

# 1 **Origin of Organic Carbon in the Topsoil of Wadden Sea Salt Marshes**

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3 **Running head:** Organic carbon origin in salt marshes

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11

## 12 **ABSTRACT**

13 Blue carbon ecosystems, including salt marshes, play an important role in the global carbon  
14 cycle because of their high efficiency to store soil organic carbon (OC). Few studies focus on  
15 the origin of OC stored in salt-marsh soils, which comes from either allochthonous or  
16 autochthonous sources. The origin, however, has important implications for carbon crediting  
17 approaches because the alternative fate of allochthonous OC (AlIOC), i.e. if it had not  
18 accumulated in the Blue C ecosystem, is unclear. Here, we assessed the origin of OC in two  
19 mainland salt-marsh sites of the European Wadden Sea, analyzing  $\delta^{13}\text{C}$  of topsoil (0-5 cm)  
20 samples, freshly deposited sediment (allochthonous source), and of above- and belowground  
21 biomass of vegetation (autochthonous sources). We tested for effects of geomorphological  
22 factors, including elevation and the distance to sediment sources, and of livestock grazing, as  
23 the most important land-use form, on the relative contributions of allochthonous versus  
24 autochthonous sources to the topsoil OC stock. A negative effect of distance to the creek on  
25 the relative contribution of AlIOC was found at only one of the two salt marshes, probably

26 due to differences in micro-topography between the two salt marshes. Additionally, the  
27 relative contribution of AlloC increased with increasing distance to the marsh edge in areas  
28 without livestock-grazing, while it decreased in grazed areas. Our findings demonstrate that  
29 spatial factors such as surface elevation and distance to a sediment source, which have been  
30 found to determine the spatial patterns of sediment deposition, also are important factors  
31 determining the relative contribution of AlloC to topsoil OC stocks of salt marshes.  
32 Furthermore, we provide first evidence that livestock-grazing can reduce the relative  
33 contribution of AlloC to the soil OC stock. These findings thereby yield important  
34 implications for C crediting and land-use management.

35

36 **KEY WORDS**

37 stable isotopes, carbon sequestration, livestock grazing, habitat management, Blue carbon,  
38 allochthonous, autochthonous, tidal wetland, North Sea

## 39 INTRODUCTION

40 Salt marshes and other tidal wetlands play an important role in climate change mitigation  
41 because they are more efficient at storing soil organic carbon (OC) than most terrestrial  
42 ecosystems (Chmura et al. 2003, Mcleod et al. 2011). The outstanding capacity of tidal  
43 wetlands for long-term carbon (C) sequestration has often been attributed to three main  
44 factors, namely high rates of OC input, reduced rates of decomposition, and constant burial of  
45 OC with rising sea level (Bridgham et al. 2006, Mcleod et al. 2011). Tidal wetlands are  
46 characterized by high rates of autochthonous net primary production, and they are effective in  
47 trapping OC from allochthonous marine or riverine sources (Duarte et al. 2013). Moreover,  
48 reduced conditions of wetland soils can inhibit microbial metabolism and slow down the  
49 decay of organic matter (OM), such that OC can accumulate and remain stable over centuries  
50 and millennia in many tidal wetlands (Kirwan & Megonigal 2013). As tidal wetland soils  
51 accrete vertically with rising sea level, they do not become C saturated like most terrestrial  
52 soils. Rates of C sequestration can therefore be maintained over long time scales, potentially  
53 millennia, as evident by deep, C-rich deposits found in tidal-wetland ecosystems worldwide  
54 (Mcleod et al. 2011).

55 The recognition of the important role these coastal ecosystems play in the global C  
56 cycle has led to the concept of ‘Blue C’ and a strong interest to include these ecosystems in C  
57 crediting programs (Herr et al. 2017). For instance, protection, restoration, or construction of  
58 Blue C ecosystems could be credited as greenhouse-gas-offset activities in the context of  
59 climate-change policy (Callaway et al. 2012, Needelman et al. 2018). Such measures,  
60 however, need to be based on a thorough understanding of the processes driving C  
61 sequestration in these ecosystems.

62 The origin of OC in tidal wetland soils, i.e. allochthonous OC (AlIOC) vs.  
63 autochthonous OC (AutOC), can have important implications for C crediting and greenhouse-

64 gas-offset activities, because the alternative fate of AlloC, i.e. if it had not accumulated in the  
65 tidal wetland, is unclear. For instance, C credits for the sequestration of AlloC are only due  
66 in protection, restoration, or construction projects if it would have returned to the atmosphere  
67 in the project baseline scenario (Needelman et al. 2018). It is well established that the relative  
68 contribution of AlloC vs. AutoC to soil OC stocks can vary considerably across sites and  
69 regions (Middelburg et al. 1997, Bouillon et al. 2003). Furthermore, based on studies  
70 assessing patterns of C-stable isotope signatures in soils or sedimentary processes within  
71 sites, it can be expected that the geomorphology of tidal wetlands and land use play key roles  
72 in the relative importance of AlloC vs. AutoC (Ember et al. 1987, Chen et al. 2015,  
73 Kelleway et al. 2017, Mueller et al. 2017). Yet, estimates of the relative contributions of  
74 AlloC to soil OC stocks derived from such assessments have rarely been conducted.

75 Numerous studies investigated which geomorphological factors affect the spatial  
76 pattern of sediment deposition in tidal wetlands, which is the main pathway for AlloC input.  
77 Those factors include surface elevation and distance to sediment sources (i.e. distance to the  
78 creek, distance to the marsh edge) (Fagherazzi et al. 2012). In general, lower elevations and  
79 closer proximity to the sediment source result in higher sediment deposition (Esselink et al.  
80 1998, Temmerman et al. 2003, Chmura & Hung 2004). Additionally, vegetation height, stem  
81 density, and other plant traits are factors known to increase sediment deposition (Morris et al.  
82 2002, Fagherazzi et al. 2012).

83 Live-stock grazing is a common form of land use or habitat management throughout  
84 European salt marshes, but also in other regions, e.g. China and South America (Bakker et al.  
85 2002, Di Bella et al. 2014, Yang et al. 2017). A large number of studies assessed the effects  
86 of livestock grazing on soil OC stocks, mineralization, and sequestration in tidal wetlands and  
87 produced a wide range of outcomes (Yu & Chmura 2009, Olsen et al. 2011, Elschot et al.  
88 2015, Davidson et al. 2017, Mueller et al. 2017). Because livestock grazing exerts strong

89 control on vegetation dynamics in tidal wetlands, effects on the contribution of AlloC inputs  
90 to soil OC stocks can be expected. Specifically, livestock grazing reduces vegetation height  
91 (Elschot et al. 2013), and therefore sediment deposition rate might also decrease (Nolte et al.  
92 2015), likely resulting in lower AlloC inputs. Grazing also affects biomass production  
93 (Morris & Jensen 1998, Di Bella et al. 2014), which is the source of AutoC. Both reduction  
94 in aboveground biomass production (Morris & Jensen 1998) and increases in belowground  
95 biomass production (Elschot et al. 2015) under livestock grazing have been reported. In a  
96 previous study we hypothesized that the contribution of AlloC could be reduced under high  
97 grazing pressures due decreased sediment trapping by shorter vegetation (Mueller et al.  
98 2017). However, a systematic assessment and an understanding of livestock-grazing effects  
99 on the relative contribution of AlloC versus AutoC to soil OC stocks in tidal wetlands are  
100 yet missing.

101         The aim of the present study is to identify important spatial factors controlling the  
102 relative contributions of AlloC to topsoil (0-5 cm) OC stocks in two salt marshes at the  
103 Wadden Sea mainland coast of Germany.  $\delta^{13}\text{C}$  analyses were used to assess the origin of OC,  
104 as commonly used in coastal environments (e.g. Thornton & McManus 1994, Kemp et al.  
105 2010, Saintilan et al. 2013). We expect that the relative contribution of AlloC to the soil OC  
106 pool is driven by geomorphological factors and additionally mediated by livestock grazing.  
107 Specifically, we hypothesize (1) that a higher relative contribution of AlloC would be found  
108 in lower elevations within the tidal frame. We hypothesize (2) that the AlloC contribution  
109 would decrease with distance to potential sediment sources, namely the distance to creek and  
110 the distance to marsh edge. Lastly, we hypothesize (3) that livestock grazing decreases the  
111 relative contribution of AlloC.

112

113

114 **MATERIALS AND METHODS**

115 *Study sites and sampling design*

116 The study was conducted from Dec 2014 to Sep 2015 in two salt marshes at the mainland  
117 coast of the Schleswig-Holstein Wadden Sea National Park, Germany (Fig. 1 A). The salt  
118 marshes within the National Park cover an area of ~130 km<sup>2</sup>, approximately half of which is  
119 used for livestock grazing (Stock et al. 2005, Esselink et al. 2017). Both study sites,  
120 Dieksanderkoog (DSK) and Sönke-Nissen-Koog (SNK), are minerogenic, shallow  
121 depositional salt marshes, exposed to tidal amplitudes of 3.0-3.4 m at a diurnal frequency.  
122 Soils in both sites have OM contents <15% and are dominated by grain-sizes <63 µm (>70%  
123 silts and clays)(Mueller et al. 2019). Flooding of the extensive high-marsh platforms, that this  
124 study is restricted to, only occurs during storm events predominantly in winter (Müller et al.  
125 2013a, Butzeck et al. 2015). In both sites, the presence of a rectangular network of creeks,  
126 ditches and levees reveal the anthropogenic origin of the salt marsh during land reclamation  
127 in the beginning of the 20<sup>th</sup> century (Müller et al. 2013b). The DSK (53°58'N, 8°53'E) is  
128 located in the southern part of the National Park at the mouth of the Elbe Estuary, while the  
129 SNK (54°38'N, 8°50'E) is situated in the north, ca. 35 km south of the Danish border (Fig. 1).  
130 Both sites were entirely grazed by sheep until 1988 and 1992 (SNK and DSK, respectively),  
131 when grazing was abandoned and only maintained at high stocking densities of >10 sheep ha<sup>-1</sup>  
132 until today in distinct areas of the two sites for experimental purposes (Stock et al. 2005,  
133 Mueller et al. 2017). The grazed treatment is dominated by *Festuca rubra*, *Elymus athericus*,  
134 and *Artemisia maritima* at DSK, and by *Puccinellia maritima*, *Festuca rubra*, and *Elymus*  
135 *athericus* at SNK. The ungrazed treatments are dominated by *Elymus athericus* at both sites.

136 Grazed and ungrazed treatments are located next to each other and separated by a  
137 straight main creek. In each treatment, sampling points were placed along short and long  
138 transects (Fig 1. B). The long transects covered a distance of 620 m (SNK) and 730 m (DSK)

139 along the main creek and consisted of five sampling points, starting at the seaward edge of  
140 what is considered here as the anthropogenic high-marsh platform (distance to marsh edge =  
141 0 m). The long transects kept an almost constant distance to the main creek of 54 m at SNK  
142 and 62 m at DSK. Short transects ran perpendicularly to the main creek and consisted of five  
143 points with different distances to the main creek (3 - 104 m at SNK; 2 - 107 m at DSK). The  
144 central points of the short transects were also part of the long transects (Fig.1). This sampling  
145 design resulted in N = 13 sampling points per treatment and a total of N = 52 sampling points  
146 for both marsh sites. Surface elevation relative to the German ordnance datum (NHN =  
147 Normalhöhenull) of sampling points was measured using a laser leveling device (Trimble,  
148 Sunnyvale, California, USA) and a nearby fixed benchmark with known elevation.

149

#### 150 *Sample collection*

151 We used C-stable isotope analysis to distinguish between AlloC and AutoC. To assess the  
152  $\delta^{13}\text{C}$  of the AlloC source, freshly deposited sediments were collected using circular sediment  
153 traps (Temmerman et al. 2003, Nolte et al. 2013a, 2019). The sediment traps were built of  
154 plastic plates with an internal diameter of 19 cm and a rim of 3 cm, and were equipped with a  
155 floatable lid to protect the deposited sediment in the trap from disturbances (Temmerman et  
156 al. 2003). Traps were fixed to the marsh surface with a plastic stick (Butzeck et al. 2015).  
157 Sediment traps were sampled within 24 h after two storm events during winter 2014/15. The  
158 high-marsh platform is only flooded during storm-induced high tides, so that sediment and  
159 AlloC deposition is restricted to these events (Müller et al. 2013a, Butzeck et al. 2015). As  
160 we assumed the  $\delta^{13}\text{C}$  of the freshly deposited sediment to be similar throughout the marsh,  
161 we only deployed sediment traps at five sampling points along the long transect and at two  
162 sampling points nearest to the creek in the two short transects in each treatment and site (Fig.  
163 1), resulting in the total number of 28 sediment traps. In the following, we will only refer to

164 the data of one sampling event in Dec 2014, when the majority of traps (27 out of 28)  
165 contained deposited material, after ensuring that no significant differences in C-stable isotope  
166 signatures exist between sampling events.

167 Above- and belowground plant biomass, as AutOC source, was sampled at all  
168 sampling points at the end of the growing season 2015. Aboveground biomass was harvested  
169 at each sampling position in an area of 100 cm<sup>2</sup>. Belowground biomass of the uppermost (5  
170 cm) soil layer was collected using a soil corer (Ø 2.5 cm). Dual cores were taken at each  
171 sampling position and samples were subsequently pooled. All samples were stored at -20°C  
172 until processing for further analysis.

173 To assess the  $\delta^{13}\text{C}$  of soil OC, soil samples of the uppermost 5 cm soil layer were  
174 collected at all sampling positions using a soil corer (Ø 2.5 cm) in Dec 2014. Our study  
175 investigated the origin of OC only in the uppermost 5 cm of the soil, because belowground  
176 biomass is usually concentrated at this depth in mainland high marshes of the Wadden Sea  
177 (Bartholdy et al. 2014, Redelstein et al. 2018). Additionally, down-core <sup>13</sup>C fractionation  
178 through repeated microbial cycling of OC and preferential substrate utilization make  
179 conclusions concerning the OC origin increasingly difficult with soil depth (Mueller et al.  
180 2019). Samples were transferred to the laboratory and stored at -20°C until processing.

181

### 182 *Sample processing and analysis*

183 Deposited sediment and soil samples were dried at 60°C until no further weight loss was  
184 observed. Plant materials and potential other coarse organic debris was removed by passing  
185 the sample through a 2-mm sieve. Samples were ground and homogenized using pestle and  
186 mortar. Sub-samples (1 g) were treated with 10% hydrochloric acid to remove carbonates and  
187 again dried at 60°C. Biomass samples were cleaned using tap water and given a final rinse  
188 with deionized water before drying at 60°C. Dry biomass samples were ground and



189 homogenized in a ball mill (Retsch, Haan, Germany).  $\delta^{13}\text{C}$  of dried and homogenized bulk  
190 soil OM, deposited sediment OM, and fresh above- and belowground biomass were  
191 determined using an isotope ratio mass spectrometer (Nu Horizon, Nu Instruments,  
192 Wrexham, UK). Samples were analyzed in tandem with both laboratory (BBOT 2,5-Bis-(5-  
193 tert-butyl-2-benzo-oxazol-2-yl) thiophene) and international standards (IAEA-600 Caffeine).  
194 The precision of the isotopic analysis was  $< 0.1\%$ . The isotopic compositions of all samples  
195 are reported using the standard  $\delta$ -notation versus Vienna PeeDee Belemnite (VPDB) as parts  
196 per thousand (‰).

197 The relative proportions of the AlIOC and AuOC were determined by applying a  
198 two-end-member mixing model based on the stable isotope composition of OC (e.g. Hedges  
199 et al. 1988, Belicka and Harvey 2009):

$$200 \quad f_{\text{Al}}(\%) = \frac{\delta^{13}\text{C}_{\text{Soil}} - \delta^{13}\text{C}_{\text{Au}}}{\delta^{13}\text{C}_{\text{Al}} - \delta^{13}\text{C}_{\text{Au}}} \quad (1)$$

201 where:  $f_{\text{Al}}(\%)$  is the percentage contribution of the Al OC in soil;  $\delta^{13}\text{C}_{\text{Soil}}$  is  $\delta^{13}\text{C}$  measured in  
202 the soil;  $\delta^{13}\text{C}_{\text{Au}}$  is  $\delta^{13}\text{C}$  of the AuOC source (plant biomass); and  $\delta^{13}\text{C}_{\text{Al}}$  is  $\delta^{13}\text{C}$  of Al OC.

203 The calculated contributions of AlIOC vs. AuOC sources depend on the type of plant  
204 biomass (i.e. above- vs. belowground biomass) considered in the mixing-model calculations.  
205 As the contributions of above- vs. belowground plant biomass to the soil OM pool were  
206 unknown, we calculated  $f_{\text{Al}}(\%)$  under the assumption of three different end-member terms  
207 for Au  $\delta^{13}\text{C}$ : (1)  $\delta^{13}\text{C}$  of the aboveground biomass (Model A), (2)  $\delta^{13}\text{C}$  of the belowground  
208 biomass (Model B), and (3) the calculated  $\delta^{13}\text{C}$  of a 1:1 mixed contribution of above- and  
209 belowground biomass (Model M).

210

211 *Statistical analyses*

212 Two-way ANOVAs were used to test for differences in the isotopic composition of sediment  
213 and soil between sites and treatments. To test if  $\delta^{13}\text{C}$  of ALIOC in deposited sediment differs  
214 in response to distance to the marsh edge, linear regressions were used separately for each  
215 site. To test if  $\delta^{13}\text{C}$  of ALIOC deposited in sediment traps positioned close to the creek differ  
216 from those positioned far from the creek, one-way ANOVAs were used separately for each  
217 site. Three-way ANOVA was used to test for the effects of site, treatment, and type of plant  
218 tissue (above- vs. belowground biomass) on plant  $\delta^{13}\text{C}$ , and subsequent two-way ANOVAs  
219 were conducted to test for the effects of treatment and site on the  $\delta^{13}\text{C}$  of above- and  
220 belowground biomass separately. Tukey's HSD tests were used for pairwise comparisons.  
221 Normal distribution of residuals (as checked visually) and equal sample sizes across groups  
222 assured robustness for parametric testing (McGuinness 2002).

223 ANCOVA was conducted to test for effects of site, treatment, distance to marsh edge,  
224 distance to creek, and relative elevation (elevation in comparison to mean surface elevation at  
225 each site) on the relative contribution of ALIOC to the soil. Only pairwise interactions of  
226 factors were considered in the models. As model simplification is an essential part of  
227 ANCOVA designs, the most insignificant parameters were dropped step by step, and as few  
228 parameters as possible were kept in the model (Crawley 2005). The more complex models  
229 were retained only if the p-value (ANOVA based) comparing the two models was  $< 0.05$   
230 (Crawley 2005). To exclude that identified effects on the contribution of ALIOC are artifacts  
231 driven by spatial variability in the plant isotopic signature, we additionally conducted the  
232 same ANCOVA procedures to test for effects of spatial factors and grazing on the  
233 autochthonous  $\delta^{13}\text{C}$  end members (i.e. aboveground, belowground, mixed). All analyses were  
234 carried out using the software package 'R' version 3.3.1 (The R Foundation for Statistical  
235 Computing 2016).

236

237 **RESULTS**

238  *$\delta^{13}\text{C}$  of AlloC, AutoC, and soil samples*

239  $\delta^{13}\text{C}$  of AlloC in deposited sediment differed significantly between sites ( $F_{1,23} = 88.6$ ,  $p <$   
240  $0.0001$ ). AlloC was more depleted in  $\delta^{13}\text{C}$  at DSK ( $-24.22\% \pm 0.35\%$ ) vs. SNK ( $-23.10\% \pm$   
241  $0.25\%$ ) (Table 1). Within sites, no significant differences in  $\delta^{13}\text{C}$  of the AlloC were detected  
242 between grazed and ungrazed treatments (Table 1). There was no significant interaction  
243 effect of site and treatment on the  $\delta^{13}\text{C}$  of AlloC ( $F_{1,23} = 0.7$ ,  $p > 0.4$ ).  $\delta^{13}\text{C}$  of AlloC did not  
244 differ in response to distance to the marsh edge (in each site:  $R^2 < 0.25$ ,  $p > 0.1$ ).  $\delta^{13}\text{C}$  of  
245 AlloC in traps positioned close to the creek did not differ from those positioned far from the  
246 creek (DSK:  $F_{1,6} = 2.2$ ,  $p > 0.1$ ; SNK:  $F_{1,6} = 1.9$ ,  $p > 0.2$ ). Therefore, the mean  $\delta^{13}\text{C}$  of each  
247 site was chosen for  $\delta^{13}\text{C}_{\text{Al}}$  in Equation 1.

248 The  $\delta^{13}\text{C}$  values of AutoC differed between sites ( $F_{1,93} = 17.1$ ,  $p < 0.0001$ ), treatments  
249 ( $F_{1,93} = 11.6$ ,  $p < 0.001$ ), and type of plant tissue, i.e. above- vs. belowground biomass ( $F_{1,93} =$   
250  $5.9$ ,  $p < 0.05$ ). There was a significant interaction effect of treatment and type of plant tissue  
251 on  $\delta^{13}\text{C}$  of AutoC ( $F_{1,93} = 4.0$ ,  $p < 0.05$ ). Subsequent two-way ANOVAs showed the effects  
252 of site and treatment on  $\delta^{13}\text{C}$  of aboveground biomass, belowground biomass and the 1:1  
253 mixed above- and belowground biomass (Table 1). The aboveground biomass  $\delta^{13}\text{C}$  values  
254 were significantly different between sites ( $F_{1,47} = 11.5$ ,  $p < 0.005$ ), with  $\delta^{13}\text{C}$  being more  
255 depleted in DSK ( $-27.72\% \pm 0.74\%$ ) vs. SNK ( $-26.65\% \pm 1.38\%$ ) (Table 1). Treatment had  
256 no effect on  $\delta^{13}\text{C}$  of aboveground biomass, and there was also no interaction of site and  
257 treatment effect on the  $\delta^{13}\text{C}$  of aboveground biomass (Table 1).  $\delta^{13}\text{C}$  values of belowground  
258 biomass were affected significantly by site ( $F_{1,46} = 6.0$ ,  $p < 0.05$ ), being more depleted in  
259 DSK ( $-27.00\% \pm 1.05\%$ ) vs. SNK ( $-26.32\% \pm 1.24\%$ ) (Table 1).  $\delta^{13}\text{C}$  of belowground  
260 biomass were constantly more depleted under grazing ( $F_{1,46} = 16.5$ ,  $p < 0.001$ ) at both sites.  
261 The interaction of site and treatment had no effect on belowground biomass  $\delta^{13}\text{C}$ . Similarly,

262 the 1:1 mixed biomass  $\delta^{13}\text{C}$  was affected significantly by site ( $F_{1,47} = 15.8$ ,  $p < 0.0005$ ) and  
263 treatment ( $F_{1,47} = 9.7$ ,  $p < 0.01$ ), but not by the interaction of site and treatment. These  
264 differences in  $\delta^{13}\text{C}$  between above- and belowground biomass, treatments, and sites did not  
265 allow using any sort of mean  $\delta^{13}\text{C}$  value for the autochthonous end-member term in mixing  
266 models. Instead, we used the specific  $\delta^{13}\text{C}$  values of above-, belowground, and mixed  
267 biomass of each sampling point for the respective mixing-model calculations (Equation 1).

268 Soil OC had  $\delta^{13}\text{C}$  values varying from  $-28.00\text{‰}$  to  $-23.24\text{‰}$  and differed significantly  
269 by site ( $F_{1,48} = 34.7$ ,  $p < 0.001$ ).  $\delta^{13}\text{C}$  of DSK soil OC ( $-26.75\text{‰} \pm 0.35 \text{‰}$ ) was more  
270 depleted than SNK soil OC ( $-25.61\text{‰} \pm 0.85 \text{‰}$ ). Treatments had an effect on soil OC  $\delta^{13}\text{C}$ ,  
271 ( $F_{1,48} = 10.4$ ,  $p < 0.01$ ), with more depleted  $^{13}\text{C}$  under grazing ( $-26.49\text{‰} \pm 0.95 \text{‰}$ ) compared  
272 to ungrazed treatments ( $-25.87\text{‰} \pm 0.82 \text{‰}$ ) (Table 1). There was no significant interaction  
273 effect between site and treatment on soil OC  $\delta^{13}\text{C}$  values (Table 1).

274

#### 275 *Relative contribution of AlloC in salt-marsh topsoils*

276 The three mixing models in which different AutoC end-member assumptions (only  
277 aboveground plant biomass (A), only belowground plant biomass (B), or 1:1 mixed plant  
278 biomass (M)) were used to calculate contributions of AlloC to the salt-marsh topsoil OC  
279 yielded different results (Fig. 2). Depending on the model used, AlloC contributions in  
280 grazed treatments ranged from  $17.72 \pm 3.85$  to  $23.64 \pm 4.84$  (%). In comparison, a higher  
281 range was found in ungrazed treatments (from  $11.79 \pm 4.95$  to  $38.99 \pm 5.31$ (%)). Here, we  
282 found that a mixing model using the aboveground  $\delta^{13}\text{C}$  resulted in much higher contributions  
283 of AlloC to the topsoil OC stock compared to using the belowground  $\delta^{13}\text{C}$  (Fig. 2).

284 The ANCOVA results for the three mixing models, which were based on different  
285 autochthonous OC end-member assumptions (see above) also differed (Table 2). For further  
286 interpretation, we therefore considered only those effects as robust which were found to be

287 significant in at least two models (Table 2). The interaction effect between site and distance to  
288 the creek was found to be significant in Model A and Model M (Table 2). In DSK, we found  
289 the highest ALIOC contributions close to the creek. With increasing distance to the creek,  
290 there was a steep drop until a more or less stable value was reached (Fig. 3 A). In contrast,  
291 the contribution of ALIOC in SNK showed a different pattern and remained constant with  
292 increasing distance to the creek (Fig. 3 A). The interaction between treatment and distance to  
293 the marsh edge also had significant effects on the contribution of ALIOC in Model A and  
294 Model M (Table 2). Close to the marsh edge (0 to 300 m), the percentages of ALIOC  
295 contribution were quite similar between treatments (Fig. 4 A). In the area of greater distance  
296 to the marsh edge, however, we found opposite patterns for the two treatments. Here, the  
297 percentage of ALIOC increased with increasing distance to the marsh edge in ungrazed  
298 treatments, while it decreased in grazed treatments (Fig. 4 A).

299 ANCOVAs assessing the effects of geomorphological factors and grazing on the  
300 autochthonous  $\delta^{13}\text{C}$  end members (i.e. aboveground, belowground, mixed biomass) showed  
301 neither interaction effects of distance to the marsh edge and treatment, nor of site and  
302 distance to the creek, excluding that the identified effects on the ALIOC contribution to the  
303 soil are artefacts driven by spatial variability in plant  $\delta^{13}\text{C}$  (Table A1).

304

305 **DISCUSSION**

306 The present study assessed the relative contributions of AlIOC to topsoil OC stocks in two  
307 salt-marsh sites of the European Wadden Sea. A large number of studies on the importance of  
308 AlIOC in marsh soils have been performed (e.g. Boschker et al. 1999, Tanner et al. 2010,  
309 Saintilan et al. 2013, Chen et al. 2015, Van de Broek et al. 2018). However, estimates of the  
310 relative contribution of AlIOC vs. AutoOC to OC stocks have rarely been provided. This is  
311 possibly due to large uncertainties associated with the application of two-end-member mixing  
312 models, which were also encountered in the present study (see discussed below in  
313 ‘Methodological considerations’). For instance, mean AlIOC contributions to topsoil OC  
314 stocks of the high-marsh platforms ranged between 18 and 31% of total soil OC depending  
315 on autochthonous  $\delta^{13}\text{C}$  end-member assumptions. The central aim of our investigation was  
316 therefore not to provide accurate budgets of AlIOC vs. AutoOC contributions, but to identify  
317 important spatial factors influencing the balance of the two sources. Specifically, we  
318 hypothesized that surface elevation, distance to sediment sources, and livestock grazing  
319 negatively affect the relative contribution of AlIOC to the soil OC stock. Our findings can  
320 provide partial support for these hypotheses because complex interactions between the  
321 assessed factors existed and seem to exert important control on the distribution pattern of  
322 AlIOC.

323         Relative elevation as a single factor did not affect the relative contribution of AlIOC  
324 to the topsoil. We did, however, identify a negative effect of distance to the creek on the  
325 relative contribution of AlIOC to the topsoil. This effect was only found at DSK and not at  
326 SNK, which we attribute to differences in relative elevation patterns within sites, and more  
327 specifically, in patterns of levee formation along the main creek (Fig. 3 B). At SNK,  
328 sampling points closest to the main creek are characterized by the highest relative elevation.  
329 We argue that this natural levee along the creek restricts inundation of the marsh platform

330 until tidal heights exceed the levee elevation, resulting in similar inundation frequencies and  
331 AllOC inputs across the marsh platform (Fig. 3). At DSK, by contrast, sampling points  
332 closest to the creek are lower than subsequent points with larger distance to the creek, leading  
333 to highest AllOC inputs close to the creek (Fig. 3). As previously hypothesized (Haines 1976,  
334 Ember et al. 1987, Middelburg et al. 1997), our findings provide evidence that  
335 geomorphological factors such as surface elevation and distance to the creek, which  
336 determine the spatial patterns of sediment deposition (Esselink et al. 1998, Temmerman et al.  
337 2003, Chmura & Hung 2004), also are important factors determining the relative contribution  
338 of AllOC to the topsoil OC stocks of salt marshes. Yet, our data do not allow assessing if  
339 variability in AuOC input contributed to this finding. For instance, biomass production in salt  
340 marshes is also strongly controlled by geomorphological factors, such as elevation, affecting  
341 both hydrology and nutrient supply (Morris et al. 2002, Kirwan & Guntenspergen 2012).

342        Besides distance to the creek, distance to the marsh edge is a second parameter  
343 describing the proximity of a given point to a potential sediment and thus AllOC source. We  
344 indeed demonstrate a significant effect of distance to the marsh edge. This effect, however,  
345 differed significantly between grazed and ungrazed treatments (Table 2). Specifically, our data  
346 show increasing relative contributions of AllOC with distance to the marsh edge in ungrazed  
347 treatments, whereas the opposite pattern was found in the grazed treatments (Fig. 4 A). We  
348 argue that the unexpected increase of the AllOC contribution with distance to the marsh edge  
349 in ungrazed treatments can be explained by elevational patterns of the marsh platform that are  
350 typically found across Wadden Sea salt marshes and elsewhere. Landward decreases in  
351 elevation often result from ditching, diking, and grazing processes in salt marshes (Stock 2011,  
352 Müller-Navarra et al. 2016, Esselink et al. 2017) and were also found in the two sites  
353 investigated here (Fig. 4 B). As a consequence, landward areas of the marsh platform are

354 flooded first and potentially more frequently, thus explaining higher relative contributions of  
355 AlIOC in the landward ungrazed treatments.

356         The mechanisms causing the opposite effect of distance to marsh edge on the relative  
357 contribution of AlIOC to the topsoil in grazed treatments is unknown. However, we  
358 hypothesize that they relate to pronounced differences in grazing pressure between landward  
359 and seaward locations (Kiehl et al. 2001, Nolte et al. 2013b, Mueller et al. 2017). Because  
360 livestock tends to remain close to the freshwater source near the seawall (Kiehl et al. 2001),  
361 grazing pressure increases with distance to the marsh edge, which is also reflected in decreasing  
362 vegetation height and increasing soil bulk density with increasing distance to the marsh edge  
363 in our study sites (Nolte 2014, Mueller et al. 2017). At least three non-exclusive mechanisms,  
364 supported by literature, could explain lower relative AlIOC contributions at high grazing  
365 pressures. First, grazing-induced reductions in vegetation height could lead to lower plant-  
366 mediated sediment trapping (Morris et al. 2002, Fagherazzi et al. 2012) and thus AlIOC input  
367 in (Yang et al. 2008, Mueller et al. 2017). Second, livestock grazing is known to increase  
368 belowground AutOC inputs through higher root production (Elschot et al. 2015), which would  
369 translate into lower relative contributions of AlIOC. Third, grazing could lead to a more  
370 effective preservation of AutOC in the soil, thereby decreasing the relative contribution of  
371 AlIOC. That is, grazing-induced soil compaction is known to lower soil oxygen availability  
372 and thus reduce microbial activity (Elschot et al. 2015, Mueller et al. 2017). However, recent  
373 studies from the North Sea region suggest AlIOC inputs are highly resistant to decay, being  
374 old-aged materials that stabilized in the marine environment before entering the marsh OC pool  
375 (Van de Broek et al. 2018, Mueller et al. 2019). It therefore seems likely that primarily the  
376 decay of autochthonous OM is susceptible to grazing effects on oxygen availability and  
377 microbial activity.



378           Although our findings cannot identify the mechanism(s) responsible for the observed  
379 grazing effect, they yield important implications for ecosystem management and C crediting.  
380 Recent meta-analyses and large-scale studies suggest livestock grazing has no effect on soil  
381 OC stocks of salt marshes (Davidson et al. 2017, Ford et al. 2019). Our study provides first  
382 evidence of grazing effects on the balance between allochthonous and autochthonous soil  
383 OC. Because it is uncertain if C credits are due for the sequestration of ALIOC, livestock  
384 grazing may actually increase the C value of salt-marsh area, by reducing the relative  
385 contribution of ALIOC to the soil OC stock. However, there are important caveats concerning  
386 these implications; our study did not quantify absolute rates of ALIOC vs. AutOC input and  
387 accumulation, but only reports on the relative contributions of the two sources to the soil OC  
388 stock. Yet, the majority of studies concerned with grazing effects on salt-marsh C dynamics  
389 are likewise restricted to OC stock assessments, but did not quantify rates of sequestration  
390 (Davidson et al. 2017, but see Elschot et al. 2015). In addition, recent studies provide  
391 evidence of a more effective preservation of allochthonous OM inputs in marsh soils than in  
392 situ produced OM, leading to an increasing relative contribution of ALIOC vs. AutOC with  
393 soil depth or time in NW European salt marshes (Van de Broek et al. 2018, Mueller et al.  
394 2019). Future research therefore needs to assess whether the here identified grazing effects on  
395 the balance between ALIOC and AutOC remain with increasing soil depth and are thus  
396 relevant for considerations on long-term C dynamics.

397

#### 398 *Methodological considerations*

399 Large differences were found in the estimates of ALIOC contribution when the three mixing  
400 model approaches based on different plant  $\delta^{13}\text{C}$  end-members assumptions were compared,  
401 particularly in the ungrazed treatments (Fig. 2). We therefore stress the importance to  
402 consider differences in the isotopic composition of different plant tissues in mixing-model

403 approaches used to calculate the relative contributions of AlloC vs. AutoC, which is  
404 surprisingly left unconsidered in many studies (but see Kelleway et al. 2018). In the present  
405 study, we found large differences in  $\delta^{13}\text{C}$  between above- and belowground biomass (Table  
406 1). Because the relative contribution of above- vs. belowground biomass to the top soil is  
407 unknown, also the absolute values presented here need to be considered with caution.  
408 Depending on the end-member assumptions used (i.e. Model A vs. B vs. M), calculated  
409 AlloC contributions to the topsoil can be as low as 0% in some sampling points (Fig. 4 A).  
410 This seems unrealistically low, and may point to the fact that the actual ratio of above- to  
411 belowground contributions to the soil OC pool diverge from the end-member assumption. For  
412 instance, it is reasonable to assume small aboveground and large belowground contributions  
413 under grazing, considering the negligible aboveground litter accumulation (Mueller et al.  
414 2017). Indeed, Model B (assuming no aboveground contributions) yields more realistic  
415 AlloC contributions of 4% for the sampling points yielding a 0% in Model M (Fig. 4 A).

416 Unless relative contributions of different plant tissues to the soil OC pool are known,  
417 simple two-end-member mixing models can only yield an approximation of AlloC vs.  
418 AutoC contributions to soil OC stocks. The main goal of this study was therefore not to  
419 calculate precise budgets of AlloC vs. AutoC inputs, but to identify important factors  
420 controlling their relative distributions. Here, we used three different assumptions for our  
421 autochthonous end members to assess the robustness of the results obtained. Above, we only  
422 discussed effects that were significant in at least two of the three models tested. Additionally,  
423 however, there were other significant effects that were only detected by one of the three  
424 models, which provide additional support for the factors identified to be important and  
425 discussed above. That is, negative effects of grazing, distance to the creek, and relative  
426 elevation on the relative contribution of AlloC to the top soil were additionally detected by  
427 Models A and M (Table 1). Furthermore, a significant interaction of grazing and relative

428 elevation was detected by Model B, supporting the interpretation that the interaction effect of  
429 grazing and distance to the marsh edge is mediated by differences in surface elevation.

430 In order to exclude artifacts caused by spatial variability in  $\delta^{13}\text{C}$  of plant biomass (i.e.  
431 our autochthonous  $\delta^{13}\text{C}$  end members), we also tested for effects of site, treatment, distance  
432 to the marsh edge, distance to the creek, and relative elevation on the autochthonous  $\delta^{13}\text{C}$  end  
433 members used. We found neither interaction effects of distance to marsh edge and treatment,  
434 nor of site and distance to creek on the autochthonous  $\delta^{13}\text{C}$  end member, excluding that the  
435 identified interaction effects on the AIOC contribution are artifacts driven by spatial  
436 variability in the plant  $\delta^{13}\text{C}$  (Table A1). We argue that such sensitivity analyses are crucial to  
437 assess the reliability of conclusions derived from stable-isotope mixing-model approaches.

438

#### 439 *Conclusions and perspective*

440 The present study partly supported previous research showing that more AIOC is found  
441 closer to the sediment sources and in lower elevations (Middelburg et al. 1997, Spohn et al.  
442 2013, Hansen et al. 2017, Yuan et al. 2017). However, while previous studies have shown  
443 that the contribution of AIOC to soil OC varies among different zones of tidal marshes  
444 (Spohn et al. 2013) or across sites and regions (Middelburg et al. 1997, Hansen et al. 2017,  
445 Yuan et al. 2017), our study is the first to evaluate geomorphology and land-use effects on the  
446 small scale variability in the relative contribution of AIOC to soil OC stocks. Our findings  
447 demonstrate that spatial factors such as surface elevation and distance to a sediment source,  
448 which have been found to determine the spatial patterns of sediment deposition (Esselink et  
449 al. 1998, Temmerman et al. 2003, Chmura & Hung 2004), also are important factors  
450 determining the relative contribution of AIOC to the topsoil OC stocks of salt marshes.  
451 Furthermore, we provide first evidence that livestock-grazing can reduce the relative  
452 contribution of AIOC to the soil OC stock. These findings could yield important implications

453 for C crediting and land-use management. Future research is required to quantify absolute  
454 rates of AlIOC vs. AutOC inputs and to assess whether the here identified effects on the  
455 balance between AlIOC and AutOC remain with increasing soil depth and are thus relevant  
456 for considerations on long-term C dynamics.

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477 sediment: management and ecosystem functions of Wadden Sea salt marshes).

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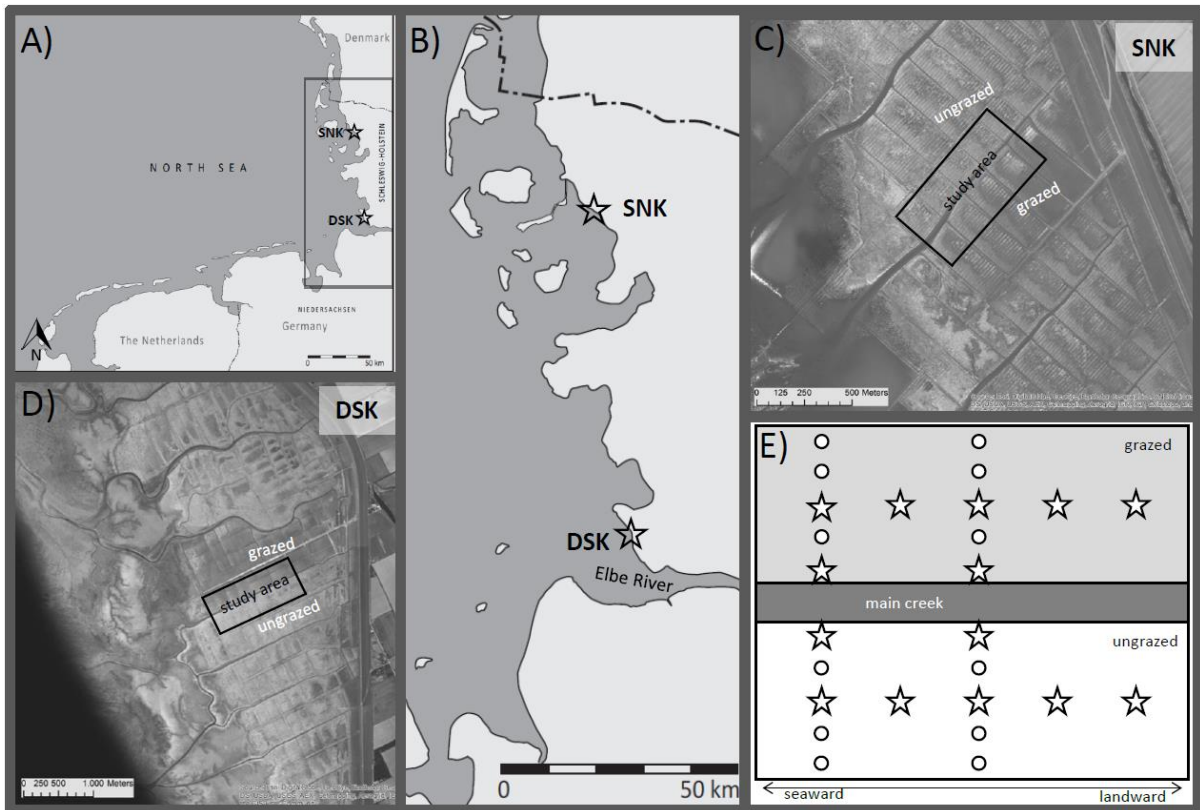
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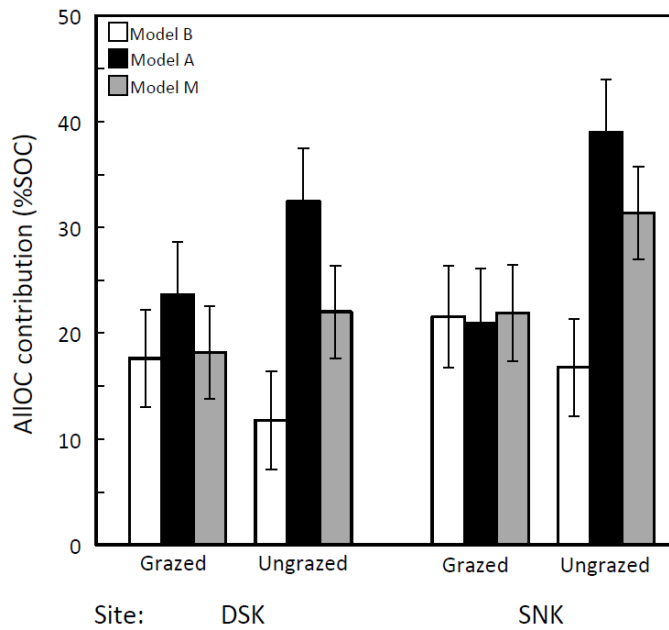
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663 **FIGURES**  
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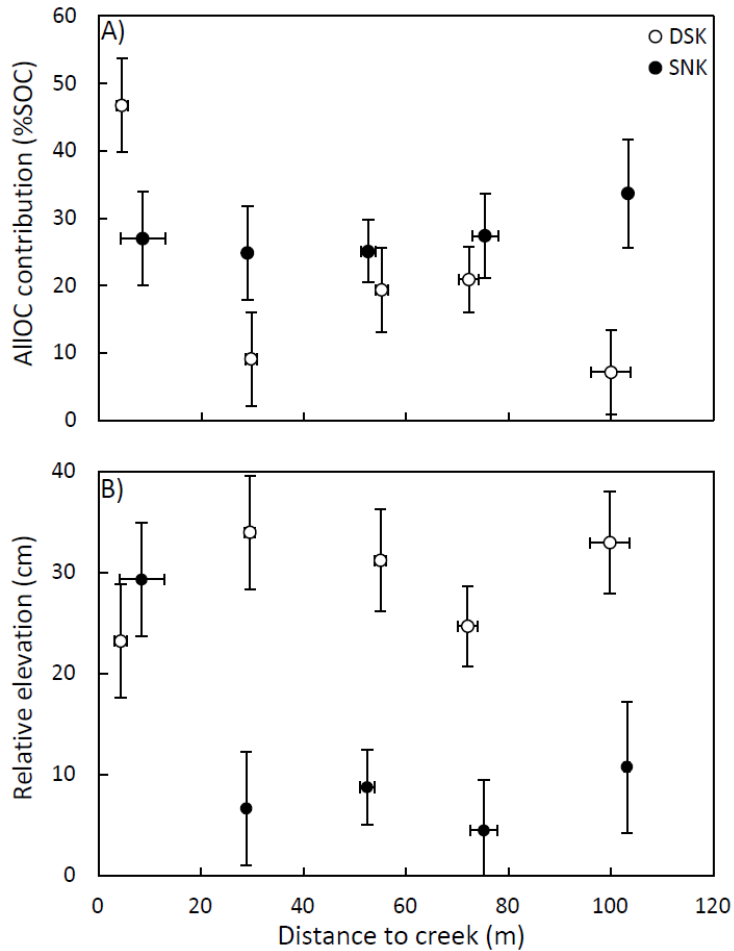


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 667 **Figure 1 (A +B)** Location of the study sites Sönke-Nissen-Koog (SNK) and Dieksanderkoog  
 668 (DSK) within the Wadden Sea area. **(C + D)** Aerial photos of the study sites. **(E)** Sampling  
 669 design: sampling points were organized along short transects (distance to the creek) and long  
 670 transects (distance to the edge of the anthropogenic high-marsh platform) in grazed vs.  
 671 ungrazed treatments. Soil and biomass samples were collected at all points ,while deposited  
 672 sediment samples were only collected at points marked with star symbols. Aerial photos source: Esri,  
 673 DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus CS USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the  
 674 GIS User Community.  
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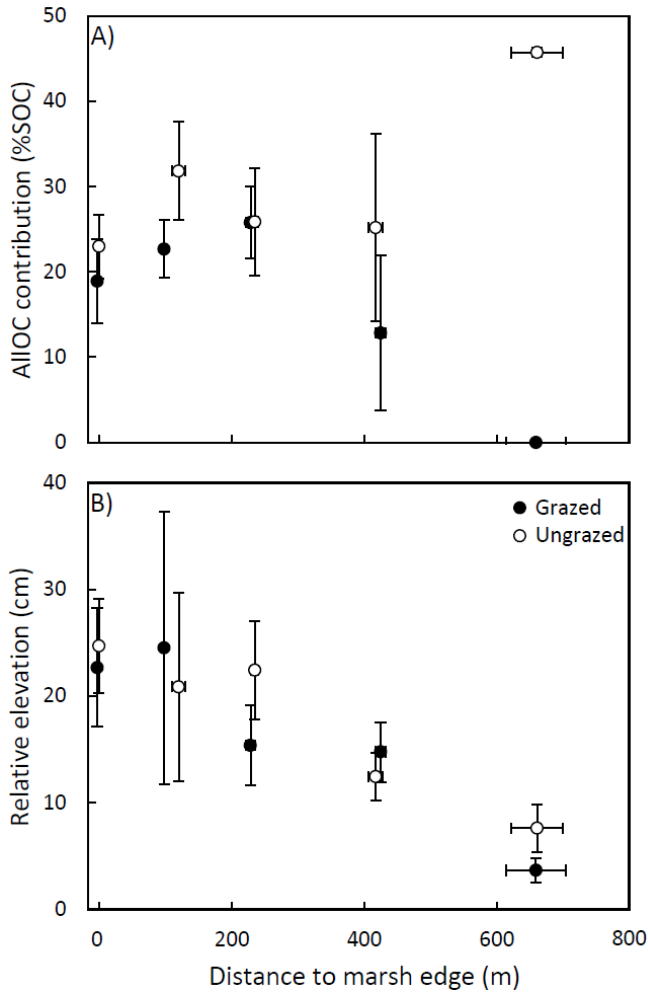
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678 **Figure 2** Allochthonous organic carbon (AlIOC) contribution in percent of soil organic  
 679 carbon (%SOC) in the topsoil of grazed and ungrazed treatments of two Wadden Sea salt-  
 680 marsh sites, Dieksanderkoog (DSK) and Sönke-Nissen-Koog (SNK). Values presented are  
 681 based on mixing-model calculations using three different end-member assumptions for the  
 682  $\delta^{13}\text{C}$  of autochthonous organic carbon: **Model B** ( $\delta^{13}\text{C}$  of the belowground biomass), **Model**  
 683 **A** ( $\delta^{13}\text{C}$  of the aboveground biomass), **Model M** (the calculated  $\delta^{13}\text{C}$  of a 1:1 mixed  
 684 contribution of aboveground and belowground biomass). Values are means  $\pm$  SE.



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**Figure 3** (A) Allochthonous organic carbon (AlIOC) contribution [percentage of soil organic carbon (%SOC)] in relation to distance to the creek in two Wadden Sea salt-marsh sites, Dieksanderkoog (DSK) and Sönke-Nissen-Koog (SNK). Values are based on Model M (i.e. the calculated  $\delta^{13}\text{C}$  of a 1:1 mixed contribution of aboveground and belowground biomass was used for the  $\delta^{13}\text{C}$  of autochthonous organic carbon in mixing-model calculations). (B) Relative elevation of the sampling points in relation to distance to the creek in DSK and SNK. Values are means  $\pm$  SE.



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697 **Figure 4** (A) Allochthonous organic carbon (ALIOC) contribution [percentage of soil organic  
 698 carbon (%SOC)] in relation to distance to the marsh edge in grazed vs. ungrazed treatments  
 699 of two Wadden Sea salt-marsh sites. (B) Relative elevation of the sampling points in relation  
 700 to distance to the marsh edge in. Values are means  $\pm$  SE, pooled by site.

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709 **TABLES**

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711 **Table 1**  $\delta^{13}\text{C}$  of allochthonous and autochthonous OC sources (AlIOC, AutOC) and topsoil  
 712 samples in grazed und ungrazed treatments of two Wadden Sea salt-marsh sites. Values are  
 713 means  $\pm$  SD given in  $\delta$ -notation versus Vienna PeeDee Belemnite (‰). Values not connected  
 714 by the same letter within one row are significantly different at  $p < 0.05$  based on Tukey's  
 715 HSD tests.

Sample material	Dieksanderkoog (DSK)		Sönke-Nissen-Koog (SNK)	
	Grazed	Ungrazed	Grazed	Ungrazed
Deposited sediment (AlIOC)	-24.29 <sup>a</sup> $\pm$ 0.42	-24.14 <sup>a</sup> $\pm$ 0.26	-23.08 <sup>b</sup> $\pm$ 0.20	-23.12 <sup>b</sup> $\pm$ 0.31
Plant biomass (AutOC)				
<i>Aboveground</i>	-27.81 <sup>a</sup> $\pm$ 0.74	-27.62 <sup>a,b</sup> $\pm$ 0.76	-26.86 <sup>a,b</sup> $\pm$ 1.16	-26.45 <sup>b</sup> $\pm$ 1.58
<i>Belowground</i>	-27.38 <sup>a</sup> $\pm$ 1.16	-26.60 <sup>a,b</sup> $\pm$ 0.77	-27.10 <sup>a</sup> $\pm$ 1.05	-25.60 <sup>b</sup> $\pm$ 0.95
<i>1:1 mixed</i>	-27.60 <sup>a</sup> $\pm$ 0.98	-27.11 <sup>a</sup> $\pm$ 0.91	-26.97 <sup>a</sup> $\pm$ 1.09	-26.03 <sup>b</sup> $\pm$ 1.35
Topsoil	-27.04 <sup>a</sup> $\pm$ 0.57	-26.46 <sup>a,b</sup> $\pm$ 0.59	-25.94 <sup>b</sup> $\pm$ 0.96	-25.28 <sup>b</sup> $\pm$ 0.58

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717 **Table 2** Results of ANCOVA analyses testing for effects of single and interacting factors on  
 718 the allochthonous organic carbon contribution (percentage of soil organic carbon) to the  
 719 topsoil of two Wadden Sea salt-marsh sites. ANCOVA analyses were run using three  
 720 different end-member assumptions for the  $\delta^{13}\text{C}$  of autochthonous organic carbon in mixing-  
 721 model calculations: **Model A** ( $\delta^{13}\text{C}$  of the aboveground biomass), **Model B** ( $\delta^{13}\text{C}$  of the  
 722 belowground biomass), **Model M** (the calculated  $\delta^{13}\text{C}$  of a 1:1 mixed contribution of  
 723 aboveground and belowground biomass). (-) indicates that factor dropped out of the  
 724 ANCOVA model, ns. = not significant.

Factors	Model A		Model B		Model M	
	<i>F value</i>	<i>p value</i>	<i>F value</i>	<i>p value</i>	<i>F value</i>	<i>p value</i>
Site	0.28	ns.	1.07	ns.	2.93	<0.10
Treatment	9.41	<0.01	1.58	ns.	2.82	0.10
Distance to marsh edge	0.01	ns.	0.03	ns.	0.08	ns.
Distance to creek	5.12	<0.05	0.46	ns.	2.93	<0.10
Relative elevation		-	3.07	<0.10	4.14	<0.05
Site x treatment		-		-		-
Site x dist. marsh edge		-	3.51	<0.10		-
Site x dist. creek	9.21	<0.01		-	4.27	<0.05
Site x rel. elevation		-		-		-
Treat. x dist. marsh edge	5.71	<0.05	3.42	<0.10	5.17	<0.05
Treat. x dist. creek		-		-		-
Treat. x rel. elevation		-	4.93	<0.05		-
Dist. marsh edge x dist. creek		-		-		-
Dist. marsh edge x rel. elevation		-		-		-
Dist. creek x rel. elevation		-		-		-

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727 **APPENDIX**

728 **Appendix Table A1** Results of ANCOVA analyses testing for effects of single and  
 729 interacting factors on the  $\delta^{13}\text{C}$  of aboveground plant biomass, belowground plant biomass,  
 730 and mixed biomass (1:1 aboveground : belowground), referring to the three end-member  
 731 assumptions used in mixing-model calculations. (-) indicates that factor dropped out of the  
 732 ANCOVA model, ns. = not significant.

Factors	$\delta^{13}\text{C}$ aboveground		$\delta^{13}\text{C}$ belowground		$\delta^{13}\text{C}$ mixed	
	<i>F value</i>	<i>p value</i>	<i>F value</i>	<i>p value</i>	<i>F value</i>	<i>p value</i>
Site	11.42	<b>&lt;0.01</b>	6.45	<b>&lt;0.05</b>	16.03	<b>&lt;0.001</b>
Treatment		-	17.76	<b>&lt;0.001</b>	12.07	<b>&lt;0.01</b>
Distance to marsh edge		-	0.22	ns.	2.57	ns.
Distance to creek		-		-		-
Relative elevation	1.65	ns.		-		-
Site x treatment		-		-		-
Site x dist. marsh edge		-	6.12	<b>&lt;0.05</b>		-
Site x dist. creek		-		-		-
Site x rel. elevation	3.86	<0.10		-	3.39	<0.10
Treat. x dist. marsh edge		-		-		-
Treat. x dist. creek		-		-		-
Treat. x rel. elevation		-		-		-
Dist. marsh edge x dist. creek		-		-		-
Dist. marsh edge x rel. elevation		-		-		-
Dist. creek x rel. elevation		-		-		-

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