). The preservation of OM is a critical process by which tidal wetlands gain elevation thereby allowing them to keep pace with SLR (Kirwan and Megonigal 2013). Consequently, global changes that decrease OM preservation in tidal wetland soils not only affect C sequestration, but also put ecosystem stability at risk against accelerated SLR. Therefore, identifying global change factors that affect the transformation of organic inputs to stable SOM in tidal wetlands and assessing the magnitude of their effects is critical.

Litter-bag techniques assessing the mass loss of plant material over time are widely used to assess effects of climate parameters on decomposition rates in situ across

ecosystems (Zhang et al. 2008, Prescott 2010). However, complex interactions between climate and litter-quality effects make it difficult to predict the relevance of potential global-change drivers for decomposition (Prescott 2010). To separately assess climate effects on decomposition at a global scale, standardizing litter quality is necessary. Furthermore, implications of litter-decay data for C sequestration need to be considered cautiously, as the link among decomposition rate, SOM formation, and ultimately C sequestration is not straightforward (Prescott 2010, Cotrufo et al. 2015). Because plant tissues are not resistant to decay per se, it is critical to understand their biogeochemical transformation into stable compounds that leads to the formation of humus or SOM (i.e. humification or stabilization) rather than understanding the pace at which decomposition proceeds (Prescott 2010). Keuskamp and others (2013) developed an efficient approach for studying litter decomposition and OM transformation at a global scale, using commercially available tea as standardized material. Their TBI approach has gained considerable attention among the scientific community and media alike. By deploying two types of tea that considerably differ in their OM quality, the method allows for the determination of the decomposition constant (*k*), as in classic litter-bag approaches, and a stabilization factor (*S*), which describes the fraction of labile and decomposable OM that becomes stabilized during deployment.

In this study, we assessed the impacts of temperature and relative sea level on both *k* and *S* in tidal wetland soils by conducting a worldwide TBI field study (Figure 1; Methods Table 1). First, we aimed to capture temperature effects on *k* and *S* by covering a large gradient of $\Delta T > 15$ °C, thereby improving our understanding of how global warming affects carbon turnover and ultimately sequestration. Second, by conducting paired measurements in both high and low elevated zones within each site, we aimed to gain insights into the effects of increased flooding frequency and duration and thus potential effects of accelerated SLR on *k* and *S*.

We found that both temperature and relative sea level strongly reduced stabilization, but surprisingly, we found subtle and no effects of temperature and relative sea level, respectively, on decomposition rate (Figure 2). Other factors such as salinity (fresh, brackish, or salt water) or soil (mineral vs. organic) and ecosystem type (marsh vs. mangrove) neither affected *k*, whereas temperature effects caused *S* to be lower in mangroves than in marshes, and lower in organic than in mineral systems (Table 1; Figure S1).

Table 1 Spearman's rank coefficients between the variables temperature, latitude, tidal amplitude, salinity class, *k*, and *S* (coefficients are bold typed at $p \leq 0.05$) and comparisons of temperature (°C), latitude (°), amplitude (m), *k*, and *S* between ecosystem types (mangrove vs. marsh) and soil type (mineral vs. organic) shown as means ± SE

	temperature	latitude	amplitude	salinity	*S*	*k*
Spearman's rank correlations						
temperature		**-0.78**	**-0.69**	-0.06	**-0.47**	0.00
latitude	**-0.78**		**0.56**	0.06	**0.42**	0.12
amplitude	**-0.69**	**0.56**		**0.34**	0.10	0.06
salinity	-0.06	0.06	**0.34**		-0.01	-0.07
S	**-0.47**	**0.42**	0.10	-0.01		**-0.48**
k	0.00	0.12	0.06	-0.07	**-0.48**	
group means ± SE						
soil type	***	*	**		*	ns
mineral	17.6±4.6	45.4±8.1	1.73±0.22		0.22±0.03	0.010±0.001
organic	23.0±4.5	31.1±15.7	0.91±0.22		0.13±0.03	0.010±0.001
ecosystem	***	***	**		*	ns
marsh	18.4±4.2	44.3±7.1	1.58±0.18		0.20±0.03	0.010±0.001
mangrove	28.1±0.7	11.4±3.7	0.23±0.02		0.07±0.01	0.010±0.000

Asterisks show results of Mann-Whitney U tests and denote significant differences as: $p \leq 0.05$ = *, $p \leq 0.01$ = **, $p \leq 0.001$ = ***, not significant = ns

We neither found linear (Figure 2A) nor monotonic (Table 2) relationships between temperature and *k*. This is plausible because relationships between single parameters and *k* are often not linear or monotonic (Prescott 2010). Instead, critical thresholds exist at which certain predictors become influential, and classification and regression tree analysis (CRTA) can identify such thresholds (Rothwell et al. 2008, Prescott 2010). Indeed, CRTA reveals that temperature is a secondary predictor for *k* (Figure S1). However, temperature seems to positively affect *k* only in meso-tidal systems (amplitude >2.1 m; ~35% of sites), with sites ≥ 14.5°C during deployment supporting higher *k* than sites characterized by lower temperatures (Figure S1). CRTA suggests that tidal amplitude is an important predictor for *k*. However, this result needs to be interpreted cautiously because no linear ($p > 0.68$; $r^2 = 0.004$) or monotonic relationship (Table 1) exists between tidal amplitude and *k*, and effects of tidal amplitude are not independent from other factors because strong correlations exist with ecosystem and soil type, temperature, and latitude (Table 1).

The weak temperature response of *k* is surprising in the context of basic biokinetic theory. Following typical Q10 values for biological systems of 2-3 (Davidson and Janssens 2006), *k* should have at least doubled over a gradient of $\Delta T > 15$°C. However, findings from studies conducted at single-marsh to regional scales are not conclusive either, ranging from no or small (Charles and Dukes 2009, Kirwan et al. 2014, Janousek et al. 2017) to strong effects with a Q10 >3.4 as found within a single site (Kirwan and Blum 2011). Although temperature sensitivity of litter types is variable (Craine et al. 2010, Wilson et al. 2016), the

profound temperature sensitivity of the TBI materials has sufficiently been demonstrated (Keuskamp et al. 2013). We therefore conclude that other parameters exerted overriding influence on *k*, mainly masking temperature effects, and have not been captured by our experimental design. For instance, we do not have data on plant-biomass parameters that are thought to exert extraordinary control on decomposition through priming effects (Mueller et al. 2016b, Bernal et al. 2017).

Flooding and progressively anaerobic soil conditions are supposed to be a strong suppressor of decomposition (Davidson and Janssens 2006). Despite this dominant paradigm, we clearly demonstrate that *k* is not reduced in low vs. high elevated zones (Figure 2C). This finding is in accordance with an increasing number of case studies (Kirwan et al. 2013, Mueller et al. 2016b, Janousek et al. 2017) demonstrating negligible direct effects of sea level on decomposition in tidal wetland soils. A SLR-induced reduction in decomposition with positive feedback on tidal wetland stability (Reed 1995) is therefore an unlikely scenario.

In contrast to missing or subtle effects of sea level and temperature on *k*, OM stabilization was strongly affected by temperature. The strong negative correlation between *S* and temperature ($p < 0.0001$; $r^2 = 0.288$; Figure 2B) agrees well with the CRTA identifying temperature as the only predictor for *S* (Figure S1). Overall, we demonstrate a decrease in *S* by nearly 50% over a 10 °C temperature increase – a 18% reduction considering an end-of-century projection of $\Delta T = 3.8$ °C (IPCC 2013). Additionally, paired comparisons reveal that *S* is significantly reduced by 27% in low vs. high elevation zones ($p < 0.01$; Figure 2D).

The mechanism by which *S* is decreased in the more flooded zones is unknown. Because we did not observe consistent salinity effects on *S* and *k* in our data (Figure S1, S2), we do not suppose that regular exposure of litter to salt water explains the unexpected finding. Instead, more favorable soil moisture conditions in low vs. high elevated zones could have decreased OM stabilization, if higher flooding frequencies did not induce redox conditions low enough to suppress microbial activity (Pfauder and Zimmer 2005). In support of this, flooding-frequency induced changes in moisture conditions have been reported as primary driver of surface litter break down, leading to more than four-fold increased litter mass loss in low vs. high marsh zones of a New Jersey salt marsh (Halupa and Howes 1995).

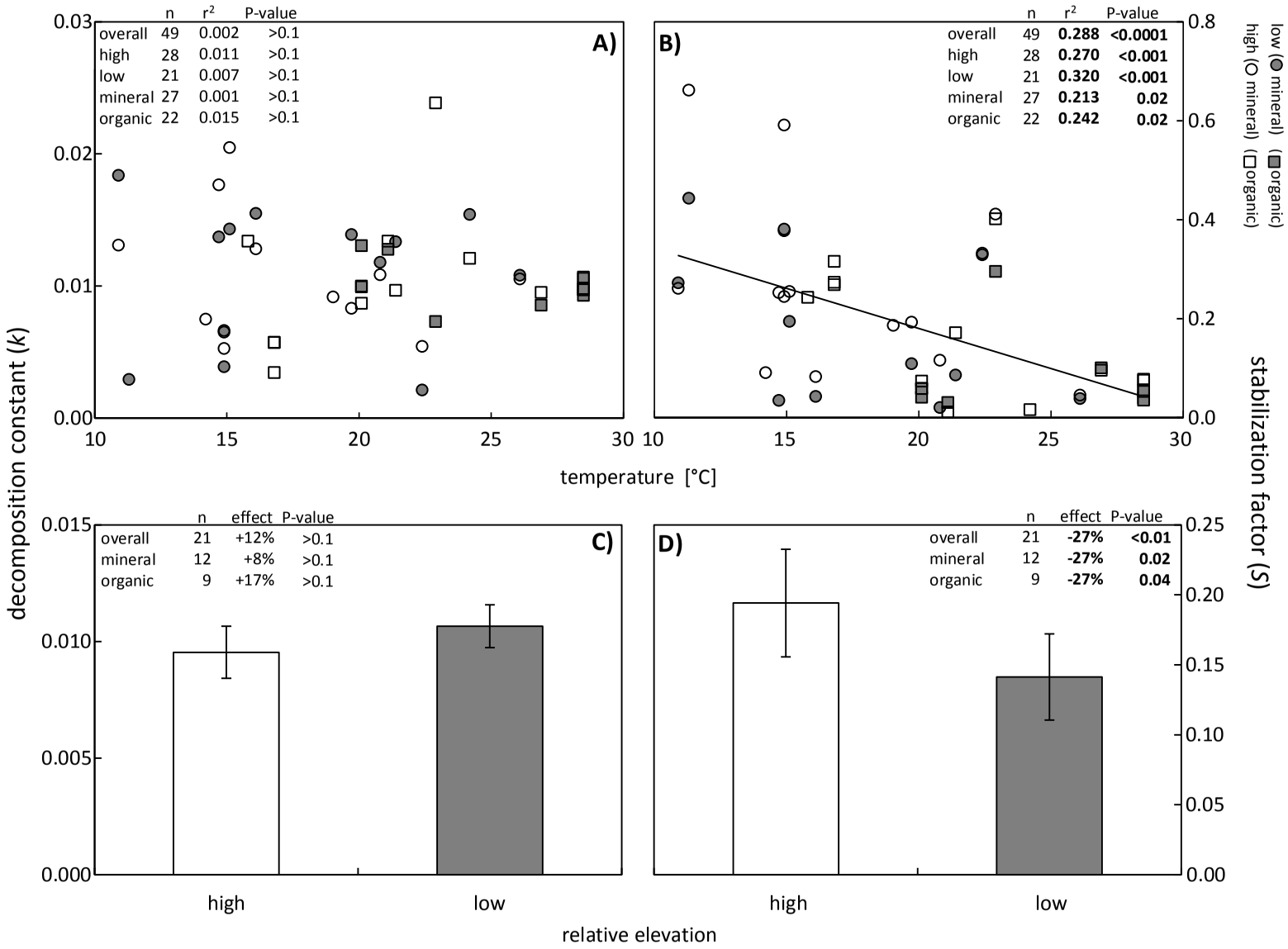

Figure 2 (**A and B**) Decomposition (*k*) and stabilization (*S*) versus mean atmospheric temperature during deployment period. Shown are results of linear regression analyses across and within elevation zones and soil types. (**C and D**) *k* and *S* in high and low elevated zones of salt marsh and mangrove sites (compare Methods Table 1). Zones are characterized by distinct high or low marsh plant-species assemblages or by different stature of mangroves along the flooding gradient within each site. Shown are means ± SE, mean effect size as decrease or increase (%) relative to the high zone, and results of paired t-tests for all sites (overall; two-tailed) and subsequent within mineral and organic systems (one-tailed)

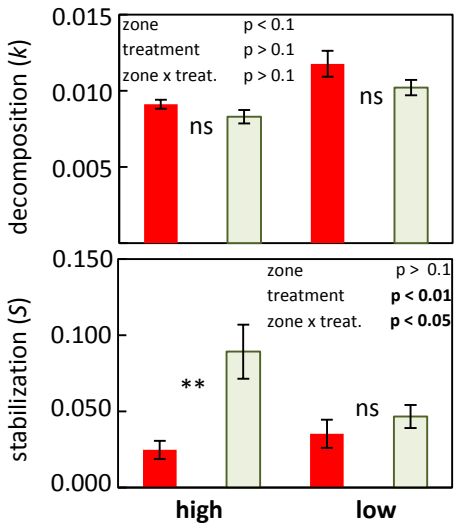

Figure 3 Effects of marsh elevation (zone) and nutrient fertilization on both decomposition rate (*k*) and stabilization (*S*) in long-term N fertilized (filled bars) and reference areas (open bars) in the high marsh (*Sparina patens* zone) and low marsh (*Spartina alterniflora* zone) of the TIDE project site at the Plum Island Sound Estuary, Massachusetts, US. Shown are means ± SE and results of two-way ANOVAs and pairwise comparisons (Tukey's HSD test)

Greater nutrient availability and less nutrient-limited microbial communities in more frequently flooded zones could have contributed to this effect (Deegan et al. 2012, Kirwan et al. 2013). Strong effects of both high quality marine-derived OM and nutrient amendments on microbial structure and activity have been reported (Deegan et al. 2012, Keuskamp et al. 2015, Mueller et al. 2017), suggesting that regular marine OM and inorganic nutrient inputs can positively affect OM break down. Indeed, we also observed a dramatic effect of nutrient amendment in the high marsh of the TIDE project site (Methods Table 1), decreasing *S* by 72%, while *S* in the low marsh likewise was low as in the fertilized high marsh and not further reduced by fertilization. Similar to the absent response of *k* to relative sea level, fertilization did not affect *k* (Figure 3).

Our study emphasizes that particularly OM stabilization rather than decay rate per se is strongly affected by temperature. Furthermore, we provide alarming evidence that accelerated SLR is unlikely to slow down decomposition rate and additionally may strongly decrease OM stabilization. Awareness about potential global-warming impacts on OM preservation and their resulting threat to future tidal wetland stability has been raised (Kirwan and Mudd 2012). In

comparison, concepts on the vulnerability of tidal wetlands to accelerated SLR mainly focus on plant-productivity responses and their biophysical feedbacks (Kirwan et al. 2016). However, potentially negative effects of accelerated SLR on OM preservation are overlooked probably because stimulation of decomposition processes through increasing flooding is counterintuitive (Mueller et al. 2016b). While this study addresses the influence of temperature and relative sea level on the initial transformation of biomass to SOM, it does not encompass their effects on the existing SOM pool. However, aspects of *S* and *k* are key components of many tidal wetland resiliency models (Schile et al. 2014, Swanson et al. 2014) that have highlighted the critical role of the organic contribution to marsh elevation gain. A reduction in the future organic contribution to accretion would reduce the accumulation rate and thus increase wetland vulnerability to accelerated SLR. The combined negative effects of temperature and relative sea level on OM stabilization therefore yield the potential to strongly reduce the fraction of net primary production and other OM sources that are transformed to stable SOM, thus reducing the C sequestration potential and ecosystem stability against accelerated SLR of tidal wetlands.

Methods

Study sites and experimental design

The worldwide study was conducted in 25 tidal wetlands during the 2015 growing season (Figure 1; Methods Table 1). Nine sites were situated along the European coasts of the North Sea, Mediterranean, and Baltic, ten sites were located along the East and West coasts of North America including the Gulf of St. Lawrence, Chesapeake Bay, and San Francisco Bay, and four mangrove sites were along the Caribbean coast of Central America in Belize and Panama. Additionally, one Chinese site (Yangtze Estuary) and one Argentinian site were included in our study. Fifteen of the sites are salt marshes, six are tidal freshwater and brackish sites, and four sites were mangroves. At 21 sites, we could compare high and low elevated zones, which were characterized by distinct plant species compositions or stature for mangroves. Decomposition and stabilization were measured in ten replicates per zone (see Methods Table 1 for deviations from this). Spacing between replicates within a zone or treatment was ≥2 m. However, as sites differed considerably in their areal extent, the distribution and thus spacing between points had to be adjusted by the responsible researcher (Methods Table 1) to be representative for the given system. Mean temperature data for the period of deployment was obtained from the online service of *Accuweather* (accuweather.com; accessed 12/25/2016) for towns within a distance of 15 km to the site for most sites, but not further than 60 km for some remote sites. Information on vegetation composition (dominant species), soil type (mineral vs. organic), tidal amplitude, salinity (classes: fresh, brackish, or salt), and land use were obtained from the responsible researchers and/or previously published works conducted in the respective sites (Methods Table 1).

Decomposition and stabilization measurements

Decomposition (*k*) and stabilization (*S*) were assessed following the *Tea Bag Index* protocol (Keuskamp et al. 2013). Briefly, two nylon tea bags (200 µm mesh size) containing either green tea (EAN: 8 722700 055525; Lipton, Unilever + PepsiCo, UK) or rooibos (8 722700 188438, Lipton, Unilever + PepsiCo, UK) were deployed as pairs in 8 cm soil depth separated by approx. 5 cm. The initial weight of the contents was determined by subtracting the mean weight of 10 empty bags (bag + string + label) from the weight of the intact tea bag prior to deployment (content + bag + string + label). The tea bags were retrieved approx. 90 days after deployment. Upon retrieval, tea bags were opened, tea materials were carefully separated from clay particles and fine roots, dried for 48 h at 70°C, and weighed.

Methods Table 1 Overview of study sites

		Zonation		Salinity						
Region	Site name	high	low	fresh	brac.	salt	N	Ecosystem	Soil[c]	Contact[site ref.]
Europe										
Germany	Dieksanderkoog, Elbe Est.	x	x			x	20	marsh	mineral	Mueller[1]
	Sönke-Nissen-Koog	x	x			x	20	marsh	mineral	Mueller[1]
	Spiekeroog	x	x			x	20	marsh	mineral	Dinter[2]
Netherlands	Ameland	x	x			x	20	marsh	mineral	de Groot[3]
	Noord-Friesland Buitendijks	x	x			x	20	marsh	mineral	Esselink[4]
	Schiermonnikoog	x				x	b	marsh	mineral	Smit[5]
Italy	Venice Lagoon	x	x			x	20	marsh	mineral	D'Alpaos[6]
Spain	Ebro Delta	x	x	x	x	x	30	marsh	organic	Ibáñez[7]
Poland	Mechelińskie Łąki	x			x		b	marsh	organic	Lazarus
North America										
Canada, QC	Sacré-Coeur	x	x			x	20	marsh	mineral	Neumeier[8]
Maine, US	Long Marsh	x		x	x	x	15	marsh	organic	Johnson
Massachusetts, US	Laws Point, Plum Island Est.	x	x			x	20	marsh	organic	Mozdzer[9]
	TIDE project, Plum Island Est.	x	x			x	40[a]	marsh	organic	Mozdzer[10]
Maryland, US	Patuxent	x	x	x			20	marsh	organic	Baldwin[11]
	Rhode River	x	x		x		36	marsh	organic	Schile[12]
Virginia, US	Wachapreague	x	x			x	20	marsh	mineral	Schile[13]
California, US	Coon Island	x	x		x		20	marsh	mineral	Schile[13]
	Rush Ranch, Suisun City	x	x		x		20	marsh	mineral	Schile[13]
	China Camp, Novato	x				x	10	marsh	mineral	Schile[13]
Central America										
Belize	Twin Cays	x	x			x	20	mangrove	organic	Schile[14]
Panama	Isla Solarte, Bocas del Toro	x	x			x	20	mangrove	organic	Schile[15]
	Isla Cristóbal, Bocas del Toro	x	x			x	20	mangrove	organic	Schile[15]
	Isla Popa, Bocas del Toro	x	x			x	20	mangrove	organic	Schile[15]
South America										
Argentina	Mar Chiquita	x	x		x		b	marsh	mineral	Montemayor[16]
Asia										
China	Dongtan, Yangtze Est.	x	x			x	18	marsh	mineral	Wu[17]

(a) additional fertilization treatment was included, compare reference 10; (b) low retrieval rates of paired bags only allowed for calculation of site or zone averages (c) If unclear, soil type was judged as mineral at organic matter contents < 35% (Soil Survey Staff 2014); Site references: (1) Nolte et al. (2013), (2) Flemming and Davis (1994), (3) Dijkema et al. (2010), (4) Bos et al. (2014), (5) Howison et al. (2015), (6) Roner et al. (2016), (7) Benito et al. (2014), (8) Neumeier and Cheng (2015), (9) Morris et al. (2013), (10) Deegan et al. (2012), (11) Neff et al. (2009), (12) Langley and Megonigal (2010), (13) Vasey et al. (2012), (14) Mckee et al. (2007), (15) Lovelock et al. (2005), (16) Fanjul et al. (2007), (17) Yang et al. (2017)

For calculations, the recommended hydrolysable fractions (H_g = 0.842 and H_r = 0.552 g g^{-1} for green and rooibos tea, respectively) were not used because a considerable number of observations (49.7% of >500 observations; independent of the conducting researcher) would have resulted in slightly negative values for *S*. *S* becomes negative when the mass loss from green tea is greater than the predicated maximum loss based on its hydrolysable fraction. As a consequence, we tested if the quality of the tea material used in this study differs from the results reported in Keuskamp et al. (2013) and calculations were conducted using the hydrolysable fractions presented in Methods Table 2.

In accordance with Keuskamp et al. (2013), extractions followed Ryan et al. (1990). However, instead of using the *forest products protocol*, we conducted the alternative *forage fiber protocol* for the determination of the hydrolysable fraction because both protocols include a final extraction step with 72% sulfuric acid (H_2SO_4) before sample ignition (Ryan et al. 1990). This also allowed to test if different extraction protocols can lead to differences in the estimated hydrolysable fraction as already suggested by Ryan et al. (1990). Briefly, 1 g of dried tea material (70°C for 24 h) was boiled in cetyltrimethyl ammonium bromide (CTAB) solution (1 g CTAB in 100 ml 0.5 M H_2SO_4) for 1 h (Ryan et al. 1990, Brinkmann et al. 2002). The extract was filtered through a 16-40-µm sinter-filter crucible (Duran, Wertheim, Germany) using a water-jet vacuum pump and washed with 150 ml of hot water, followed by addition of acetone until no further decoloration occurred (Brinkmann et al. 2002). The remaining material was left in the sinter, dried for 12 h at 70°C, cooled in a desiccator, and weighed. 20 mL of 72% H_2SO_4 was added to the sinter and filtered off after an incubation of 3 h, followed by washing with hot water to remove remaining acid. The sinter was dried at 70°C for 12 h, cooled in a desiccator, and weighed to determine the non-hydrolysable fraction. Finally, the sinter containing the remaining sample was ignited at 450°C for 3 h in order to determine the ash content of the material.

Methods Table 2 Hydrolysable and mineral fractions of green tea (n = 5 batches) and rooibos tea (n = 3 batches) and C and N contents (n = 2 batches). Samples of each batch were analyzed as duplicates

	Green Tea		Rooibos Tea	
	mean	SD	mean	SD
H [g g^{-1}]	0.933	0.01	0.676	0.04
Total C [%]	47.9	2.8	50.1	0.7
Total N [%]	3.9	0.2	1.1	0.1
Mineral fraction [%]	<0.5		<0.1	

In addition to the determination of the hydrolysable fraction, we measured total C and N contents of the tea material on an elemental analyzer (HEKAtech, Wegberg, Germany). We did this in order to assess if possible deviations from the hydrolysable fraction reported in Keuskamp et al. (2013) are due to actual differences in quality or caused by the extraction method. While total C and N contents of the tea material are in well agreement, the hydrolysable fraction of both green and rooibos tea was higher than reported in Keuskamp et al. (2013) (Methods Table 2). We therefore conclude that deviations from the hydrolysable fraction as reported previously are due to the less conservative extraction assessment in the present study and not due to actual changes in the quality of the material.

Data Analyses

Analyses were conducted using mean values of each *site by elevation zone by salinity class* combination. Relationships between single parameters and litter decomposition are often not linear. Instead, critical thresholds seem to exist at which a certain predictor (e.g. mean annual temperature) becomes influential (Prescott 2010). In the present study, we therefore used classification and regression tree analysis (CRTA) to identify important predictors for *k* and *S*. CRTA is a non-parametric procedure for the step-wise splitting of the data set with any number of continuous or categorical predictor variables (Breiman et al. 1984, Rothwell et al. 2008), and it has been recommended to identify thresholds and to handle large-scale decomposition data sets (Rothwell et al. 2008, Prescott 2010). We conducted CRTA separately for *k* and *S* using temperature, salinity class, tidal amplitude, ecosystem type, soil type, and relative elevation as predictor variables. V-fold cross validation was set at 5 (as commonly conducted, compare Rothwell et al. (2008)), and the minimum number for observations per child node was set at n = 4, corresponding to at least two sites or 8% of the total data set (N = 49).

Spearman rank correlations were used to test for correlations between the variables salinity class (salt/brackish/fresh), temperature, latitude, tidal amplitude, *k*, and *S* (Table 1). Mann-Whitney U tests were conducted to test for differences in *k* and *S* between marshes and mangroves and between mineral and organic soil types.

We additionally tested for linear effects of temperature on *k* and *S* using simple linear regression analyses. Two-tailed paired t-tests were used to test for effects of relative elevation on *k* and *S* across sites. Subsequent one-tailed paired t-tests were conducted to test for the same effect within mineral and organic systems separately. We tested for effects of eutrophication on *k* and *S* in the data from the TIDE site (Massachusetts, US) using two-way ANOVA with

fertilization treatment and marsh zone as predictors. Finally, we compared the initial weights of green and rooibos tea contents across observers in order to test for potential effects on k and S caused by differences in the initial moisture content: Mean initial weight of green tea is not correlated with S ($p > 0.8$; $r^2 = 0.001$), and the mean initial weight of rooibos is not correlated with k ($p > 0.4$; $r^2 = 0.013$), indicating that moisture differences in the initial material did not affect our results. All analyses were conducted using STATISTICA 10 (StatSoft Inc., Tulsa, OK, USA).

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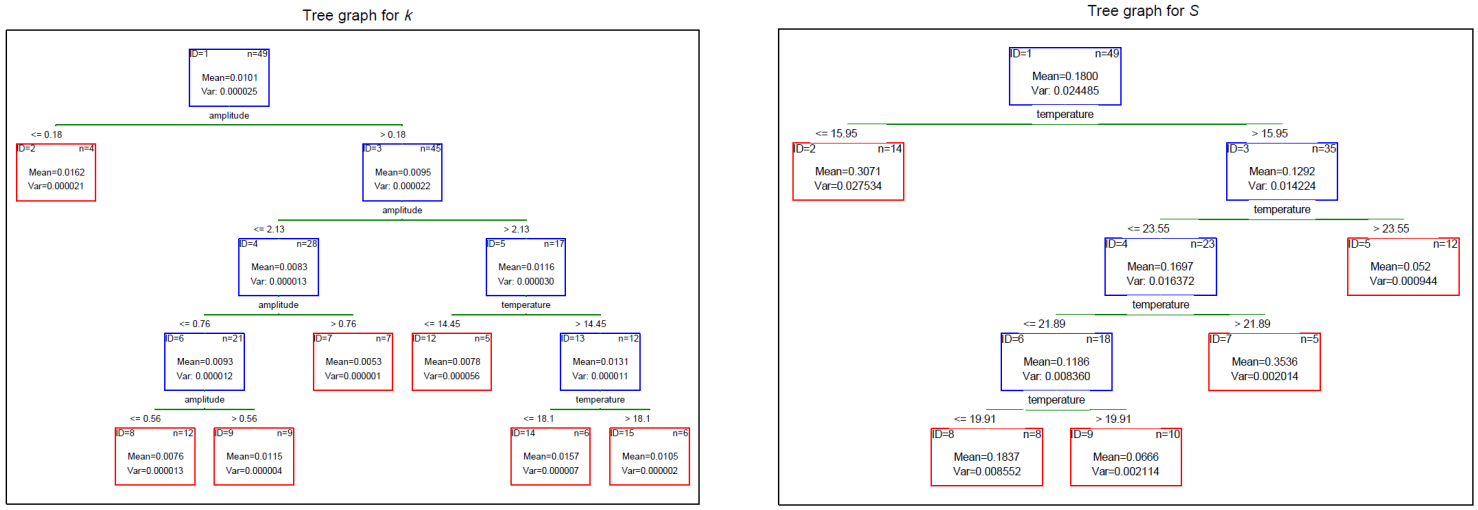

Figure S1 Classification and regression trees for decomposition constant (*k*) (left panel) and stabilization factor (*S*) (right panel). Step-wise splitting of the data set was conducted based on the predictor variables temperature, tidal amplitude, salinity class, soil type, ecosystem type, and elevation zone. Minimum size of child nodes was set at 4 corresponding to at least two sites

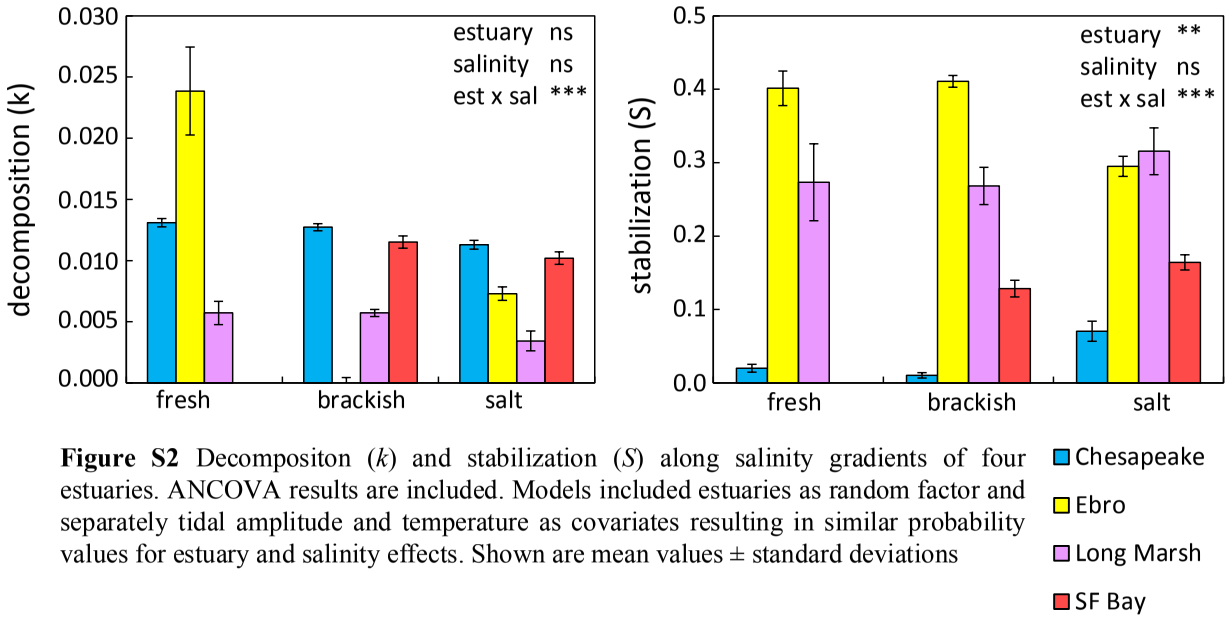

Figure S2 Decompositon (*k*) and stabilization (*S*) along salinity gradients of four estuaries. ANCOVA results are included. Models included estuaries as random factor and separately tidal amplitude and temperature as covariates resulting in similar probability values for estuary and salinity effects. Shown are mean values ± standard deviations

7 Synthesis

In the first part of this synthesis, I will discuss the findings of the previous five chapters in the common context of global change effects on C turnover in tidal wetlands. In the second part, I will present two concepts building on my findings: Concept I uses the findings of chapters 2 and 3 on plant-mediated effects on C-turnover processes to hypothesize unrecognized mechanisms of plant invasion and their feedbacks on tidal wetland C turnover. Concept II revisits the surprising finding of chapter 6 that organic matter stabilization is reduced in low elevated zones of tidal wetlands. It puts this finding into context with the results of chapter 5 and additional new data suggesting substrate control of microbial structure and function. I will close this synthesis by lining out future research perspectives.

Global change is a multifaceted process that is expected to affect C cycling in tidal wetlands through increasing temperatures and atmospheric CO_2 concentrations, nutrient pollution, land use changes, encroachment of invasive species, and accelerated relative sea level rise (RSLR) with potentially strong feedbacks on ecosystem stability (Kirwan and Megonigal 2013). In the previous chapters, I addressed the effects of invasive plant species on CH_4 emissions (Chapter 2), effects of RSLR and warming on decomposition processes (Chapters 3 and 6), and land use effects on C turnover and sequestration (Chapters 4 and 5).

In accordance with the central hypothesis of my thesis, which states that plant-trait responses to global change are the main drivers of change in C turnover and GHG fluxes from tidal wetland soils, I provide evidence for strong, if not overriding, effects of morphological and physiological plant traits on GHG emissions and decomposition (Chapters 2 and 3). Additionally, I propose a novel indirect mechanism by which plants can influence the organic matter composition and thus the microbial community of the top soil via changes in vegetation structure (Chapter 5).

Effects of invasive species on GHG emissions

There is a growing body of research focusing on the impact of invasive plant species on tidal wetland carbon sequestration and GHG emissions, particularly with respect to changes in the balance of sequestration and methane (CH_4) emissions (Valéry et al. 2004, Emery and Fulweiler 2014, Yuan et al. 2015; also compare Concept I).

The so far most comprehensive research on the impact of an invasive wetland species on GHG emissions comes from the invasion of non-native *Spartina alterniflora* in East Chinese coastal wetlands. Here, *in situ* static chamber quantification of CH_4 emissions (Tong et al. 2012, Yuan et al. 2015) and mesocosm experiments (Cheng et al. 2007, Zhang et al. 2010) suggest *S. alterniflora* invasion increases CH_4 emissions in comparison to the respective native community (dominated by *Phragmites australis*, *Suaeda salsa*, and *Scirpus mariqueter*).

Also *Phragmites* invasion in North America has been associated with increased CH_4 emissions from tidal wetlands (Martin and Moseman-Valtierra 2015). However, often patterns in plant invasion co-vary with other environmental gradients, making the attribution to ecosystem effects to invasion difficult in observational studies. Chapter 2 presents remarkable differences in the impact of *Phragmites* invasion into native shortgrass communities on CH_4 emissions in two neighboring marsh sites and in a complementary mesocosm experiment. Although *Phragmites* tended to increase CH_4 emissions in all assessments, the data suggest that the relative impact of *Phragmites* on CH_4 emissions is only profound in soils with innately high rates of CH_4 production. This effect is likely attributable to *Phragmites*' capacity to develop a deep root system in anoxic soils and the effective plant-supported ventilation of soil CH_4 to the atmosphere (Brix et al. 1992, Olsson et al. 2015). In contrast to the findings of previous studies that have focused on invader-ecosystem effects on GHG emissions, I show that such invader-ecosystem effects are site-specific. Furthermore, seasonality and changes in light availability can cause contrasting responses of CH_4 emissions from different vegetation types. Generalizations with respect to invader-ecosystem processes should therefore be interpreted with caution.

Sea level and warming effects on C turnover

Relative sea level rise (RSLR) is expected to alter the accumulation and cycling of organic matter in tidal wetlands with important consequences for tidal wetland stability and ecosystem services such as C sequestration (Kirwan and Megonigal 2013). Feedbacks between sea level and marsh surface elevation alter the hydrological and biogeochemical conditions that control

C inputs via plant production (Morris et al. 2002, Kirwan and Guntenspergen 2012) and C outputs via decomposition (Kirwan et al. 2013), the two major factors controlling the rate of C sequestration. Plant-production responses to RSLR are well understood and represented in century-scale forecast models of soil surface elevation change. In comparison, we understand far less about the response of decomposition to RSLR.

In this thesis, I address the effect of RSLR on decomposition processes in two studies (Chapters 3 and 6), assessing the response of different organic matter pools. Chapter 3 is focused on the decomposition of soil organic matter (SOM), the pool that decays most slowly and contributes the most to surface elevation change (Rybzcyk and Callaway 2009). Chapter 6 is concerned with early stages of litter decomposition and stabilization, thereby focusing on the processes that precede and determine the formation of SOM, respectively. Both studies have in common that relative elevation or sea level is used as a proxy for flooding frequency and duration, the two factors that will increase with RSLR.

A wide held assumption in wetland ecosystem ecology is that flooding, water-logging, and consequently anaerobia leads to slow decomposition (Davidson and Janssens 2006). However, neither of my studies suggests a significant reduction of decomposition with increasing rates of flooding depth and duration. Specifically, chapter 3 shows that SOM decomposition is completely insensitive to changes in flooding frequency and duration if plant-mediated effects are excluded. In the presence of plants, SOM decomposition was not reduced by increasing flooding, but followed the plant-biomass response to flooding. That is, SOM decomposition was strongly correlated with aboveground biomass, which in turn was regulated by flooding frequency and duration. This resulted in lowest SOM decomposition in the least frequently flooded elevation and similar rates of decomposition in elevations experiencing intermediate and high rates of flooding. Chapter 6 supports this finding by showing no difference in the decomposition of standardized litter between high and low elevated zones across 25 marsh and mangrove sites worldwide. More importantly, organic matter stabilization, the process describing the biogeochemical transformation of decomposable organic matter into stable compounds that leads to the formation of SOM (Prescott 2010), is in fact strongly reduced in low vs. high elevated zones.

Besides addressing sea level effects, chapter 6 also provides important insights into warming effects on decomposition processes in tidal wetlands. Although measurements were conducted over a large temperature gradient ($\Delta T > 15°C$), I show that effects on decomposition rate per se are minor and may be masked by other factors such as rhizosphere priming effects

(Chapter 5). In contrast, organic matter stabilization is strongly influenced by temperature and decreases by ~5% per 1°C temperature increase.

Taken together, the data of chapter 3 and 6 suggest that a RSLR-induced reduction in decomposition with positive feedback on tidal wetland stability is an unlikely scenario. Instead, the combined negative effects of temperature and relative sea level on organic matter stabilization yield the potential to strongly reduce the fraction of net primary production and other organic matter sources that are transformed to stable SOM, thus reducing the C sequestration potential and ecosystem stability of tidal wetlands against accelerated rates of RSLR.

Land use effects on C turnover and sequestration

Effects of land use change are central in the study of global change and of major importance for policy agendas on GHG-emission mitigation and C-crediting programs (Guo and Gifford 2002, IPCC 2013). The salt marshes of the European Wadden Sea region are a particularly interesting system to study land use effects for at least three reasons:

- Large parts of the extensive natural marsh area have been converted to agricultural land during the last centuries (Esselink et al. 2009)
- In turn, the majority of the existing mainland salt marshes are artificial systems that resulted from the conversion of tidal flat ecosystems through accretion enhancement techniques (Hofstede 2003)
- There is ongoing debate on the management of the existing salt-marsh area, particularly with respect to the benefits and risks of livestock grazing (Bakker et al. 2002, Esselink et al. 2009, Wanner et al. 2014, Klink et al. 2016)

In chapter 4, I provide first data on rates of C sequestration in the artificial salt marshes of the European Wadden Sea. Unlike the organogenic brackish marsh systems of the US Atlantic coast that were my study sites for chapters 2 and 3, the salt marshes of the Wadden Sea are minerogenic systems and thus may not primarily rely on slow C turnover and sequestration to build up vertically with rising sea level (Allen 2000). Indeed, I demonstrate that large amounts of organic C are lost down-core in these well-aerated marsh soils. Still, long-term (i.e. potentially over centuries) and mid-term (over decades) rates of C sequestration are 1.12 - 1.49 Mg C ha^{-2} yr^{-1}, respectively, and thus well in range with rates reported from other blue C systems. Importantly, my data further suggest a much greater capacity of the vegetated

ecosystem to sequester C than the tidal flat ecosystem it was converted from. The Wadden Sea salt marshes thereby provide a powerful man-made C sink with an estimated OC stock of 24.7 Mio. tons in the top 0.5 m of the foreland mainland marshes alone.

Livestock grazing is conducted in salt marshes worldwide (Yang et al. 2008, Gedan et al. 2009, Di Bella et al. 2014). Particularly in Europe, large parts of the salt-marsh area are used for livestock grazing. Here, the proportion of grazed vs. ungrazed area as well as the grazing intensity is regulated through habitat-management directives (e.g. Esselink et al. 2009). In chapter 5, I describe mechanisms and develop concepts by which large herbivores can affect organic matter decomposition and ultimately C sequestration in tidal wetlands through both direct animal-microbe and indirect animal-plant-microbe interactions. I propose a novel and indirect form of animal-plant-microbe interaction: grazing affects aboveground vegetation structure, which in turn determines marine organic matter trapping and thus the microbial structure. Livestock grazing thereby increases relative fungal abundance and therefore potentially exerts important control over C turnover and sequestration rates. Furthermore, I provide first evidence that grazing affects functioning of the microbial community by slowing down exo-enzyme activity and thus decomposition through changes in soil redox chemistry. It remains unclear if this is a direct grazing effect, and indeed trampling-driven as hypothesized in earlier studies, or if the grazing effect on redox chemistry is plant mediated and thus also indirect. Linking to chapter 3, which illustrated the overriding role of plant effects on C turnover in tidal wetland soils, chapter 5 is the first study to show that top-down or consumer control of plant community composition and biomass might exert indirect, but important control over both microbial structure and function. Taken together, my findings on changes in microbial structure and function suggest a decelerated C turnover under grazing. In the context of blue C, livestock grazing therefore yields the potential to significantly increase the C value of salt-marsh area.

Concept I: Linking nutrient availability, priming, and plant invasion

In chapters 2 and 3, I demonstrate strong plant-mediated effects on the GHG balance and C turnover of a brackish marsh. Probably, the reported effects are simply the result of wetland plant adaptations to cope with anoxic soil conditions.

The key adaptation of vascular wetland plants to cope with soil anoxia is the formation of long-distance apoplastic gas-transport pathways – called aerenchyma – that enable O_2 supply

to belowground tissues (Ashford and Allaway 1995, Jackson and Armstrong 1999). Aerenchyma create a bridge between anoxic and oxic environments that supports aerobic metabolism of plant tissues and microbes in an otherwise anoxic environment and enables the detoxification and removal of reduced phytotoxins – predominantly sulfides – through internal and external oxidation and ventilation of gaseous H_2S to the atmosphere (Drew 1997, Lee et al. 1999, Lamers et al. 2013).

Another strong stressor on wetland plant growth is nutrient deficiency caused by the impairment of nutrient uptake in anoxic soils (Mendelssohn et al. 1981). This effect is often explained by energetic constraints of the root cells caused by low O_2 availability or the phytotoxic impact of reduced species of sulfur, iron, and manganese (Lee et al. 1999, Steffens et al. 2005, Lamers et al. 2013). The impairment of N uptake in anoxic soils is also thought to be the responsible mechanism for the paradoxical phenomenon of strong N deficiency of salt marsh plants rooting in soil environments with high porewater concentrations of dissolved inorganic N (Mendelssohn 1979, Howes et al. 1981). However, plants with the ability to modify their soil environment by rhizosphere oxygenation are thought to alleviate the stress of impaired nutrient uptake (Bradley and Morris 1990, Bertness 1991) and may even induce facilitative effects on more flooding-sensitive species (Schat 1984).

My key finding of chapter 3, that rhizosphere priming effects are the primary control of SOM decomposition in tidal wetlands, may add another important point to this concept of plant nutrient acquisition. Specifically, I show that the presence of roots can greatly accelerate decomposition of SOM by priming the microbial community with energy-rich C sources or by introducing oxygen into anoxic soil layers. Because N, P, and other nutrients are also released in the mineralization process (Blagodatskaya and Kuzyakov 2008, Phillips et al. 2011), I hypothesize that priming can increase the nutrient supply through enhanced mineralization of SOM (Mozdzer et al. 2016). This concept also implies that deep-rooting plants could access a larger nutrient pool than shallow rooting plants by mobilizing nutrients in deep SOM pools that would remain highly inert otherwise. This conclusion may be particularly relevant in the context of global-change induced shifts in plant species composition, such as plant invasions (Chapter 2).

Indeed, in Mozdzer et al. (2016; not part of this thesis) I illustrate the large SOM-priming potential of the deep-rooting invasive grass *Phragmites australis*, and we further include this finding into a conceptual framework to explain the invasion success of *Phragmites* and other deep-rooting invaders (e.g. *Spartina alterniflora; Phalaris arundinacea; Arundo*

donax) in wetland ecosystems (Figure 1). It needs to be mentioned here, that I demonstrated the priming effect in an artificial system with old SOM moved up to a depth where it could also be reached by shallow roots of *Phragmites*.

However, a follow-up investigating by Bernal et al. (2017) could clearly demonstrate that *Phragmites* induces deep-soil priming of SOM below the root zone of the native vegetation. Thus, deep-rooting does not only increase the nutrient availability for the invasive species, it also mobilizes the C pool that was previously sequestered deep under the native vegetation, thereby threatening the ecosystem's function as a long-term C sink.

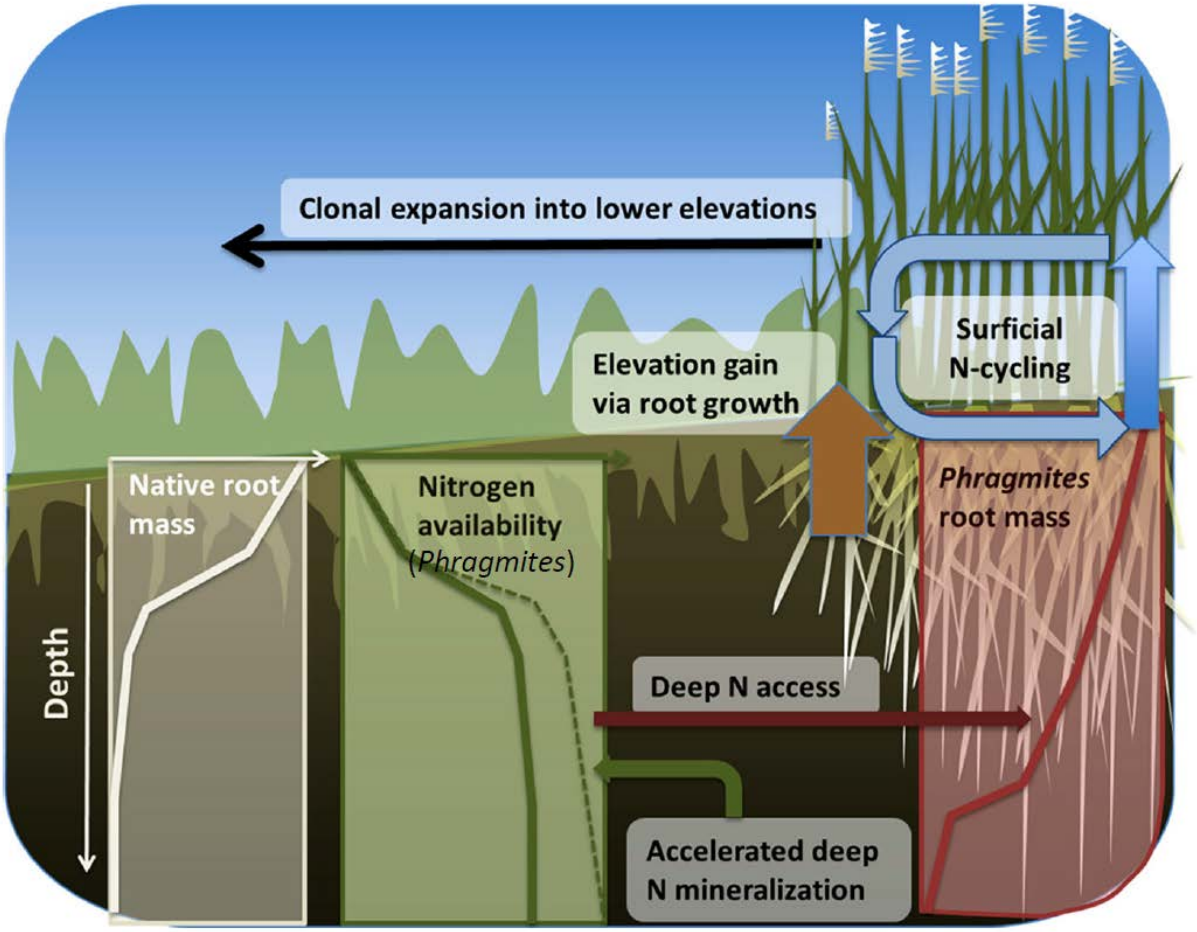

Figure 1 Conceptual diagram illustrating the interpretation on how deep-rooting invasive plants gain access to nutrients below the rooting depth of native plants. Priming of the microbial community deep within the soil profile further increases nutrient availability, thereby increasing plant growth and facilitating invasion into the ecosystem via competitive exclusion. Belowground growth builds soils, engineering the ecosystem to be drier and more suitable for *Phragmites* than the native plant community as *Phragmites* invades into lower elevations. Once deep nutrients are brought to the surface, *Phragmites* self-fertilizes the ecosystem resulting in a positive feedback loop of high productivity stimulating further invasion. Source: modified after Mozdzer et al. (2016)

Concept II: Redox vs. organic matter quality effects on C turnover in tidal wetlands

Figure 2 Conceptual diagram of opposing gradients in organic matter quality and oxygen availability along an intertidal flat to salt-marsh ecotone stretching over an elevational gradient from intertidal flat to high marsh. Photos show sampling positions and typical plant communities along the zonation of a naturally developed salt marsh at Dieksanderkoog, German Wadden Sea (Photos: P. Mueller)

In chapter 6, I found that the stabilization of organic matter is significantly reduced in low elevated and thus more frequently flooded zones compared to high elevated zones of tidal wetlands worldwide. The dominant paradigm in wetland ecosystem ecology that flooding and thus lower oxygen availability slows down organic matter turnover is obviously not supported by this finding. However, the mechanisms explaining this unexpected response of organic matter stabilization to relative sea level are not understood, either. I argued that differences in the microbial community, nutrient availability, and quality of the organic material between high and low elevated zones could be relevant factors explaining the result.

Indeed, in chapter 5 I found that the source of the organic matter has a strong effect on the microbial structure. Specifically, the data suggest that higher contributions of marine-derived allochthonous organic matter favor relative bacterial over fungal abundance. In a way, this is not a particularly surprising result because marine-derived organic matter is known to be of higher quality due to its lower C/N ratio (and minimal contents of structural components such as lignocelluloses) compared to terrigenous organic matter, and bacteria are known to have a higher N demand than fungi (Strickland and Rousk 2010). What is surprising, however, is the fact that different contributions of marine-derived organic matter and changes in the microbial structure hardly translated into the activity of microbial exo-enzymes (EEA). Instead, soil redox was found to be the dominant driver of EEA in chapter 5, even though the microbial structure was unaffected by redox.

In order to reveal further insight into the relative importance of redox vs. quality effects on EEA, the microbial structure, and consequently decomposition and C turnover in tidal wetlands, I conducted a follow-up investigation along an entire salt-marsh zonation stretching from intertidal flat to high marsh, assuming that redox would consistently increase and organic matter quality consistently decrease over this zonational gradient (Figure 2). I studied ß-glucosidase activity (GLU), a widely used indicator for the microbial C-acquisition activity, as it correlates with the activity of enzymes that hydrolyze related groups of compounds like cellulose and hemicellulose (Moorhead et al. 2013). The activity of the indicator enzymes leucine-aminopeptidase (LAP) and chitinase (CHI) was studied to assess microbial N-acquisition activity (Sinsabaugh et al. 2008, Moorhead et al. 2013). The overall methodology followed chapter 5 with slight modifications.

I found that redox is consistently lower in the intertidal flat than in the high marsh zone; however, redox does not consistently increase over the zonational gradient (Figure 3A). Depending on hydrology, redox was found to be lowest in either the intertidal flat or the *Spartina* and *Aster* dominated low marsh zones. Surprisingly, redox in the *Salicornia* dominated pioneer zone is consistently high and – depending on hydrology – higher than in the low marsh zones dominated by *Spartina*, *Aster*, *Puccinellia*, and *Atriplex* (Figure 3A, also compare Box A). My data suggest that redox of the top soil/sediment does not necessarily increase with relative elevation or sea level in tidal wetlands. Obviously, other factors such as drainage properties of the soil and organic matter content can also induce strong control on soil redox conditions.

In accordance with my initial assumption, top-soil/sediment organic matter quality – as based on soil C/N ratios – strictly decreased over the zonational gradient from intertidal flat to high marsh (Figure 3B). Furthermore, I demonstrate that this change in C/N is primarily driven by the source of the organic matter. That is, C/N is strongly and negatively correlated with organic matter $\delta^{13}C$ (Figure 3B), suggesting that decreasing contributions of marine-derived organic matter cause lower organic matter quality along the gradient from intertidal flat to high marsh.

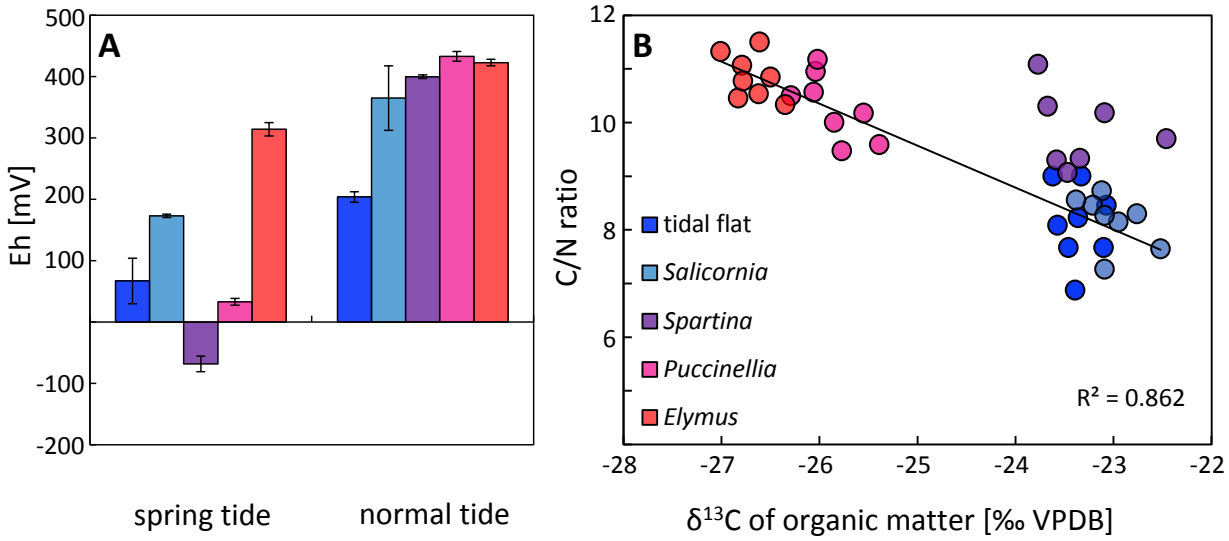

Figure 3 [A] Soil redox at 5 cm depth measured during low water on three sampling campaigns between Oct and Nov 2016 along an intertidal flat to salt-marsh ecotone at Dieksanderkoog (Figure 2). Two campaigns captured redox conditions after normal high water events and one campaign captured redox after spring tide. Redox after spring tide is shown on the left [spring tide] and the average redox of two campaigns after normal high water is shown on the right [normal tide]. Presented are mean values ± standard errors (n = 3). **[B]** Organic C to total nitrogen ratio and organic matter $\delta^{13}C$ of the bulk-soil samples in the top soil/sediment [5 cm] along the same intertidal flat to salt-marsh ecotone. Data points of the *Spartina*-dominated zone were excluded from regression analyses due to the inherent $\delta^{13}C$ divergence of C4 from C3 plant biomass. Presented are color coded single data points of each zone (n = 8)

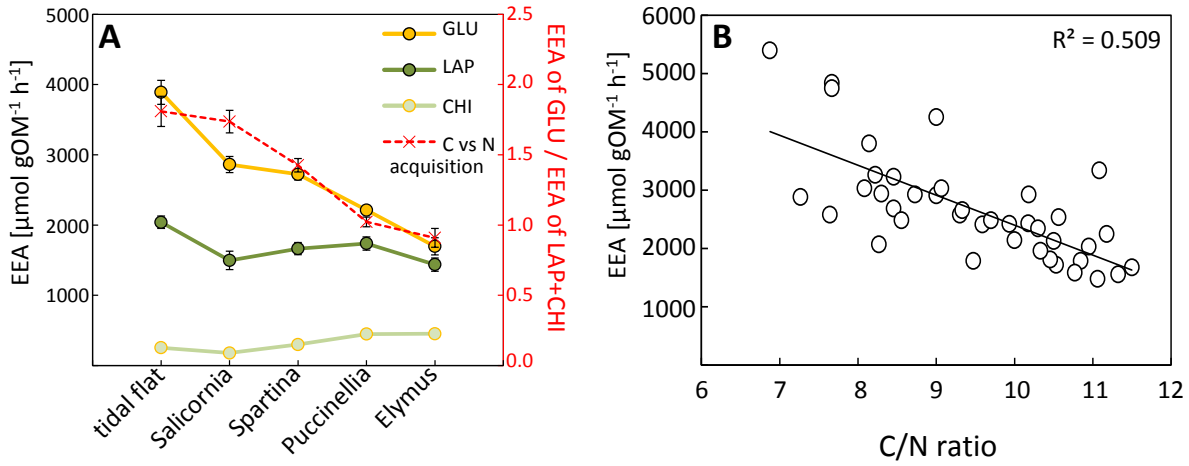

Figure 4 [A] Exo-enzyme activity (EEA) of ß-glucosidase (GLU), leucine-aminopeptidase (LAP), and chitinase (CHI), and ratio of C vs. N acquisition activities (Sinsabaugh et al. 2008) along an intertidal flat to salt-marsh ecotone (Figure 2). Presented are mean values ± standard errors (n = 8). **[B]** EEA of ß-glucosidase in relation to organic C to total N ratio of bulk-soil/sediment samples, (N = 40)

I found that GLU strongly decreases along the gradient from intertidal flat to high marsh, despite considerably higher soil redox in high vs. low marsh and intertidal flat (Figure 4A). In fact, the decrease in GLU over the zonational gradient is largely driven by substrate quality (C/N) and thus by decreasing relative contributions of marine-derived organic matter to the organic matter pool of the top soil (Figure 4B). LAP is highest in the intertidal flat and lower in pioneer zone, low marsh, and high marsh; however, it does not consistently decline along the zonational gradient as GLU does (Figure 4A). In contrast, CHI is generally low and increases slightly from intertidal flat to high marsh (Figure 4A). As a result, the microbial C vs. N acquisition activity declines consistently from intertidal flat to high marsh (Figure 4A). Taken together, these data suggest that EEA in the top soil – as the initial rate-limiting step of decomposition (Chapter 1, Figure 2) – can be strongly governed by organic matter quality and source, while soil redox conditions exert little leverage.

The findings on microbial abundance and structure are in well agreement with the results of chapter 5. That is, bacterial gene abundance is little affected by changes in redox conditions and organic matter quality. Here I demonstrate that, in fact, bacterial abundance per unit organic matter does not differ along the entire zonational gradient (one-way ANOVA: $p > 0.1$; Figure 5A).

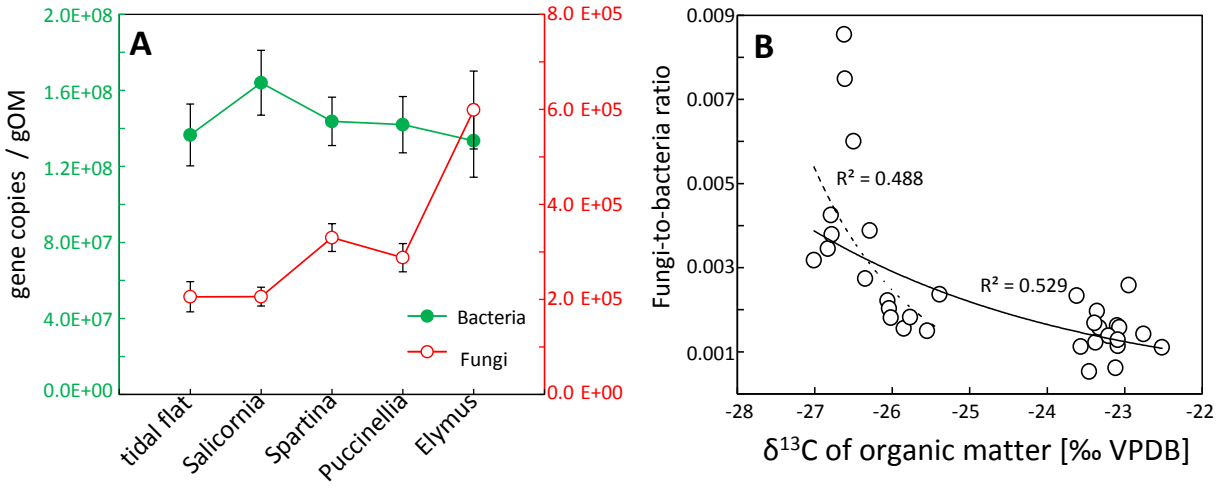

Figure 5 [A] Bacterial and fungal gene abundance along a zonational gradient from intertidal flat to high marsh (Figure 2). Presented are mean values ± standard errors, n = 8. **[B]** Fungi-to-bacteria gene copy ratio in relation to organic matter $\delta^{13}C$ of bulk-soil/sediment samples. Solid line shows overall fit of an exponential function (N = 32), dashed line shows exponential fit for low and high marsh data points only (N = 16). Data points of the *Spartina*-dominated zone were excluded from regression analyses due to the inherent $\delta^{13}C$ divergence of C4 from C3 plant biomass

In contrast, fungal abundance increased from intertidal flat and pioneer zone to *Spartina* and *Puccinellia* dominated low marsh and even stronger from low to high marsh (1-way ANOVA: $p < 0.001$; Figure 5A). Thus, also the fungi-to-bacteria ratio (F/B) increases significantly along the zonational gradient (1-way ANOVA: $p < 0.001$, data not shown); although it needs to be noted here that F/B is extremely low compared to the previous findings (Chapter 5). In accordance with the findings of chapter 5, organic matter $\delta^{13}C$ is a strong predictor for F/B suggesting that the relative contributions of marine- vs. terrestrial-derived organic matter may be an important factor shaping the microbial structure in salt marsh soils (Figure 5B). In contrast to chapter 5, however, the present assessment cannot clearly separate quality from redox effects on relative and absolute fungal abundance, as both decreasing quality and changes in redox over the zonational gradient could have favored fungal growth (see discussed in Chapter 5). Despite differences in redox conditions that should have been large enough to induce changes in microbial activity and decomposition rate (Yu and Patrick 2003, Yu et al. 2007), my present findings suggest that organic matter quality (as driven by its source) exerts the primary control over EEA in this intertidal flat to salt-marsh ecotone. Specifically, microbial communities in the lower and more frequently flooded zones seem to be less N limited than those of the high marsh, as evident by the strong decline in C- vs. N-acquisition activity over the zonational gradient (Figure 4A). This effect could also have contributed to the unexpected finding that the stabilization of organic material is generally lower in low elevated than in high elevated zones of tidal wetlands worldwide (Chapter 6). However, greater microbial nutrient availability and accelerated C turnover in more flooded environments does not necessarily depend on high quality organic matter inputs, as also inorganic nutrient supply can greatly increase decomposition and EEA in marsh and mangrove systems (Deegan et al. 2012, Keuskamp et al. 2015).

Future research perspectives

My work presented in chapter 3 is one of the first to invoke the importance of rhizosphere priming effects in non-terrestrial systems. I show that SOM decomposition and the plant-mediated increase in SOM decomposition (priming effect) scale positively with aboveground biomass. Both substrate-induced and O_2-driven priming can be expected to scale positively with aboveground biomass (Megonigal et al. 2004, Dijkstra et al. 2006, Jones et al. 2009, Lai et al. 2012). However, while plant-driven rhizosphere oxygenation as a control of decomposition

is a relatively well established concept in wetland biogeochemistry (Brix 1994, Wolf et al. 2007), little is known about substrate-induced priming in aquatic, marine, and tidal wetland systems (Guenet et al. 2010). Yet, some of the results in chapter 3 suggest that rhizosphere oxygenation through ROL is not the sole mechanism driving the observed increase in SOM decomposition because SOM-derived CO_2 flux and priming were not related to soil redox conditions. However, the mechanism by which these plant-mediated increases in SOM decomposition are induced still remains unclear. Future studies should therefore address this knowledge gap. This would include spatial and temporal assessments of O_2 in the rhizosphere (compare Chapter 1, Figure 4) in combination with C-isotope based C-flux partitioning. Furthermore, we need to understand the role of rhizodeposits and primarily root exudates in tidal-wetland biogeochemistry by quantifying exudation rates, identifying the exuded substances (Pan et al. 2011, Wu et al. 2011, Zhai et al. 2013), and linking this to changes in rhizosphere C and nutrient fluxes. Another promising technique to capture the interaction of ROL and root exudation with rhizosphere C and nutrient fluxes is the mapping of microbial exo-enzymes using "zymography" as demonstrated in terrestrial study systems (Spohn and Kuzyakov 2013, 2014, Sanaullah et al. 2016). This technique allows measuring the distribution and activity of exo-enzymes in rhizospheres using specific fluorogenically labeled substrate molecules (German et al. 2011, also compare Chapter 5 and Concept II) coated on nylon membranes that incubate on exposed rhizospheres. However, comparable mapping approaches of exo-enzyme distributions have not been conducted in a wetland setting or under waterlogged, anoxic soil conditions, presumably posing some additional methodological challenges to the approach.

Chapter 5 and the data presented in concept II illustrate the strong influence of allochthonous organic matter inputs on both microbial community structure and function. It is therefore reasonable to assume that high quality allochthonous organic matter is also capable to stimulate the decomposition of more recalcitrant autochthonous organic matter in tidal wetlands. In fact, recent studies demonstrated priming of terrestrial-derived organic matter by fresh marine or riverine organic matter in the aquatic system (Guenet et al. 2014, Bianchi et al. 2015, Ward et al. 2016). Even though the importance of allochthonous organic matter as microbial C source in tidal wetlands has long been demonstrated (Boschker et al. 1999), effects of allochthonous organic matter inputs on rhizosphere biogeochemical processes and the microbial ecology of tidal wetland systems remain poorly understood (Bouillon et al. 2004). Particularly with regard to global warming, SLR, changes in inundation frequency,

sediment load, and allochthonous and autochthonous primary production (Boyce et al. 2010,Kirwan and Megonigal 2013, Baldwin et al. 2014), future research should address potential impacts of changes in organic source on tidal-wetland biogeochemistry and the potential "allochthonous priming effect".

The majority of studies on C turnover in tidal wetlands, including most of which that has been presented in this thesis, is focused on processes occurring in the top soil or the rhizosphere. Considering the higher decomposability of the organic material and the numerous feedbacks between plants, soil fauna, and soil C cycling, the C pool of the rhizosphere is probably the most responsive to global change factors (Kirwan and Megonigal 2013, Wilson et al. 2016). However, the by far largest C pool of tidal wetlands is the one stored in the often several meters deep soils and sediments below the rhizosphere of the current vegetation (Mcleod et al. 2011). Little is known about the stability and the response of this deep C stock to global change (Bernal et al. 2016, 2017). An improved understanding of the response of this vast C stock to global-change factors, such as changes in the availability of terminal electron acceptors (i.e. through SLR induced salt water intrusion, or increasing nitrate concentrations through eutrophication) and global warming is therefore critical to predict the stability of tidal wetlands and their role in climate change mitigation.

References

Allen, J. R. L. 2000. Morphodynamics of Holocene salt marshes: A review sketch from the Atlantic and Southern North Sea coasts of Europe. Quaternary Science Reviews 19:1155–1231.

Alve, E., and J. W. Murray. 1999. Marginal marine environments of the Skagerrak and Kattegat: A baseline study of living (stained) benthic foraminiferal ecology. Palaeogeography, Palaeoclimatology, Palaeoecology 146:171–193.

Arkebauer, T. J., J. P. Chanton, S. B. Verma, and J. Kim. 2001. Field measurements of internal pressurization in Phragmites australis (Poaceae) and implications for regulation of methane emissions in a midlatitude prairie wetland. American Journal of Botany 88:653–658.

Armstrong, J., W. Armstrong, P. M. Beckett, J. E. Halder, S. Lythe, R. Holt, and A. Sinclair. 1996. Pathways of aeration and the mechanisms and beneficial effects of humidity- and venturi-induced convections in Phragmites australis (Cav.) Trin. ex Steud. Aquatic Botany 54:177–197.

Arrouays, D., J. Balesdent, A. Mariotti, and C. Girardin. 1995. Modelling organic carbon turnover in cleared temperate forest soils converted to maize cropping by using 13C natural abundance measurements. Plant and Soil 173:191–196.

Ashford, A. E., and W. G. Allaway. 1995. There is a continuum of gas space in young plants of Avicennia marina. Hydrobiologia 295:5–11.

Bachar, A., A. Al-Ashhab, M. I. M. Soares, M. Y. Sklarz, R. Angel, E. D. Ungar, and O. Gillor. 2010. Soil microbial abundance and diversity along a low precipitation gradient. Microbial Ecology 60:453–461.

Bakker, E. S., H. Olff, M. Boekhoff, J. M. Gleichman, and F. Berendse. 2004. Impact of herbivores on nitrogen cycling: Contrasting effects of small and large species. Oecologia 138:91–101.

Bakker, J. P., D. Bos, and Y. De Vries. 2002. To graze or not to graze, that's the question. Grassland Science in Europe 13:706–716.

Bakker, J. P., J. de Leeuw, K. S. Dijkema, P. C. Leendertse, H. H. T. Prins, and J. Rozema. 1993. Salt marshes along the coast of The Netherlands. Hydrobiologia 265:73–95.

Báldi, A. 1999. Microclimate and vegetation edge effects in a reedbed in Hungary. Biodiversity and Conservation 8:1697–1706.

Baldock, J. A., C. A. Masiello, Y. Gélinas, and J. I. Hedges. 2004. Cycling and composition of organic matter in terrestrial and marine ecosystems. Marine Chemistry 92:39–64.

Baldwin, A. H., K. Jensen, and M. Schönfeldt. 2014. Warming increases plant biomass and reduces diversity across continents, latitudes, and species migration scenarios in experimental wetland communities. Global Change Biology 20:835–850.

Ballantine, K., and R. Schneider. 2014. Fifty-five years of soil development in restored years of soil development freshwater wetlands depressional. Ecological Applications 19:1467–1480.

Barbier, E. B., S. D. Hacker, C. Kennedy, E. Koch, A. C. Stier, and B. R. Silliman. 2011. The value of estuarine and coastal ecosystem services. Ecological Monographs 81:169–193.

Bardgett, R. D., S. Keiller, R. Cook, and A. S. Gilburn. 1998a. Dynamic interactions between soil animals and microorganisms in upland grassland soils amended with sheep dung: A microcosm experiment. Soil Biology and Biochemistry 30:531–539.

Bardgett, R. D., D. a Wardle, and G. W. Yeates. 1998b. Linking above-gound and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil

Biology and Biochemistry 30:1867–1878.
Bart, D., D. Burdick, R. Chambers, and J. M. Hartman. 2006. Human facilitation of Phragmites australis invasions in tidal marshes: A review and synthesis. Wetlands Ecology and Management 14:53–65.
Di Bella, C. E., E. Jacobo, R. A. Golluscio, and A. M. Rodríguez. 2014. Effect of cattle grazing on soil salinity and vegetation composition along an elevation gradient in a temperate coastal salt marsh of Samborombón Bay (Argentina). Wetlands Ecology and Management 22:1–13.
Benito, X., R. Trobajo, and C. Ibáñez. 2014. Modelling habitat distribution of Mediterranean coastal wetlands: The Ebro delta as case study. Wetlands 34:775–785.
Benner, R., A. E. Maccubbin, and R. E. Hodson. 1984. Anaerobic biodegradation of the lignin and polysaccharide components of lignocellulose and synthetic lignin by sediment microflora. Appl. Envir. Microbiol. 47:998–1004.
Benner, R., M. A. Moran, and R. E. Hodson. 1985. Effects of pH and plant sourceon lignocellulose biodegradation rates in two wetland ecosystems, the Okefenokee Swamp and a Georgia salt marsh. Limnology and Oceanography 30:489–499.
Benner, R., M. A. Moran, and R. E. Hodson. 1986. Biogeochemical cycling of lignocellulosic carbon in marine and freshwater ecosystems: Relative contributions of prokaryotes and eukaryotes. Limnology and Oceanography 31:89-100
Bernal, B., D. C. McKinley, B. A. Hungate, P. M. White, T. J. Mozdzer, and J. P. Megonigal. 2016. Limits to soil carbon stability; Deep, ancient soil carbon decomposition stimulated by new labile organic inputs. Soil Biology and Biochemistry 98:85–94.
Bernal, B., J. P. Megonigal, and T. J. Mozdzer. 2017. An Invasive Wetland Grass Primes Deep Soil Carbon Pools. Global Change Biology:2104–2116.
Bertness, M. D. 1991. Zonation of Spartina patens and Spartina alterniflora in a New England salt marsh. Ecology 72:138–148.
Bhomia, R. K., R. A. MacKenzie, D. Murdiyarso, S. D. Sasmito, and J. Purbopuspito. 2016. Impacts of land use on Indian mangrove forest carbon stocks: Implications for conservation and management. Ecological Applications 26:1396–1408.
Bianchi, T. S., D. C. O. Thornton, S. A. Yvon-Lewis, G. M. King, T. I. Eglinton, M. R. Shields, N. D. Ward, and J. Curtis. 2015. Positive priming of terrestrially derived dissolved organic matter in a freshwater microcosm system. Geophysical Research Letters 42:5460–5467.
Blagodatskaya, E., and Y. Kuzyakov. 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils 45:115–131.
Blum, L. K. 1993. Spartina alterniflora root dynamics in a Virginia marsh. Marine Ecology-Progress Series:169–178.
Blum, L. K., and R. R. Christian. 2004. Belowground production and decomposition along a tidal gradient in a Virginia salt marsh. Pages 47–73 *in* S. Fagherazzi, M. Marani, and L. K. Blum, editors.The ecogeomorphology of tidal marshes. American Geophysical Union, Washington D.C., USA.
Bodelier, P. L. E., M. Stomp, L. Santamaria, M. Klaassen, and H. J. Laanbroek. 2006. Animal-plant-microbe interactions: Direct and indirect effects of swan foraging behaviour modulate methane cycling in temperate shallow wetlands. Oecologia 149:233–244.
Boelter, D. H. 1969. Physical properties of peats as related to degree of decomposition. Soil Science Society of America Journal 33:606.
Boesch, D. F., and R. E. Turner. 1984. Dependence of fishery species on salt marshes: the role of food and refuge. Estuaries 7:460–468.
Bos, D., S. Boersma, M. Engelmoer, R. M. Veeneklaas, J. P. Bakker, and P. Esselink. 2014.

Utilisation of a coastal grassland by geese after managed re-alignment. Journal of Coastal Conservation 18:471–479.
Boschker, H. T. S., J. F. C. de Brouwer, and T. E. Cappenberg. 1999. The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: Stable carbon isotope analysis of microbial biomarkers. Limnology and Oceanography 44:309–319.
Bossio, D. A., and K. M. Scow. 1998. Impacts of carbon and flooding on soil microbial communities: phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecology 35:265–278.
Boström, B., D. Comstedt, and A. Ekblad. 2007. Isotope fractionation and 13C enrichment in soil profiles during the decomposition of soil organic matter. Oecologia:89–98.
Bouillon, S., T. Moens, N. Koedam, F. Dahdouh-Guebas, W. Baeyens, and F. Dehairs. 2004. Variability in the origin of carbon substrates for bacterial communities in mangrove sediments. FEMS Microbiology Ecology 49:171–179.
Bouillon, S., A. V. V. S. Rao, N. Koedam, and F. Dehairs. 2003. Sources of organic carbon in mangrove sediments: variability and possible ecological implications. Hydrobiologia 495:33–39.
Boyce, D. G., M. R. Lewis, and B. Worm. 2010. Global phytoplankton decline over the past century. Nature 466:591–596.
Boyle, S. A., R. R. Yarwood, P. J. Bottomley, and D. D. Myrold. 2008. Bacterial and fungal contributions to soil nitrogen cycling under Douglas fir and red alder at two sites in Oregon. Soil Biology and Biochemistry 40:443–451.
Bradley, P. M., and J. T. Morris. 1990. Influence of oxygen and sulfide concentration on nitrogen uptake kinetics in Spartina alterniflora. Ecology 71:282–287.
Breiman, L., J. H. Friedman, R. A. Olshen, and C. J. Stone. 1984. Classification and Regression Trees. Wadsworth International Group, Blemont, CA, USA.
Bridgham, S. D., J. P. Megonigal, J. K. Keller, N. B. Bliss, and C. Trettin. 2006. The carbon balance of North American wetlands. Wetlands 26:889–916.
Brinkmann, K., L. Blaschke, and A. Polle. 2002. Comparison of different methods for lignin determination as a basis for calibration of near-infrared reflectance spectroscopy and implications of lignoproteins. Journal of chemical ecology 28:2483–501.
Brix, H. 1994. Functions of macrophytes in constructed wetlands. Water Science and Technology 29:71–78.
Brix, H., B. K. Sorrel, and P. T. Orr. 1992. Internal pressurization and convective gas flow in some freshwater macrophytes. Lim 37:1420–1433.
Brix, H., B. K. Sorrell, and H.-H. Schierup. 1996. Gas fluxes achieved by in situ convective flow in Phragmites australis. Aquatic Botany 54:151–163.
BSH. 2011. (Federal Maritime and Hydrographic Agency) Gezeitentafeln 2012. Europäische Gewässer. Bundesamt für Seeschifffahrt und Hydrographie.
Buckeridge, K. M., S. Banerjee, S. D. Siciliano, and P. Grogan. 2013. The seasonal pattern of soil microbial community structure in mesic low arctic tundra. Soil Biology and Biochemistry 65:338–347.
Butzeck, C., A. Eschenbach, A. Gröngröft, K. Hansen, S. Nolte, and K. Jensen. 2014. Sediment deposition and accretion rates in tidal marshes are highly variable along estuarine salinity and flooding gradients. Estuaries and Coasts:434–450.
Caffrey, J. M., and W. M. Kemp. 1991. Seasonal and spatial patterns of oxygen production, respiration and root-rhizome release in Potamogeton perfoliatus L. and Zostera marina L. Aquatic Botany 40:109–128.
Cahoon, D. R., J. C. Lynch, and A. N. Powell. 1996. Marsh vertical accretion in a Southern

California Estuary, U.S.A. Estuarine, Coastal and Shelf Science 43:19–32.
Callaway, J. C., J. A. Nyman, and R. D. DeLaune. 1996. Sediment accretion in coastal wetlands: A review and a simulation model of processes. Current Topics in Wetland Biogeochemistry 2:2–23.
Callaway, J., J. C. Callaway, E. L. Borgnis, R. E. Turner, and C. S. Milan. 2012. Carbon sequestration and sediment accretion in San Francisco Bay tidal wetlands 35:1163–1181.
Castenson, K. L., and M. C. Rabenhorst. 2006. Indicator of Reduction in Soil (IRIS). Soil Science Society of America Journal 70:1222–1226.
Cebrián, J., and C. M. Duarte. 1995. Plant growth-rate dependence of detrital carbon storage in ecosystems. Science 268:1606–8.
Chambers, L. G., R. Guevara, J. N. Boyer, T. G. Troxler, and S. E. Davis. 2016. Effects of salinity and inundation on microbial community structure and function in a mangrove peat soil. Wetlands 36:361–371.
Chambers, R. M., L. A. Meyerson, and K. Saltonstall. 1999. Expansion of Phragmites australis into tidal wetlands of North America. Aquatic Botany 64:261–273.
Chambers, R. M., T. J. Mozdzer, and J. C. Ambrose. 1998. Effects of salinity and sulfide on the distribution of Phragmites australis and Spartina alterniflora in a tidal saltmarsh. Aquatic Botany 62:161–169.
Charles, H., and J. S. Dukes. 2009. Effects of warming and altered precipitation on plant and nutrient dynamics of a New England salt marsh. Ecological Applications 19:1758–1773.
Chemidlin Prévost-Bouré, N., R. Christen, S. Dequiedt, C. Mougel, M. Lelièvre, C. Jolivet, H. R. Shahbazkia, L. Guillou, D. Arrouays, and L. Ranjard. 2011. Validation and application of a PCR primer set to quantify fungal communities in the Soil environment by Real-Time quantitative PCR. PLoS ONE 6:e24166.
Cheng, X., R. Peng, J. Chen, Y. Luo, Q. Zhang, S. An, J. Chen, and B. Li. 2007. CH4 and N2O emissions from Spartina alterniflora and Phragmites australis in experimental mesocosms. Chemosphere 68:420–427.
Chmura, G. L., S. C. Anisfeld, D. R. Cahoon, and J. C. Lynch. 2003. Global carbon sequestration in tidal, saline wetland soils. Global Biogeochemical Cycles 17:22-1-22–11.
Churchland, C., S. J. Grayston, and P. Bengtson. 2013. Spatial variability of soil fungal and bacterial abundance: Consequences for carbon turnover along a transition from a forested to clear-cut site. Soil Biology and Biochemistry 63:5–13.
Cifuentes, L. A., and G. G. Salata. 2001. Significance of carbon isotope discrimination between bulk carbon and extracted phospholipid fatty acids in selected terrestrial and marine environments. Organic Geochemistry 32:613–621.
Codron, D., M. Sponheimer, J. Codron, S. Hammer, A. Tschuor, U. Braun, S. M. Bernasconi, and M. Clauss. 2012. Tracking the fate of digesta 13C and 15N compositions along the ruminant gastrointestinal tract: Does digestion influence the relationship between diet and faeces? European Journal of Wildlife Research 58:303–313.
Colberg, P. J., and L. Y. Young. 1982. Biodegradation of lignin-derived molecules under anaerobic conditions. Canadian Journal of Microbiology 28:886–889.
Colmer, T. D. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant Cell and Environment 26:17–36.
Connell, E. L., T. D. Colmer, and D. I. Walker. 1999. Radial oxygen loss from intact roots of Halophila ovalis as a function of distance behind the root tip and shoot illumination. Aquatic Botany 63:219–228.
Connor, R. F., G. L. Chmura, and C. B. Beecher. 2001. Carbon accumulation in Bay of Fundy salt marshes: Implications for restoration of reclaimed marshes. Global Biogeochemical Cycles 15:943–954.

Cooper, A. 1982. The Effects of Salinity and on the Growth and Cation Uptake of Salt Marsh Plants. New Phytologist 90:263–275.

Cornwell, W. K., J. H. C. Cornelissen, K. Amatangelo, E. Dorrepaal, V. T. Eviner, O. Godoy, S. E. Hobbie, B. Hoorens, H. Kurokawa, N. Pérez-Harguindeguy, H. M. Quested, L. S. Santiago, D. A. Wardle, I. J. Wright, R. Aerts, S. D. Allison, P. Van Bodegom, V. Brovkin, A. Chatain, T. V. Callaghan, S. Díaz, E. Garnier, D. E. Gurvich, E. Kazakou, J. A. Klein, J. Read, P. B. Reich, N. A. Soudzilovskaia, M. V. Vaieretti, and M. Westoby. 2008. Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecology Letters 11:1065–1071.

Cotrufo, M. F., J. L. Soong, A. J. Horton, E. E. Campbell, M. L. Haddix, D. H. Wall, and W. J. Parton. 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience 8:776–779.

Cowie, G. L., and J. I. Hedges. 1993. A comparison of organic matter sources, diagenesis and preservation in oxic and anoxic coastal sites. Chemical Geology 107:447–451.

Craft, C. 2007. Freshwater input structures soil properties, vertical accretion, and nutrient accumulation of Georgia and U.S tidal marshes. Limnology and Oceanography 52:1220–1230.

Craft, C. B., E. D. Seneca, and S. W. Broome. 1991. Loss on ignition and kjeldahl digestion for estimating organic carbon and total nitrogen in estuarine marsh soils: Calibration with dry combustion. Estuaries 14:175–179.

Craft, C., J. Clough, J. Ehman, S. Jove, R. Park, S. Pennings, H. Guo, and M. Machmuller. 2009. Forecasting the effects of accelerated sea-level rise on tidal marsh ecosystem services. Frontiers in Ecology and the Environment 7:73–78.

Craft, C., J. P. Megonigal, S. Broome, J. Stevenson, J. Cornell, L. Zheng, J. Sacco, and R. Freese. 2003. The Pace of Ecosystem Development of Constructed Spartina alterniflora Marshes. Ecological Applications 13:1417–1432.

Craft, C., J. Reader, J. N. Sacco, S. W. Broome, S. E. Applications, and N. Nov. 1999. Twenty-five years of ecosystem development of constructed Spartina alterniflora (Loisel) marshes. Ecological Applications 9:1405–1419.

Craine, J. M., N. Fierer, and K. K. McLauchlan. 2010. Widespread coupling between the rate and temperature sensitivity of organic matter decay. Nature Geoscience 3:854–857.

Crooks, S., S. Emmet-Mattox, and J. Findsen. 2010. National Blue Ribbon Panel on the Development of a Greenhouse Gas Offset Protocol for Tidal Wetlands Restoration and Management: Action Plan to guide protocol development.

Crosby, S. C., D. F. Sax, M. E. Palmer, H. S. Booth, L. A. Deegan, M. D. Bertness, and H. M. Leslie. 2016. Salt marsh persistence is threatened by predicted sea-level rise. Estuarine, Coastal and Shelf Science 181:93–99.

Cussler, E. L. 1984. Diffusion: Mass transfer in fluid systems. Cambridge University Press, Cambridge, UK.

Davidson, E. A., and I. A. Janssens. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440:165–73.

Davy, A. J., M. J. H. Brown, H. L. Mossman, and A. Grant. 2011. Colonization of a newly developing salt marsh: Disentangling independent effects of elevation and redox potential on halophytes. Journal of Ecology 99:1350–1357.

Deegan, L. A., D. S. Johnson, R. S. Warren, B. J. Peterson, J. W. Fleeger, S. Fagherazzi, and W. M. Wollheim. 2012. Coastal eutrophication as a driver of salt marsh loss. Nature 490:338–392.

Dijkema, K. S., A. S. Kers, and W. E. Van Duin. 2010. Salt marshes: applied long-term monitoring salt marshes. Wadden Sea Ecosystem:35–40.

Dijkstra, F. A., W. Cheng, and D. W. Johnson. 2006. Plant biomass influences rhizosphere priming effects on soil organic matter decomposition in two differently managed soils. Soil Biology and Biochemistry 38:2519–2526.

Dingemans, B. J. J., E. S. Barker, and P. L. E. Bodelier. 2011. Aquatic herbivores facilitate the emission of methane from wetlands. Ecology 92:1166–1173.

Doody, J. P. 2008. Saltmarsh conservation, management and restoration. Saltmarsh Conservation, Management and Restoration. Springer International Publishing.

Drew, M. C. 1997. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. Annual Review of Plant Physiology and Plant Molecular Biology 48:223–250.

Duarte, C. M., J. Borum, F. T. Short, and D. I. Walker. 2008. Seagrass ecosystems: their global status and prospects. Pages 281–294*in* N. V. C. Polunin, editor.Aquatic ecosystems: trends and global prospects. Cambridge University Press, Cambridge, UK.

Duarte, C. M., and J. Cebrián. 1996. The fate of marine autotrophic production. Limnology and Oceanography 41:1758–1766.

Duarte, C. M., I. J. Losada, I. E. Hendriks, I. Mazarrasa, and N. Marba. 2013. The role of coastal plant communities for climate change mitigation and adaptation. Nature Clim. Change 3:961–968.

Duarte, C. M., J. J. Middelburg, and N. Caraco. 2005. Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences 2:1–8.

Edwards, R. J., A. J. Wright, and O. Van De Plassche. 2004. Surface distributions of salt-marsh foraminifera from Connecticut, USA: Modern analogues for high-resolution sea level studies. Marine Micropaleontology 51:1–21.

Elschot, K., J. P. Bakker, S. Temmerman, J. Van De Koppel, and T. J. Bouma. 2015. Ecosystem engineering by large grazers enhances carbon stocks in a tidal salt marsh. Marine Ecology Progress Series 537:9–21.

Emery, H. E., and R. W. Fulweiler. 2014. Spartina alterniflora and invasive Phragmites australis stands have similar greenhouse gas emissions in a New England marsh. Aquatic Botany 116:83–92.

Esselink, P., J. Petersen, S. Arens, B. J.P., J. Bunje, K. S. Dijkema, N. Hecker, U. Hellwig, A.-V. Jensen, A. S. Kers, P. Körber, E. J. Lammerts, M. Stock, R. M. Veeneklaas, M. Vreeken, and M. Wolters. 2009. Salt Marshes. Wadden Sea Ecosystem No. 25 Quality Status Report 2009 Thematic Report No. 8 46:27–30.

Esselink, P., W. Zijlstra, K. S. Dijkema, and R. Van Diggelen. 2000. The effects of decreased management on plant-species distribution patterns in a salt marsh nature reserve in the Wadden Sea. Biological Conservation 93:61–76.

Ewers Lewis, C. J., P. E. Carnell, J. Sanderman, J. A. Baldock, and P. I. Macreadie. 2017. Variability and vulnerability of coastal “blue carbon” stocks: A case study from Southeast Australia. Ecosystems: in press.

Fagervold, S. K., S. Bourgeois, A. M. Pruski, F. Charles, P. Kerherve, G. Vetion, and P. E. Galand. 2014. River organic matter shapes microbial communities in the sediment of the Rhone prodelta. Isme J 8:2327–2338.

Fanjul, E., M. A. Grela, and O. Iribarne. 2007. Effects of the dominant SW Atlantic intertidal burrowing crab Chasmagnathus granulatus on sediment chemistry and nutrient distribution. Marine Ecology Progress Series 341:177–190.

Fatela, F., and R. Taborda. 2002. Confidence limits of species proportions in microfossil assemblages. Marine Micropaleontology 45:169–174.

Feagin, R. A., S. M. Lozada-Bernard, T. M. Ravens, I. Moller, K. M. Yeager, and A. H. Baird. 2009. Does vegetation prevent wave erosion of salt marsh edges? Proceedings of the

National Academy of Sciences 106:10109–10113.
Feinstein, L. M., J. S. Woo, and C. B. Blackwood. 2009. Assessment of bias associated with incomplete extraction of microbial DNA from soil. Applied and Environmental Microbiology 75:5428–5433.
Fierer, N., J. A. Jackson, R. Vilgalys, and R. B. Jackson. 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71:4117–4120.
Flemming, B. W., and R. A. Davis. 1994. Holocene evolution, morphodynamics and sedimentology of the Spiekeroog barrier island system (southern North Sea). Senckenbergia maritima 24:117–155.
Ford, H., J. Rousk, A. Garbutt, L. Jones, and D. L. Jones. 2013. Grazing effects on microbial community composition, growth and nutrient cycling in salt marsh and sand dune grasslands. Biology and Fertility of Soils 49:89–98.
Frank, D. A., P. M. Groffman, R. D. Evans, and B. F. Tracy. 2000. Ungulate stimulation of nitrogen cycling and retention in Yellowstone Park grasslands. Oecologia 123:116–121.
Freeman, C., N. Ostle, and H. Kang. 2001. An enzymic “latch” on a global carbon store. Nature 409:149–150.
Frenzel, P., U. Bosse, and P. H. Janssen. 1999. Rice roots and methanogenesis in a paddy soil: Ferric iron as an alternative electron acceptor in the rooted soil. Soil Biology and Biochemistry 31:421–430.
Frenzel, P., F. Rothfuss, and R. Conrad. 1992. Oxygen profiles and methane turnover in a flooded rice microcosm. Biology and Fertility of Soils 14:84–89.
Fu, S., and W. Cheng. 2002. Rhizosphere priming effects on the decomposition of soil organic matter in C4 and C3 grassland soils. Plant and Soil 238:289–294.
Gedan, K. B., B. R. Silliman, and M. D. Bertness. 2009. Centuries of human-driven change in salt marsh ecosystems. Annual review of marine science 1:117–141.
Gehrels, R. W., and S. W. G. Newman. 2004. Salt-marsh foraminifera in Ho Bugt, western Denmark, and their use as sea-level indicators. Geografisk Tidsskrift Danish Journal of Geography 104:97–106.
Ghashghaie, J., and F. W. Badeck. 2014. Opposite carbon isotope discrimination during dark respiration in leaves versus roots - a review. The New phytologist 201:751–69.
Gomez-Casanovas, N., R. Matamala, D. R. Cook, and M. A. Gonzalez-Meler. 2012. Net ecosystem exchange modifies the relationship between the autotrophic and heterotrophic components of soil respiration with abiotic factors in prairie grasslands. Global Change Biology 18:2532–2545.
Green, E. P., and F. Short. 2003. World Atlas of Seagrasses. University of California Press, Berkeley and Los Angeles, CA, USA.
Guenet, B., M. Danger, L. Abbadie, and G. Lacroix. 2010. Priming effect: bridging the gap between terrestrial and aquatic ecology. Ecology 91:2850–2861.
Guenet, B., M. Danger, L. Harrault, B. Allard, M. Jauset-Alcala, G. Bardoux, D. Benest, L. Abbadie, and G. Lacroix. 2014. Fast mineralization of land-born C in inland waters: First experimental evidences of aquatic priming effect. Hydrobiologia 721:35–44.
Gunnarsson, U. 2005. Global patterns of *Sphagnum* productivity. Journal of Bryology 27:269–279.
Guo, L. B., and R. M. Gifford. 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8:345–360.
Hackney, C. T. 1987. Factors affecting accumulation or loss of macroorganic matter in salt marsh sediments. Ecology 68:1109–1113.
Haichar, F. E. Z., C. Santaella, T. Heulin, and W. Achouak. 2014. Root exudates mediated

interactions belowground. Soil Biology and Biochemistry 77:69–80.
Halupa, P. J., and B. L. Howes. 1995. Effects of tidally mediated litter moisture content on decomposition of Spartina alterniflora and S. patens. Marine Biology 123:379–391.
Hansson, G., and N. Molin. 1981. End product inhibition in methane fermentations: effects of carbon dioxide on methanogenic bacteria utilizing acetate. European journal of applied microbiology and biotechnology 13:236–241.
He, Q., and B. R. Silliman. 2016. Consumer control as a common driver of coastal vegetation worldwide. Ecological Monographs 86:278–294.
van der Heijden, M. G. A., R. D. Bardgett, and N. M. Van Straalen. 2008. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310.
Hemminga, M. A., and G. J. C. Buth. 1991. Decomposition in salt marsh ecosystems of the S. W. Netherlands: the effects of biotic and abiotic factors. Vegetatio 92:73–83.
Hemminga, M. A., C. J. Kok, and W. de Munck. 1988. Decomposition of Spartina anglica roots and rhizomes in a salt marsh of the Westerschelde Estuary. Marine Ecology Progress Series 48:175–184.
Hemminga, M. A., J. de Leeuw, W. de Munck, and B. P. Koutstaal. 1991. Decomposition in estuarine salt marshes: the effect of soil salinity and soil water content. Vegetatio:25–33.
Herlemann, D. P., M. Labrenz, K. Jürgens, S. Bertilsson, J. J. Waniek, and A. F. Andersson. 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. The ISME journal 5:1571–9.
Hofstede, J. L. A. 2003. Integrated management of artificially created salt marshes in the Wadden Sea of Schleswig-Holstein, Germany. Wetlands Ecology and Management 11:183–194.
Howes, B. L., R. W. Howarth, J. M. Teal, and I. Valiela. 1981. Oxidation-reduction potentials in a salt marsh: Spatial patterns and interactions with primary production. Limnology and Oceanography 26:350–360.
Howison, R. A., H. Olff, R. Steever, and C. Smit. 2015. Large herbivores change the direction of interactions within plant communities along a salt marsh stress gradient. Journal of Vegetation Science 26:1159–1170.
Hulthe, G., S. Hulth, and P. O. J. Hall. 1998. Effect of oxygen on degradation rate of refractory and labile organic matter in continental margin sediments - A comparative survey of certain organic and inorganic compounds in an oxic and anoxic Baltic basin. Geochimica et Cosmochimica Acta 62:1319–1328.
Huston, M. A., and S. Wolverton. 2009. The global distribution of net primary production: Resolving the paradox. Ecological Monographs 79:343–377.
IPCC. 2013. IPCC Fifth Assessment Report The Physical Science Basis (AR5). Page IPCC. Cambridge University Press, Cambridge, UK, and New York, USA.
Jackson, M. B., and W. Armstrong. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. Plant Biology 1:274–287.
Janousek, C. N., K. J. Buffington, G. R. Guntenspergen, K. M. Thorne, B. D. Dugger, and J. Y. Takekawa. 2017. Inundation, vegetation, and sediment effects on litter decomposition in Pacific Coast tidal marshes. Ecosystems:2014–2015.
Jenkinson, B. J. 2002. A visual method for the identification of hydric soils. PhD thesis, Purdue University.
Jones, D. L., A. Hodge, and Y. Kuzyakov. 2004. Plant and mycorrhizal regulation of rhizodeposition. New Phytologist 163:459–480.
Jones, D. L., C. Nguyen, and R. D. Finlay. 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant and Soil 321:5–33.

Jordan, T. E., D. L. Correll, and D. F. Whigham. 1983. Nutrient flux in the Rhode River - Tidal exchange of nutrients by brackish marshes. Estuarine, Coastal and Shelf Science 17:651–667.

Jowsey, P. C. 1966. An improved peat sampler. New Phytologist 65:245–248.

Kauffman, J. B., C. Heider, J. Norfolk, and F. Payton. 2014. Carbon stocks of intact mangroves and carbon emissions arising from their conversion in the Dominican Republic. Ecological Applications 24:518–527.

Kauffman, J. B., A. S. Thorpe, and E. N. J. Brookshire. 2004. Livestock exclusion and belowground ecosystem responses in riparian meadows of eastern Oregon. Ecological Applications 14:1671–1679.

Keller, J. K., A. E. Sutton-Grier, A. L. Bullock, and J. P. Megonigal. 2012. Anaerobic metabolism in tidal freshwater wetlands: I. Plant removal effects on iron reduction and methanogenesis. Estuaries and Coasts 36:457–470.

Keller, J. K., A. A. Wolf, P. B. Weisenhorn, B. G. Drake, and J. P. Megonigal. 2009. Elevated CO2 affects porewater chemistry in a brackish marsh. Biogeochemistry 96:101–117.

Kelleway, J. J., N. Saintilan, P. I. Macreadie, and P. J. Ralph. 2016. Sedimentary factors are key predictors of carbon storage in SE Australian saltmarshes. Ecosystems 19:865–880.

van Kessel, C., B. Boots, M. A. de Graaff, D. Harris, H. Blum, and J. Six. 2006. Total soil C and N sequestration in a grassland following 10 years of free air CO2 enrichment. Global Change Biology 12:2187–2199.

Keuskamp, J. A., B. J. J. Dingemans, T. Lehtinen, J. M. Sarneel, and M. M. Hefting. 2013. Tea Bag Index: a novel approach to collect uniform decomposition data across ecosystems. Methods in Ecology and Evolution 4:1070–1075.

Keuskamp, J. A., I. C. Feller, H. J. Laanbroek, J. T. A. Verhoeven, and M. M. Hefting. 2015. Short- and long-term effects of nutrient enrichment on microbial exoenzyme activity in mangrove peat. Soil Biology and Biochemistry 81:38–47.

Khan, N. S., C. H. Vane, and B. P. Horton. 2015. Stable carbon isotope and C/N geochemistry of coastal wetland sediments as a sea-level indicator. Handbook of Sea-Level Research:295–311.

Kirchner, G., and H. Ehlers. 1998. Seditnent Geochronology in Changing Coastal Environments: Potentials and Limitations of the 137CS and 210Pb Methods. Journal of coastal research 14:483–492.

Kirwan, M. L., and L. K. Blum. 2011. Enhanced decomposition offsets enhanced productivity and soil carbon accumulation in coastal wetlands responding to climate change. Biogeosciences 8:987–993.

Kirwan, M. L., and G. R. Guntenspergen. 2012. Feedbacks between inundation, root production, and shoot growth in a rapidly submerging brackish marsh. Journal of Ecology 100:764–770.

Kirwan, M. L., G. R. Guntenspergen, and J. A. Langley. 2014. Temperature sensitivity of organic-matter decay in tidal marshes. Biogeosciences 11:4801–4808.

Kirwan, M. L., J. A. Langley, G. R. Guntenspergen, and J. P. Megonigal. 2013. The impact of sea-level rise on organic matter decay rates in Chesapeake Bay brackish tidal marshes. Biogeosciences 10:1869–1876.

Kirwan, M. L., and J. P. Megonigal. 2013. Tidal wetland stability in the face of human impacts and sea-level rise. Nature 504:53–60.

Kirwan, M. L., and S. M. Mudd. 2012. Response of salt-marsh carbon accumulation to climate change. Nature 489:550–3.

Kirwan, M. L., S. Temmerman, E. E. Skeehan, G. R. Guntenspergen, and S. Faghe. 2016. Overestimation of marsh vulnerability to sea level rise. Nature Climate Change 6:253–

260.
Klindworth, A., E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn, and F. O. Glöckner. 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research 41:e1.
Klink, R. van, S. Nolte, F. S. Mandema, G. Lagendijk, M. F. WallisDeVries, J. P. Bakker, P. Esselink, and C. Smit. 2016. Effects of grazing management on biodiversity across trophic levels - The importance of livestock species and stocking density in salt marshes. Agriculture, Ecosystems and Environment 235:329–339.
Kohl, L., J. Laganière, K. a. Edwards, S. a. Billings, P. L. Morrill, G. Van Biesen, and S. E. Ziegler. 2015. Distinct fungal and bacterial δ13C signatures as potential drivers of increasing δ13C of soil organic matter with depth. Biogeochemistry:13–26.
Koop-Jakobsen, K., and F. Wenzhöfer. 2015. The dynamics of plant-mediated sediment oxygenation in Spartina anglica rhizospheres - A planar optode study. Estuaries and Coasts 38:951–963.
Kristensen, E., S. I. Ahmed, and A. H. Devol. 1995. Aerobic and anaerobic decomposition of organic matter in marine sediment: Which is fastest? Limnology and Oceanography 40:1430–1437.
Kuehn, K. A., D. Steiner, and M. O. Gessner. 2004. Diel mineralization patterns of standing-dead plant litter: implications for CO2 flux from wetlands. Ecology 85:2504–2518.
Kuehn, K. A., and K. Suberkropp. 1998. Decomposition of standing litter of the freshwater emergent macrophyte Juncus effusus. Freshwater Biology 40:717–727.
Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. Journal of Plant Nutrition and Soil Science 165:382.
Kuzyakov, Y., and W. Cheng. 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology and Biochemistry 33:1915–1925.
Kuzyakov, Y., and W. Cheng. 2004. Photosynthesis controls of CO2 efflux from maize rhizosphere. Plant and Soil:85–99.
Kuzyakov, Y., J. K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32:1485–1498.
Laanbroek, H. J. 2010. Methane emission from natural wetlands: interplay between emergent macrophytes and soil microbial processes. A mini-review. Annals of botany 105:141–153.
Lai, W. L., Y. Zhang, and Z. H. Chen. 2012. Radial oxygen loss, photosynthesis, and nutrient removal of 35 wetland plants. Ecological Engineering 39:24–30.
Lamers, L. P. M., L. L. Govers, I. C. J. M. Janssen, J. J. M. Geurts, M. E. W. Van der Welle, M. M. Van Katwijk, T. Van der Heide, J. G. M. Roelofs, and A. J. P. Smolders. 2013. Sulfide as a soil phytotoxin—a review. Frontiers in Plant Science 4:1–14.
Langley, J. A., K. L. McKee, D. R. Cahoon, J. A. Cherry, and J. P. Megonigal. 2009. Elevated CO2 stimulates marsh elevation gain, counterbalancing sea-level rise. Proceedings of the National Academy of Sciences of the United States of America 106:6182–6186.
Langley, J. A., and J. P. Megonigal. 2010. Ecosystem response to elevated CO2 levels limited by nitrogen-induced plant species shift. Nature 466:96–9.
Langley, J. A., T. J. Mozdzer, K. A. Shepard, S. B. Hagerty, and J. P. Megonigal. 2013. Tidal marsh plant responses to elevated CO2 , nitrogen fertilization, and sea level rise. Global change biology 19:1495–503.
Lavorel, S. 2013. Plant functional effects on ecosystem services. Journal of Ecology 101:4–8.
Lee, R. W. ., D. W. . Kraus, and J. E. Doeller. 1999. Oxidation of sulfide by Spartina alterniflora roots. Limnology and oceanography 44:1155–1159.
Limpens, J., F. Berendse, C. Blodau, J. G. Canadell, C. Freeman, J. Holden, N. Roulet, H. Rydin, and G. Schaepman-Strub. 2008. Peatlands and the carbon cycle: from local

processes to global implications – a synthesis. Biogeosciences 5:1475–1491.
Livesley, S. J., and S. M. Andrusiak. 2012. Temperate mangrove and salt marsh sediments are a small methane and nitrous oxide source but important carbon store. Estuarine, Coastal and Shelf Science 97:19–27.
Lovelock, C. E., I. C. Feller, K. L. McKee, and R. Thompson. 2005. Variation in mangrove forest structure and sediment characteristics in Bocas del Toro, Panama. Caribbean Journal of Science 41:456–464.
MacKenzie, R. A., and M. Dionne. 2008. Habitat heterogeneity: Importance of salt marsh pools and high marsh surfaces to fish production in two Gulf of Maine salt marshes. Marine Ecology Progress Series 368:217–230.
Macreadie, P. I., A. R. Hughes, and D. L. Kimbro. 2013. Loss of "blue carbon" from coastal salt marshes following habitat disturbance. PloS one 8:e69244.
Macreadie, P. I., D. A. Nielsen, J. J. Kelleway, T. B. Atwood, J. R. Seymour, K. Petrou, R. M. Connolly, A. C. Thomson, S. M. Trevathan-Tackett, and P. J. Ralph. 2017. Can we manage coastal ecosystems to sequester more blue carbon? Frontiers in Ecology and the Environment: in press.
Mansfeldt, T. 2003. In situ long-term redox potential measurements in a dyked marsh soil. Journal of Plant Nutrition and Soil Science 166:210–219.
Martin, R. M., and S. Moseman-Valtierra. 2015. Greenhouse gas fluxes vary between Phragmites australis and native vegetation zones in coastal wetlands along a salinity gradient. Wetlands 35:1021–1031.
McCormick, M. K., K. M. Kettenring, H. M. Baron, and D. F. Whigham. 2010. Extent and reproductive mechanisms of phragmites australis spread in brackish wetlands in Chesapeake bay, Maryland (USA). Wetlands 30:67–74.
McGuinness, K. A. 2002. Of rowing boats, ocean liners and tests of the ANOVA homogeneity of variance assumption. Austral Ecology 27:681–688.
McKee, K. L., D. R. Cahoon, and I. C. Feller. 2007. Caribbean mangroves adjust to rising sea level through biotic controls on change in soil elevation. Global Ecology and Biogeography 16:545–556.
Mcleod, E., G. L. Chmura, S. Bouillon, R. Salm, M. Björk, C. M. Duarte, C. E. Lovelock, W. H. Schlesinger, and B. R. Silliman. 2011. A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO2. Frontiers in Ecology and Environment 9:552–560.
Megonigal, J. P., M. E. Hines, and P. T. Visscher. 2004. Anaerobic Metabolism : Linkages to Trace Gases and Aerobic Processes. Pages 317–424*in* W. H. Schlesinger, editor.Biogeochemistry. Elsevier-Pergamon, Oxford, UK.
Megonigal, J. P., and M. Rabenhorst. 2013. Reduction–Oxidation Potential and Oxygen. Pages 71–85 *in* R. D. DeLaune, K. R. Reddy, C. J. Richardson, and J. P. Megonigal, editors. Methods in Biogeochemistry of Wetlands. Soil Science Society of America, Inc., Madison, WI, USA.
Megonigal, J. P., and W. H. Schlesinger. 2002. Methane-limited methanotrophy in tidal freshwater swamps. Global Biogeochemical Cycles 16:35-1-35–10.
Megonigal, J., S. Whalen, D. Tissue, B. Bovard, D. Albert, and A. Allen. 1999. A plant-soil-atmosphere microcosm for tracing radiocarbon from photosynthesis through methanogenesis. Soil Science Society of America Journal 63:665–671.
Mendelssohn, I. A. 1979. The influence of nitrogen level, form, and application method on the growth response of Spartina alterniflora in North Carolina. Estuaries and Coasts 2:106–112.
Mendelssohn, I., K. McKee, and W. Patrick. 1981. Oxygen deficiency in Spartina alterniflora

roots: metabolic adaptation to anoxia. Science 214:439–441.
Middelburg, J. J., J. Nieuwenhuize, R. K. Lubberts, and O. Van De Plassche. 1997. Organic Carbon Isotope Systematics of Coastal Marshes. Estuarine, Coastal and Shelf Science 45:681–687.
Minke, M., J. Augustin, U. Hagemann, and H. Joosten. 2014. Similar methane fluxes measured by transparent and opaque chambers point at belowground connectivity of Phragmites australis beyond the chamber footprint. Aquatic Botany 113:63–71.
Minoda, T., and M. Kimura. 1994. Contribution of photosynthesized carbon to the methane emitted from paddy fields. Geophysical Research Letters 21:2007–2010.
Mitsch, W. J., and J. G. Gosselink. 2010. Wetlands. Wiley, Hoboken, NJ, USA.
Moeller, I., M. Kudella, F. Rupprecht, T. Spencer, M. Paul, B. K. van Wesenbeeck, G. Wolters, K. Jensen, T. J. Bouma, M. Miranda-Lange, and S. Schimmels. 2014. Wave attenuation over coastal salt marshes under storm surge conditions. Nature Geoscience 7:727–731.
Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. De Suiter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo, K. M. Scow, M. J. Vanni, and D. H. Wall. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters 7:584–600.
Moorhead, D. L., Z. L. Rinkes, R. L. Sinsabaugh, and M. N. Weintraub. 2013. Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: Informing enzyme-based decomposition models. Frontiers in Microbiology 4:1–12.
Morris, J. T. 2007. Estimating Net Primary Production of Salt Marsh Macrophytes. Pages 106–119 *in* T. J. Fahey and A. K. Knapp, editors.Principles and Standards for Measuring Primary Production. Oxford University Press, New York, NY, USA.
Morris, J. T., and A. Jensen. 1998. The carbon balance of grazed and non-grazed Spartina anglica saltmarshes at Skallingen, Denmark. Journal of Ecology 86:229–242.
Morris, J. T., P. V Sundareshwar, C. T. Nietch, B. Kjerfve, and D. R. Cahoon. 2002. Responses of coastal wetlands to rising sea level. Ecology 83:2869–2877.
Morris, J. T., K. Sundberg, and C. S. Hopkinson. 2013. Salt marsh primary production and its responses to relative sea level and nutrients. Oceanography 26:78–84.
Morrissey, E. M., D. J. Berrier, S. C. Neubauer, and R. B. Franklin. 2013. Using microbial communities and extracellular enzymes to link soil organic matter characteristics to greenhouse gas production in a tidal freshwater wetland. Biogeochemistry 117:473–490.
Morrissey, E. M., J. L. Gillespie, J. C. Morina, and R. B. Franklin. 2014. Salinity affects microbial activity and soil organic matter content in tidal wetlands. Global Change Biology 20:1351–62.
Mozdzer, T. J., J. Brisson, and E. L. G. Hazelton. 2013. Physiological ecology and functional traits of North American native and Eurasian introduced Phragmites australis lineages. AoB PLANTS 5:plt048-plt048.
Mozdzer, T. J., J. A. Langley, P. Mueller, and J. P. Megonigal. 2016. Deep rooting and global change facilitate spread of invasive grass. Biological Invasions 18:2619–2631.
Mozdzer, T. J., and J. P. Megonigal. 2013. Increased Methane Emissions by an Introduced Phragmites australis Lineage under Global Change. Wetlands 33:609–615.
Mozdzer, T. J., and J. C. Zieman. 2010. Ecophysiological differences between genetic lineages facilitate the invasion of non-native Phragmites australis in North American Atlantic coast wetlands. Journal of Ecology 98:451–458.
Mozdzer, T. J., J. C. Zieman, and K. J. McGlathery. 2010. Nitrogen Uptake by Native and Invasive Temperate Coastal Macrophytes: Importance of Dissolved Organic Nitrogen. Estuaries and Coasts 33:784–797.

Mueller, P., D. Granse, S. Nolte, H. T. Do, M. Weingartner, S. Hoth, and K. Jensen. 2017. Top-down control of carbon sequestration: grazing affects microbial structure and function in salt marsh soils. Ecological Applications: in press.

Mueller, P., R. N. Hager, J. E. Meschter, T. J. Mozdzer, J. A. Langley, K. Jensen, and J. P. Megonigal. 2016a. Complex invader-ecosystem interactions and seasonality mediate the impact of non-native Phragmites on CH4 emissions. Biological Invasions 18:2635–2647.

Mueller, P., K. Jensen, and J. P. Megonigal. 2016b. Plants mediate soil organic matter decomposition in response to sea level rise. Global Change Biology 22:404–414.

Müller-Navarra, K., Y. Milker, and G. Schmiedl. 2016. Natural and anthropogenic influence on the distribution of salt marsh foraminifera in the bay of Tümlau, German North Sea. The Journal of Foraminiferal Research 46:61–74.

Müller, F., E. Struyf, J. Hartmann, A. Wanner, and K. Jensen. 2013. A comprehensive study of silica pools and fluxes in Wadden Sea salt marshes. Estuaries and Coasts 36:1150–1164.

Murray, B. C. 2012. Incorporating blue carbon as a mitigation action under the United Nations Framework Convention on Climate Change technical issues to address. Report: Nicholas School of Environmental Policy Solutions.

Nannipieri, P., J. Ascher, M. T. Ceccherini, L. Lan Dl, G. Pietramellar, and G. Renell. 2003. Microbial diversity and soil functions. European Journal of Soil Science 54:655.

van der Nat, F.-J. W. A., J. J. Middelburg, D. Van Meteren, and A. Wielemakers. 1998. Diel methane emission patterns from Scirpus lacustris and Phragmites australis. Biogeochemistry 41:1–22.

Neff, K. P., K. Rusello, and A. H. Baldwin. 2009. Rapid seed bank development in restored tidal freshwater wetlands. Restoration Ecology 17:539–548.

Nellemann, C., E. Corcoran, C. M. Duarte, L. Valdés, C. De Young, L. Fonseca, and G. Grimsditch. 2009. Blue carbon: A rapid response assessment. Page Environment. United Nations Environment Programme, GRID-Arendal, www.grida.no.

Neubauer, S. C. 2008. Contributions of mineral and organic components to tidal freshwater marsh accretion. Estuarine, Coastal and Shelf Science 78:78–88.

Neubauer, S. C., K. Givler, S. K. Valentine, and J. P. Megonigal. 2005. Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. Ecology 86:3334–3344.

Neubauer, S. C., and J. P. Megonigal. 2015. Moving Beyond Global Warming Potentials to Quantify the Climatic Role of Ecosystems. Ecosystems 18:1000–1013.

Neumeier, U., and C. Cheng. 2015. Hydrodynamics and sediment dynamics in an ice-covered tidal flat. Coastal Sediments 2015:1–14.

Neutel, A. M., J. A. P. Heesterbeek, J. van de Koppel, G. Hoenderboom, A. Vos, C. Kaldeway, F. Berendse, and P. C. de Ruiter. 2007. Reconciling complexity with stability in naturally assembling food webs. Nature 449:599–602.

Nolte, S., E. C. Koppenaal, P. Esselink, K. S. Dijkema, M. Schuerch, A. V De Groot, J. P. Bakker, and S. Temmerman. 2013a. Measuring sedimentation in tidal marshes: A review on methods and their applicability in biogeomorphological studies. Journal of Coastal Conservation 17:301–325.

Nolte, S., F. Müller, M. Schuerch, A. Wanner, P. Esselink, J. P. Bakker, and K. Jensen. 2013b. Does livestock grazing affect sediment deposition and accretion rates in salt marshes? Estuarine, Coastal and Shelf Science 135:296–305.

Olsen, Y. S., A. Dausse, A. Garbutt, H. Ford, D. N. Thomas, and D. L. Jones. 2011. Cattle grazing drives nitrogen and carbon cycling in a temperate salt marsh. Soil Biology and Biochemistry 43:531–541.

Olsson, L., S. Ye, X. Yu, M. Wei, K. W. Krauss, and H. Brix. 2015. Factors influencing CO2 and CH4 emissions from coastal wetlands in the Liaohe Delta, Northeast China.

Biogeosciences 12:4965–4977.
Osland, M. J., A. C. Spivak, J. A. Nestlerode, J. M. Lessmann, A. E. Almario, P. T. Heitmuller, M. J. Russell, K. W. Krauss, F. Alvarez, D. D. Dantin, J. E. Harvey, A. S. From, N. Cormier, and C. L. Stagg. 2012. Ecosystem development after mangrove wetland creation: Plant-soil change across a 20-year chronosequence. Ecosystems 15:848–866.
Pan, X., J. Yang, D. Zhang, X. Chen, and S. Mu. 2011. Cu(II) complexation of high molecular weight (HMW) fluorescent substances in root exudates from a wetland halophyte (Salicornia europaea L.). Journal of Bioscience and Bioengineering 111:193–197.
Peiter, A. 2002. GIS-gestützte Auswertung von Monitoringdaten am Beispiel einer Habitatanalyse für Nonnengänsen (Branta leucopsis) und Ringelgänse (Branta b. bernicla) in der Salzwiese Westerhever. Diploma thesis, Hochschule Vechta.
Pfauder, A., and M. Zimmer. 2005. Intermediate tidal stress promotes the detritivore-mediated decomposition of Spartina litter. European Journal of Soil Biology 41:135–141.
Phillips, R. P., A. C. Finzi, and E. S. Bernhardt. 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO2 fumigation. Ecology letters 14:187–94.
Poffenbarger, H. J., B. A. Needelman, and J. P. Megonigal. 2011. Salinity influence on methane emissions from tidal marshes. Wetlands 31:831–842.
Prescott, C. E. 2010. Litter decomposition: What controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101:133–149.
Rabenhorst, M. C. 2006. Quick (7 day) IRIS tube paint recipe and construction procedure. Report: University of Maryland, Environmental Science and Technology.
Rabenhorst, M. C. 2008. Protocol for Using and Interpreting IRIS Tubes. Soil Survey Horizons 49:74–77.
Rawls, W. J., Y. A. Pachepsky, J. C. Ritchie, T. M. Sobecki, and H. Bloodworth. 2003. Effect of soil organic carbon on soil water retention. Geoderma 116:61–76.
Reddy, K. R., and R. D. DeLaune. 2008. Biogeochemistry of Wetlands: Science and Applications. CRC Press, Boca Raton, FL, USA.
Reed, D. J. 1995. the Response of Coastal Marshes To Sea-Level Rise : Survival or Submergence ? Earth Surface Processes and Landforms 20:39–48.
Reimold, R. J., R. A. Linthurst, and P. L. Wolf. 1975. Effects of grazing on a salt marsh. Biological Conservation 8:105–125.
Rogelj, J., M. Den Elzen, T. Fransen, H. Fekete, H. Winkler, R. Schaeffer, F. Sha, K. Riahi, and M. Meinshausen. 2016. Perspective : Paris Agreement climate proposals need boost to keep warming well below 2°C. Nature 534:631–639.
Roner, M., A. D'Alpaos, M. Ghinassi, M. Marani, S. Silvestri, E. Franceschinis, and N. Realdon. 2016. Spatial variation of salt-marsh organic and inorganic deposition and organic carbon accumulation: Inferences from the Venice lagoon, Italy. Advances in Water Resources 93:276–287.
Rooth, J. E., J. C. Stevenson, and J. C. Cornwell. 2003. Increased sediment accretion rates following invasion by Phragmites australis: The role of litter. Estuaries 26:475–483.
Rothwell, J. J., M. N. Futter, and N. B. Dise. 2008. A classification and regression tree model of controls on dissolved inorganic nitrogen leaching from European forests. Environmental Pollution 156:544–552.
Rousk, J., and S. D. Frey. 2015. Revisiting the hypothesis that fungal-to-bacterial dominance characterizes turnover of soil organic matter and nutrients. Ecological Monographs 85:457–472.
Ryan, M. G., J. M. Melillo, and A. Ricca. 1990. A comparison of methods for determining proximate carbon fractions of forest litter. Canadian Journal of Forest Research-Revue

Canadienne de Recherche Forestiere 20:166–171.
Rybzcyk, J. M., and J. C. Callaway. 2009. Surface Elevation Models. Pages 835–853 *in* G. M. E. Perillo, E. Wolanski, D. R. Cahoon, and M. M. Brinson, editors.Coastal Wetlands: an integrated ecosystem approach. Elsevier, Amsterdam, The Netherlands.
Saintilan, N., K. Rogers, D. Mazumder, and C. Woodroffe. 2013. Allochthonous and autochthonous contributions to carbon accumulation and carbon store in southeastern Australian coastal wetlands. Estuarine, Coastal and Shelf Science 128:84–92.
Sanaullah, M., B. S. Razavi, E. Blagodatskaya, and Y. Kuzyakov. 2016. Spatial distribution and catalytic mechanisms of ß-glucosidase activity at the root-soil interface. Biology and Fertility of Soils 52:505–514.
Savage, K., E. A. Davidson, and J. Tang. 2013. Diel patterns of autotrophic and heterotrophic respiration among phenological stages. Global Change Biology 19:1151–1159.
Schat, H. 1984. Oecologia of waterlogging and submergence on dune slack plants : Oecologia:279–286.
Schile, L. M., J. C. Callaway, J. T. Morris, D. Stralberg, V. Thomas Parker, and M. Kelly. 2014. Modeling tidal marsh distribution with sea-level rise: Evaluating the role of vegetation, sediment, and upland habitat in marsh resiliency. PLoS ONE 9:e88760.
Schile, L. M., J. B. Kauffman, S. Crooks, J. W. Fourqurean, J. Glavan, and J. P. Megonigal. 2017. Limits on carbon sequestration in arid blue carbon ecosystems. Ecological Applications 27:859–874.
Schlesinger, W. H. 1997. Biogeochemistry: an Analysis of Global Change. Second edition. Academic Press, San Diego, USA.
Schlesinger, W. H., and J. Lichter. 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO2. Nature 411:466–469.
Schrama, M., P. Heijning, J. P. Bakker, H. J. van Wijnen, M. P. Berg, and H. Olff. 2013. Herbivore trampling as an alternative pathway for explaining differences in nitrogen mineralization in moist grasslands. Oecologia 172:231–243.
Schuman, G., J. Reeder, J. Manley, R. Hart, and W. Manley. 1999. Impact of grazing management on the carbon and nitrogen balance of a mixed grass rangeland. Ecological Applications 9:65–71.
Scott, D. B., and F. S. Medioli. 1980. Quantitative studies of marsh foraminiferal distributions in Nova Scotia: Implications for sea level studies. Cushman Foundation for foraminiferal Research Special Publication:1–58.
Scott, D. S., and F. S. Medioli. 1978. Vertical zonations of marsh foraminifera as accurate indicators of former sea-levels. Nature 272:528–531.
Seybold, C. A., W. Mersie, J. Huang, and C. Mcnamee. 2002. Soil redox, pH, temperature, and water-table patterns of a freshwater wetland 22:149–158.
Sifleet, S., and B. C. Murray. 2011. State of the Science on Coastal Blue Carbon A Summary for Policy Makers State of the Science on Coastal Blue Carbon A Summary for Policy Makers. Report: Nicholas School of Environmental Policy Solutions.
Silliman, B. R., and J. C. Zieman. 2001. Top-down control of Spartina alterniflora production by periwinkle grazing in a Virginia salt marsh. Ecology 82:2830–2845.
Sinsabaugh, R. L., R. K. Antibus, and A. E. Linkins. 1991. An enzymic approach to the analysis of microbial activity during plant litter decomposition. Agriculture, Ecosystems & Environment 34:43–54.
Sinsabaugh, R. L., J. Belnap, S. G. Findlay, J. J. F. Shah, B. H. Hill, K. A. Kuehn, C. R. Kuske, M. E. Litvak, N. G. Martinez, D. L. Moorhead, and D. D. Warnock. 2014. Extracellular enzyme kinetics scale with resource availability. Biogeochemistry 121:287–304.
Sinsabaugh, R. L., C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. Crenshaw, A.

R. Contosta, D. Cusack, S. Frey, M. E. Gallo, T. B. Gartner, S. E. Hobbie, K. Holland, B. L. Keeler, J. S. Powers, M. Stursova, C. Takacs-Vesbach, M. P. Waldrop, M. D. Wallenstein, D. R. Zak, and L. H. Zeglin. 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11:1252–1264.

Sinsabaugh, R. L., K. Saiya-Cork, T. Long, M. P. Osgood, D. A. Neher, D. R. Zak, and R. J. Norby. 2003. Soil microbial activity in a Liquidambar plantation unresponsive to CO2-driven increases in primary production. Applied Soil Ecology 24:263–271.

Sinsabaugh, R. L., B. L. Turner, J. M. Talbot, B. G. Waring, J. S. Powers, C. R. Kuske, D. L. Moorhead, and J. J. Follstad Shah. 2016. Stoichiometry of microbial carbon use efficiency in soils. Ecological Monographs 86:172–189.

Smith, C. J., and a M. Osborn. 2009. Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. FEMS microbiology ecology 67:6–20.

Soil Survey Staff. 2014. Keys to soil taxonomy. Soil Conservation Service.

Spohn, M., and Y. Kuzyakov. 2013. Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – Coupling soil zymography with 14C imaging. Soil Biology and Biochemistry 67:106–113.

Spohn, M., and Y. Kuzyakov. 2014. Spatial and temporal dynamics of hotspots of enzyme activity in soil as affected by living and dead roots—a soil zymography analysis. Plant and Soil 379:67–77.

Sponheimer, M., T. Robinson, L. Ayliffe, B. Passey, B. Roeder, L. Shipley, E. Lopez, T. Cerling, D. Dearing, and J. Ehleringer. 2003. An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. Canadian Journal of Zoology 81:871–876.

Steffens, D., B. W. Hütsch, T. Eschholz, T. Losak, and S. Schubert. 2005. Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. Plant, Soil and Environment 51:545–552.

Stock, M. 2005. Salzwiesen an der Westküste von Schleswig-Holstein 1988–2001. Boyens Medien GmbH, Heide, Germany.

Stock, M. 2011. Patterns in surface elevation change across a temperate salt marsh platform in relation to sea-level rise. Coastline Reports 17:33–48.

Stock, M. 2012. TMAP Wadden Sea Sedimentation Database. Biodiversity and Ecology 4:372.

Strickland, M. S., and J. Rousk. 2010. Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42:1385–1395.

Suchrow, S., N. Pohlmann, M. Stock, and K. Jensen. 2012. Long-term surface elevation changes in German North Sea salt marshes. Estuarine, Coastal and Shelf Science 98:71–83.

Sundby, B., C. Vale, M. Caetano, and G. W. Luther III. 2003. Redox chemistry in the root zone of a salt marsh sediment in the Tagus Estuary, Portugal. Aquatic Geochemistry 9:257–271.

Sutton-Grier, A. E., J. K. Keller, R. Koch, C. Gilmour, and J. P. Megonigal. 2011. Electron donors and acceptors influence anaerobic soil organic matter mineralization in tidal marshes. Soil Biology and Biochemistry 43:1576–1583.

Sutton-Grier, A. E., and J. P. Megonigal. 2011. Plant species traits regulate methane production in freshwater wetland soils. Soil Biology and Biochemistry 43:413–420.

Swanson, K. M., J. Z. Drexler, D. H. Schoellhamer, K. M. Thorne, M. L. Casazza, C. T. Overton, J. C. Callaway, and J. Y. Takekawa. 2014. Wetland Accretion Rate Model of Ecosystem Resilience (WARMER) and its application to habitat sustainability for endangered species in the San Francisco Estuary. Estuaries and Coasts 37:476–492.

Tanaka, N., K. Yutani, T. Aye, and K. B. S. N. Jinadasa. 2007. Effect of broken dead culms of Phragmites australis on radial oxygen loss in relation to radiation and temperature. Hydrobiologia 583:165–172.

Temmerman, S., T. J. Bouma, G. Govers, Z. B. Wang, M. B. De Vries, and P. M. J. Herman. 2005. Impact of vegetation on flow routing and sedimentation patterns: Three-dimensional modeling for a tidal marsh. Journal of Geophysical Research: Earth Surface 110:1–18.

Toberman, H., C. Evans, and C. Freeman. 2008. Summer drought effects upon soil and litter extracellular phenol oxidase activity and soluble carbon release in an upland Calluna heathland. Soil Biology and Biochemistry 40:1519–1532.

Tong, C., W. Q. Wang, J. F. Huang, V. Gauci, L. H. Zhang, and C. S. Zeng. 2012. Invasive alien plants increase CH4 emissions from a subtropical tidal estuarine wetland. Biogeochemistry 111:677–693.

Valéry, L., V. Bouchard, and J.-C. Lefeuvre. 2004. Impact of the invasive native species Elymus athericus on carbon pools in a salt marsh. Wetlands 24:268–276.

Vann, C., and J. Megonigal. 2003. Elevated CO2 and water depth regulation of methane emissions: Comparison of woody and non-woody wetland plant species. Biogeochemistry:117–134.

Vasey, M. C., V. T. Parker, J. C. Callaway, E. R. Herbert, and L. M. Schile. 2012. Tidal wetland vegetation in the San Francisco Bay-Delta Estuary. San Francisco Estuary and Watershed Science 10:1–16.

de Vries, F. T., J. Bloem, H. Quirk, C. J. Stevens, R. Bol, and R. D. Bardgett. 2012. Extensive management promotes plant and microbial nitrogen retention in temperate grassland. PLoS ONE 7:1–12.

de Vries, F. T., E. Hoffland, N. van Eekeren, L. Brussaard, and J. Bloem. 2006. Fungal/bacterial ratios in grasslands with contrasting nitrogen management. Soil Biology and Biochemistry 38:2092–2103.

Wang, J. J., S. K. Dodla, R. D. Delaune, W. H. Hudnall, and R. L. Cook. 2011. Soil carbon characteristics in two Mississippi river deltaic Marshland profiles. Wetlands 31:157–166.

Wanner, A., S. Suchrow, K. Kiehl, W. Meyer, N. Pohlmann, M. Stock, and K. Jensen. 2014. Scale matters: Impact of management regime on plant species richness and vegetation type diversity in Wadden Sea salt marshes. Agriculture, Ecosystems and Environment 182:69–79.

Ward, N. D., T. S. Bianchi, H. O. Sawakuchi, W. Gagne-Maynard, A. C. Cunha, D. C. Brito, V. Neu, A. de Matos Valerio, R. da Silva, A. V. Krusche, J. E. Richey, and R. G. Keil. 2016. The reactivity of plant-derived organic matter and the potential importance of priming effects along the lower Amazon River. Journal of Geophysical Research: Biogeosciences 121:1522–1539.

Wardle, D. a, R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. van der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science 304:1629–33.

Weiss, M., U. Abele, J. Weckesser, W. Welte, E. Schiltz, and G. Schulz. 1991. Molecular architecture and electrostatic properties of a bacterial porin. Science 254:1627–1630.

Weston, N. B., R. E. Dixon, and S. B. Joye. 2006. Ramifications of increased salinity in tidal freshwater sediments: Geochemistry and microbial pathways of organic matter mineralization. Journal of Geophysical Research 111:G01009.

White, K. P., J. A. Langley, D. R. Cahoon, and J. P. Megonigal. 2012. C3 and C4 biomass allocation responses to elevated CO2 and nitrogen: Contrasting resource capture strategies. Estuaries and Coasts 35:1028–1035.

Wießner, A., P. Kuschk, and U. Stottmeister. 2002. Oxygen release by roots of Typha latifolia

and Juncus effusus in laboratory hydroponic systems. Acta Biotechnologica 22:209–216.
van Wijnen, H. J., and J. P. Bakker. 2001. Long-term surface elevation change in salt marshes: a prediction of marsh response to future dea-level rise. Estuarine, Coastal and Shelf Science 52:381–390.
Wilson, R. M., A. M. Hopple, M. M. Tfaily, S. D. Sebestyen, C. W. Schadt, L. Pfeifer-Meister, C. Medvedeff, K. J. McFarlane, J. E. Kostka, M. Kolton, R. K. Kolka, L. A. Kluber, J. K. Keller, T. P. Guilderson, N. A. Griffiths, J. P. Chanton, S. D. Bridgham, and P. J. Hanson. 2016. Stability of peatland carbon to rising temperatures. Nature Communications 7:#13723.
Windham-Myers, L. 2005. Dissolved inorganic nitrogen pools and surface flux under different brackish marsh vegetation types, common reed (Phragmites australis) and salt hay (Spartina patens). Biogeochemistry 75:289–304.
Windham, L., and R. Lathrop. 1999. Effect of Phragmites australis (common reed) invasion on aboveground biomass and soil properties in brackish tidal marsh of Mullica River, New Jersey. Estuaries 22:927–935.
Wolf, A. A., B. G. Drake, J. E. Erickson, and J. P. Megonigal. 2007. An oxygen-mediated positive feedback between elevated carbon dioxide and soil organic matter decomposition in a simulated anaerobic wetland. Global Change Biology 13:2036–2044.
Wu, F. Y., A. K. C. Chung, N. F. Y. Tam, and M. H. Wong. 2011. Root exudates of wetland plants influenced by nutrient status and types of plant cultivation. International Journal of Phytoremediation 14:543–553.
Wunderlich, S., and W. Borken. 2012. Partitioning of soil CO_2 efflux in un-manipulated and experimentally flooded plots of a temperate fen. Biogeosciences 9:3477–3489.
Yang, J. X., Y. Liu, and Z. H. Ye. 2012. Root-induced changes of pH, Eh, Fe(II) and fractions of Pb and Zn in rhizosphere soils of four wetland plants with different radial oxygen losses. Pedosphere 22:518–527.
Yang, S. L., H. Li, T. Ysebaert, T. J. Bouma, W. X. Zhang, Y. Y. Wang, P. Li, M. Li, and P. X. Ding. 2008. Spatial and temporal variations in sediment grain size in tidal wetlands, Yangtze Delta: On the role of physical and biotic controls. Estuarine, Coastal and Shelf Science 77:657–671.
Yang, Z., S. Nolte, and J. Wu. 2017. Tidal flooding diminishes the effects of livestock grazing on soil micro-food webs in a coastal saltmarsh. Agriculture, Ecosystems & Environment 236:177–186.
Yoneyama, T., H. Okada, P. Chongpraditnum, S. Ando, P. Prasertsak, and K. Hirai. 2006. Effects of vegetation and cultivation on del13C values of soil organic carbon and estimation of its turnover in Asian tropics: A case study in Thailand. Soil Science and Plant Nutrition 52:95–102.
Young, L. Y., and a. C. Frazer. 1987. The fate of lignin and lignin-derived compounds in anaerobic environments. Geomicrobiology Journal 5:261–293.
Yu, K., F. Böhme, J. Rinklebe, H.-U. Neue, and R. D. DeLaune. 2007. Major biogeochemical processes in soils - A microcosm incubation from reducing to oxidizing conditions. Soil Science Society of America Journal 71:1406.
Yu, K., and W. Patrick. 2003. Redox range with minimum nitrous oxide and methane production in a rice soil under different pH. Soil Science Society of America Journal 67:1952–1958.
Yu, O. T., and G. L. Chmura. 2009. Soil carbon may be maintained under grazing in a St Lawrence Estuary tidal marsh. Environmental Conservation 36:312–320.
Yuan, J., W. Ding, D. Liu, H. Kang, C. Freeman, J. Xiang, and Y. Lin. 2015. Exotic Spartina alterniflora invasion alters ecosystem-atmosphere exchange of CH4 and N2O and carbon

sequestration in a coastal salt marsh in China. Global Change Biology 21:1567–1580.
Zedler, J. B., and S. Kercher. 2004. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. Critical Reviews in Plant Sciences 23:431–452.
Zhai, X., N. Piwpuan, C. A. Arias, T. Headley, and H. Brix. 2013. Can root exudates from emergent wetland plants fuel denitrification in subsurface flow constructed wetland systems? Ecological Engineering 61:555–563.
Zhang, D. Q., D. F. Hui, Y. Q. Luo, and G. Y. Zhou. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. Journal of Plant Ecology 1:85–93.
Zhang, Y., W. Ding, Z. Cai, P. Valerie, and F. Han. 2010. Response of methane emission to invasion of Spartina alterniflora and exogenous N deposition in the coastal salt marsh. Atmospheric Environment 44:4588–4594.
Zhi-Guang, L. 1985. Oxidation-reduction potential. Pages 1–26 *in* Y. Tian-ren, editor. Physical chemistry of paddy soils. Springer, Berlin, Germany.
Zimmerman, R. J., T. J. Minello, and L. P. Rozas. 2000. Salt marsh linkages to productivity of penaeid shrimps and blue crabs in the northern Gulf of Mexico. Pages 293–314 *in* M. P. Weinstein and D. A. Kreeger, editors.Concepts and controversies in tidal marsh ecology. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Summary

Tidal wetlands, such as tidal marshes and mangroves, are global hotspots for carbon sequestration. The preservation of organic matter is a critical process by which tidal wetlands exert their strong influence over the global carbon cycle and at the same time gain elevation to keep pace with sea level rise. This thesis addresses the effects of numerous global change factors (sea level rise, warming, land use change, biological invasions) on organic matter turnover processes in tidal wetlands, aiming to assess their role in the carbon sequestration potential of these ecosystems. Besides studying direct effects of different factors on decomposition and turnover processes, this work is particularly focused on plant mediated and thus indirect effects. The thesis is structured in 7 chapters, including a general introduction to the topic (Chapter 1), five manuscripts as the main part (Chapters 2-6), and a synthesis discussing the links between the single chapters, their implications, and resulting future research perspectives (Chapter 7).

Chapter 2 assesses the potential of a deep rooting and highly productive invasive plant species – *Phragmites australis* – to change methane emissions from North American brackish marshes, and it discusses implications on the net greenhouse-gas balance of these ecosystems. The results show that the invader-ecosystem effect is both seasonally and spatially highly variable, and that the relative impact of *Phragmites* on methane emissions is particularly strong in soils with innately high rates of methane production. This effect is likely attributable to *Phragmites*' capacity to develop a deep root system in anoxic soils and the effective plant-supported ventilation of soil methane to the atmosphere.

Chapter 3 presents findings on direct and plant-mediated effects of accelerated sea level rise on soil organic matter decomposition. The findings suggest that soil organic matter decomposition in tidal marshes is not directly driven by relative sea level and its effect on oxygen diffusion through soil, but indirectly by plant responses to relative sea level and resulting changes in the rhizosphere priming effect.

Chapter 4 describes the carbon-sequestration potential of artificial salt marshes along the German mainland coast, and illustrates the importance to track soil carbon turnover over decades in order to avoid overestimation of long-term carbon sequestration. Large amounts of previously fixed carbon are lost with age and depth in the well-aerated marsh soils of the Wadden Sea region. Still, rates of carbon sequestration are well in range with rates reported

from other blue carbon systems, illustrating that the Wadden Sea salt marshes represent a powerful man-made carbon sink

Chapter 5 focuses on land use effects on carbon turnover by describing the direct and plant-mediated effects of livestock grazing on the microbial function and structure in salt marsh soils. It is shown that livestock grazing can have strong effects on the microbial structure and function of salt marsh soils by increasing the relative fungal abundance and decreasing exo-enzyme activities. Thus, livestock grazing potentially exerts important control over tidal wetland carbon turnover.

Chapter 6 presents the results of a global scale comparison of litter decomposition and stabilization rates in 25 marsh and mangrove sites, stretching a large temperature gradient, in order to improve our process understanding of temperature and sea level effects on carbon turnover. The findings suggest that rising temperatures and increasing flooding in the course of accelerated sea level rise may strongly decrease organic matter stabilization in tidal wetlands, thereby reducing both the carbon-sequestration potential and ecosystem stability against sea level rise.

Zusammenfassung

Tidebeinflusste Feuchtgebiete (TFs) wie Marschen und Mangroven sind globale Hotspots der Kohlenstofffestlegung. Der langsame Abbau und damit die Erhaltung organischen Materials in den Böden dieser Ökosysteme ist dabei ein entscheidender Prozess für diese Ökosystemleistung. Gleichzeitig ermöglicht dieser Prozess es TFs, Bodenvolumen zu generieren und so dem Meeresspiegelanstieg schrittzuhalten. Die vorliegende Arbeit setzt sich mit dem Einfluss unterschiedlicher Faktoren des Globalen Wandels auf Abbau- und Umsatzprozesse organischen Materials in TFs auseinander, um Rückschlüsse auf deren Bedeutung für das Potenzial zur Kohlenstofffestlegung dieser Ökosysteme ziehen zu können. Neben der Erfassung direkter Effekte verschiedener globaler Umweltveränderungen (Meeresspiegelanstieg, Erwärmung, Landnutzungsänderung, biologische Invasionen) auf Abbau- und Umsatzprozesse organischen Materials stehen primär pflanzenvermittelte und dadurch indirekte Effekte im Fokus dieser Arbeit. Die Arbeit ist in 7 Kapitel gegliedert, einschließlich einer generellen Einleitung in das Thema (Kapitel 1), fünf Manuskripte (Kapitel 2-6) sowie einer Synthese, welche den Zusammenhang der Kapitel darstellt und diskutiert, Implikationen ableitet und zukünftige Forschungsperspektiven aufzeigt.

Kapitel 2 untersucht das Potenzial einer tiefwurzelnden und hochproduktiven invasiven Pflanze, *Phragmites australis*, Methanemissionen in Nordamerikanischen Brackwassermarschen zu beeinflussen und es diskutiert Implikationen für die Treibhausgasbilanz dieser Ökosysteme. Die Ergebnisse zeigen, dass der Einfluss von *Phragmites* auf Methanemissionen saisonal und räumlich stark variiert und dass eine relative Steigerung der Methanemissionen durch *Phragmites* vor allem in Böden mit immanent hoher Methanproduktion auftritt. Dieser Effekt wird *Phragmites'* tiefreichendem Wurzelsystem und der effektiven pflanzenvermittelten Ventilation von bodenstammendem Methan an die Atmosphäre zugeordnet.

Kapitel 3 präsentiert Ergebnisse einer Studie zu direkten und indirekten, pflanzenvermittelten Effekten des beschleunigten Meeresspiegelanstiegs auf den Abbau organischer Bodensubstanz (OBS). Die Ergebnisse zeigen, dass der OBS-Abbau in Marschen nicht direkt durch den relativen Meeresspiegel und dessen Einfluss auf die Sauerstoffdiffusion in den Boden beeinflusst wird. Stattdessen ist der Effekt des relativen Meeresspiegels auf die pflanzliche Produktion und dadurch bedingte Veränderungen im pflanzenstimulierten OBS-Abbau (*Priming Effekt*) maßgeblich. Zusammenfassend legt diese Studie einen geringen Einfluss des beschleunigten Meeresspiegelanstiegs auf den OBS-Abbau nahe.

Kapitel 4 beschreibt das Potenzial der Salzmarschen des Europäischen Wattenmeers zur Kohlenstofffestlegung und verdeutlicht die Notwendigkeit, den Kohlenstoffumsatz längerer Zeiträume (in diesem Fall Jahrzehnte) zu berücksichtigen, um deutliche Überschätzungen in der Kohlenstofffestlegungsrate zu vermeiden. Obwohl ein Großteil des organischem Kohlenstoffs in den gut durchlüfteten Böden der Wattenmeer Salzmarschen mit der Tiefe verloren geht, sind die hier bestimmten Festlegungsraten im Bereich der aktuell publizierten Werte für Mangroven und Salzmarschen. Somit stellen die anthropogen erschaffenen Salzmarschen des Wattenmeers eine leistungsfähige Kohlenstoffsenke dar.

Kapitel 5 behandelt Landnutzungseffekte auf den Kohlenstoffumsatz in Wattenmeer Salzmarschen, indem direkte und pflanzenvermittelte Effekte von Beweidung durch Nutztiere auf die mikrobielle Funktion und Struktur im Oberboden dargestellt werden. Es wird gezeigt, dass Beweidung die relative Abundanz von Pilzen gegenüber Bakterien erhöht und gleichzeitig die Aktivität mikrobieller Exo-Enzyme im Boden reduziert. Dadurch kann die Beweidung durch Nutztiere potenziell die Kapazität zur Kohlenstofffestlegung von Marschflächen steigern.

Kapitel 6 präsentiert die Ergebnisse eines weltweiten Vergleichs von Abbauraten und Stabilisierung von frischem und standardisiertem organischen Material in 25 Marsch- und

Mangrovenflächen, um unser Verständnis über Temperatur- und Meeresspiegeleffekte auf Kohlenstoffumsatz-Prozesse in TFs zu verbessern. Die Ergebnisse zeigen, dass steigende Temperaturen und beschleunigter Meeresspiegelanstieg die Stabilisierung organischen Materials stark reduzieren können. Dadurch kann sowohl das Potenzial zur Kohlenstofffestlegung als auch die Ökosystemstabilität gegenüber dem beschleunigten Meeresspiegelanstieg von TFs herabgesetzt werden.

Key Findings (Chapters 2-6)

- Plant-supported ventilation of methane to the atmosphere by *Phragmites australis* has the potential to considerably increase methane emissions from tidal marshes. Yet, generalizations with respect to such invader-ecosystem effects should be interpreted with caution because they are highly site-specific and show large seasonal variability.

- Soil organic matter decomposition in tidal marshes is not directly driven by relative sea level and its effect on oxygen diffusion through soil, but indirectly by plant responses to relative sea level and resulting changes in the rhizosphere priming effect.

- Large amounts of previously fixed carbon are lost with age and depth in the well-aerated marsh soils of the Wadden Sea region. Still, long-term carbon sequestration rates are well in range with rates reported from other blue carbon systems.

- Livestock grazing has strong effects on the microbial structure and function of salt marsh soils by increasing the relative fungal abundance and decreasing exo-enzyme activities. Thus, livestock grazing potentially exerts important control over tidal wetland carbon turnover.

- Rising temperatures and increasing flooding in the course of accelerated sea level rise may strongly decrease organic matter stabilization in tidal wetlands, thereby reducing both the carbon sequestration potential and ecosystem stability against sea level rise.

Author contributions

Chapter 1 P. Mueller wrote this chapter. Figure 4 presents findings of a collaborative study by P. Mueller and K. Koop-Jakobsen

Chapter 2 P. Mueller designed and set up the field studies, conducted the initial methane emission measurements, analyzed all data (including statistics), and wrote the initial manuscript. R.H. Hager contributed equally to the manuscript by conducting and planning the majority of emission measurements and biomass assessments, and by commenting and editing the manuscript.

Chapter 3 P. Mueller designed and set up the field study, conducted all measurements, analyzed all data (including statistics), and wrote the initial manuscript.

Chapter 4 P. Mueller designed and set up the field study, helped with field work and lab analyses, analyzed all data (including statistics), and wrote the initial manuscript

Chapter 5 P. Mueller designed and set up the field study, conducted half of the field and lab work, analyzed all data (including statistics), and wrote the initial manuscript.

Chapter 6 P. Mueller designed the overall study, planned and conducted the research in two sites, analyzed all data (including statistics), and wrote the initial manuscript. L.M. Schile contributed equally to the manuscript by conducting and planning the research in the majority of field sites and by commenting and editing the manuscript.

Chapter 7 P. Mueller wrote this chapter. All findings presented in Concept II are based on research and data analysis by P. Mueller. D. Granse assisted with qPCR-data analysis (Concept II, Figure 5).

Co-Author affiliations

Kai Jensen, Stefanie Nolte, Dirk Granse, and Hai T. Do: Applied Plant Ecology, Biocenter Klein Flottbek, Universität Hamburg, Ohnhorststraße 18, 22609 Hamburg, Germany

J. Patrick Megonigal, Lisa M. Schile, and Justen E. Meschter: Smithsonian Environmental Research Center, 647 Contees Wharf Rd, Edgewater, MD, 21037, USA

Thomas J. Mozdzer: Bryn Mawr College, Department of Biology, 101 N. Merion Ave, Bryn Mawr, PA, 19010, USA

Adam J. Langley: Department of Biology, Villanova University, Villanova, PA 19085, USA

Rachel N. Hager: Ecology Center and Department of Watershed Sciences, Utah State University, 5210 Old Main Hill, Logan, UT 84322, USA

Lars Kutzbach, Nils Ladiges, and Alexander Jack: Center for Earth System Research and Sustainability, Institute of Soil Science, Universität Hamburg, Allende-Platz 2, 20146 Hamburg, Germany

Stefan Hoth and Magdalena Weingartner: Molecular Plant Physiology, Biocenter Klein Flottbek, Universität Hamburg, Ohnhorststraße 18, 22609 Hamburg, Germany

Gerhard Schmiedl: Center for Earth System Research and Sustainability, Institute for Geology, Universität Hamburg, Bundesstr. 55, 20146 Hamburg, Germany

Thomas Dinter and Yakov Kuzyakov: Büsgen-Institut, Georg-August Universität Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

Alma de Groot: Wageningen Marine Research, Wageningen University & Research, Den Helder, Ankerpark 27, 1781AG, The Netherlands

Peter Esselink: PUCCIMAR, Boermarke 35, 9481 HD, Vries, The Netherlands; Conservation Ecology Group, Groningen Institute for Evolutionary Life Sciences, University of Groningen, P.O. Box 11103, 9700 CC, Groningen, The Netherlands

Chris Smit: Conservation Ecology Group, Groningen Institute for Evolutionary Life Sciences, University of Groningen, P.O. Box 11103, 9700 CC, Groningen, The Netherlands

Andrea D'Alpaos: Department of Geosciences, University of Padova, Via Gradenigo 6, Padua 35131, Italy

Carles Ibáñez: IRTA Aquatic Ecosystems, Carretera Poblenou Km 5.5, 43540 Sant Carles de Ràpita, Catalonia, Spain

Magdalena Lazarus: Department of Plant Taxonomy and Nature Conservation, University of Gdansk, ul. Wita Stwosza 59, 80-308 Gdansk, Poland

Urs Neumeier: Institut des sciences de la mer de Rimouski (ISMER), Université du Québec à Rimouski, 310 allée des Ursulines C.P., 3300 Rimouski, QC, Canada

Beverly J. Johnson: Department of Geology, Bates College, 214 Carnegie Sciences Building, Lewiston, ME, 04240, USA

Andrew H. Baldwin and Stephanie A. Yarwood: Department of Environmental Science & Technology, University of Maryland, College Park, MD 20742, USA

Diana I. Montemayor: Laboratorio de Ecología, Instituto de Investigaciones Marinas y Costeras (Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Mar del Plata), Mar del Plata, Argentina

Zaichao Yang and Jihua Wu: Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering, Institute of Biodiversity Science, Fudan University, Shanghai 200438, PR China

Ketil Koop-Jakobsen: Center for Marine Environmental Sciences, University of Bremen, Loebner Str., 28359 Bremen, Germany

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Smithsonian Environmental Research Center

P.O. Box 28
Edgewater, MD 21037
Voice Mail: (443) 482-2346
Email: megonigalp@si.edu

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To whom it may concern,

As a native English Speaker, I do hereby declare that the PhD thesis: “Global Change and Land Use Effects on Carbon Turnover in Tidal Wetlands “has been written in concise and correct English (US).

Sincerely,

Dr. J. Patrick Megonigal, Senior Scientist & Associate Director of Research
Smithsonian Environmental Research Center
647 Contees Wharf Rd
Edgewater, MD 21037, USA

Information on published chapters

Chapter 2

Complex invader-ecosystem interactions and seasonality mediate the impact of non-native *Phragmites* on CH_4 emissions

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*first two authors contributed equally to the manuscript

P. Mueller designed and set up the field studies, conducted the initial methane emission measurements, analyzed all data, and wrote the initial manuscript. R.H. Hager contributed equally to the manuscript by conducting and planning the majority of emission measurements and biomass assessments, and by commenting and editing the manuscript.

Signature

Prof. Dr. Kai Jensen (first advisor)

Chapter 3

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Authors: Peter Mueller, Kai Jensen, J. Patrick Megonigal

P. Mueller designed and set up the field study, conducted all measurements, analyzed all data, and wrote the initial manuscript.

Signature

Prof. Dr. Kai Jensen (first advisor)

Chapter 5

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P. Mueller designed and set up the field study, conducted half of the field and lab work, analyzed all data, and wrote the initial manuscript.

Signature

Prof. Dr. Kai Jensen (first advisor)

Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

Hamburg, den Unterschrift (P.Müller)