Ungrazed salt marsh has well connected soil pores and less dense sediment compared with grazed salt marsh: CT scanning study

Amr Keshta, Ketil Koop-Jakobsen, Jürgen Titschack, Peter Mueller, Kai Jensen, Andrew Baldwin, Stefanie Nolte

PII: S0272-7714(20)30718-6
DOI: https://doi.org/10.1016/j.ecss.2020.106987
Reference: YECSS 106987

To appear in: Estuarine, Coastal and Shelf Science

Received Date: 29 March 2020
Revised Date: 2 August 2020
Accepted Date: 4 August 2020

Please cite this article as: Keshta, A., Koop-Jakobsen, K., Titschack, Jü., Mueller, P., Jensen, K., Baldwin, A., Nolte, S., Ungrazed salt marsh has well connected soil pores and less dense sediment compared with grazed salt marsh: CT scanning study, Estuarine, Coastal and Shelf Science (2020), doi: https://doi.org/10.1016/j.ecss.2020.106987.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier Ltd.
Author contributions statement

SN, PM, and KJ designed the original study setup with further contributions from AK, KKJ, and AB. JT conducted the CT scans and supervised AK in data processing. Lab measurements were done by AK, with contributions to grain size analysis by KKJ and JT. AK analyzed results and wrote the main draft of the manuscript. All authors contributed to the discussion of results and the writing of the manuscript.
Graphical abstract

A) Grazed saltmarsh

B) Scanning soil cores at CT scan facility

C) Grazed saltmarsh CT scan images

D) Ungrazed saltmarsh CT scan images
Title

Ungrazed salt marsh has well connected soil pores and less dense sediment compared with grazed salt marsh: CT scanning study

Authors and addresses

Amr Keshta\textsuperscript{1,2,*}, Ketil Koop-Jakobsen\textsuperscript{3,4}, Jürgen Titschack\textsuperscript{4,5}, Peter Mueller\textsuperscript{6}, Kai Jensen\textsuperscript{7}, Andrew Baldwin\textsuperscript{2}, and Stefanie Nolte \textsuperscript{8,9}

\textsuperscript{1}Botany Department, College of Science, Tanta University, 3111 Tanta, Egypt
\textsuperscript{2}Department of Environmental Science and Technology, University of Maryland, College Park, Maryland 20742, USA
\textsuperscript{3}The Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Wadden Sea Station, D-25992, Germany
\textsuperscript{4}MARUM – Center for Marine Environmental Sciences, University of Bremen, Bremen, Germany
\textsuperscript{5}Senckenberg am Meer, Marine Research Department, 26382 Wilhelmshaven, Germany
\textsuperscript{6}Smithsonian Environmental Research Center, 647 Connees Wharf Rd, Edgewater, MD 21037, United States
\textsuperscript{7}Applied Plant Ecology, Institute for Plant Sciences and Microbiology, Universität Hamburg, Hamburg, Germany
\textsuperscript{8}School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK
\textsuperscript{9}Centre for Environment, Fisheries and Aquaculture Science, Pakefield Rd, Lowestoft, UK

\textsuperscript{*}Corresponding author Tel.: +1-301-385-6206; akeshta@umd.edu

Abstract

Salt marshes provide various ecosystem functions and services including flooding protection, wildlife habitats, and carbon storage. These functions and services could, however, be strongly impacted by anthropogenic activities such as livestock grazing – a common practice in the Wadden Sea salt marshes located in North of Germany. To assess the impact of grazing on soil parameters, a total number of eight soil cores (\textphi: 18 cm; L: 50 cm) were collected in areas with and without livestock grazing, and scanned using a Computed Tomography (CT) to characterize soil parameters including soil macroporosity, sediment density, and pores connectivity. Subsequently, sub-samples were taken for determination of soil moisture content (%) and bulk...
density (g cm\(^{-3}\)). To account for the impact of grazing on soil drainage after tidal inundations, water table relative to soil surface was monitored during two flooding events. Our results demonstrated that grazed salt marsh has higher top-soil bulk density, and lower macroporosity and pore connectivity, than ungrazed marsh, due to soil compaction by livestock grazing. Moreover, grazed marsh has slower water drainage and that might keep the soil waterlogged for a longer period of time which has implications on lowering decomposition rate due to lower soil redox. This study provides evidence that grazing alters physical soil parameters in salt marsh. Consequently, grazing needs to be accounted for when evaluating how land use impacts ecosystem services and functions including carbon sequestration.

Keywords
Grazing, salt marsh; soil ecosystem services; Wadden Sea; computed tomography; soil macroporosity

1. Introduction
Coastal salt marshes make up the transition zone between the land and the sea, and over the last decades, this ecosystem has increasingly been recognized for its valuable ecosystem services, in particular in regards to its ability to sequester “blue carbon” and thus mitigate global climate change (Eid et al., 2017; Gedan et al., 2011; Keshta et al., 2020; Kirwan and Megonigal, 2013; Mueller et al., 2019b; Spalding et al., 2014). However, salt marshes are highly impacted by humans, for example through livestock grazing by sheep or cattle all over the world (Bakker et al., 2020; Di Bella et al., 2014; Yang et al., 2017). In some areas such as the Wadden Sea in northwestern Europe, livestock grazing has a long tradition and can be traced back as far as the bronze age “4900-800 BC” (Lotze et al., 2005). Livestock grazing alters the salt marsh ecosystem and its ecosystem services (Davidson et al., 2017; Mueller et al., 2017). It has direct effects in terms of defoliation, reducing the height of the vegetation (Bakker et al., 2020; Kiehl et al., 1996), which can lead to reduced rates of sediment deposition (Neuhaus et al., 1999). Yet, the effect of livestock grazing on the marshes ability to capture and accrete sediment is less clear (Elschot et al., 2013; Nolte et al., 2013b).

Livestock grazing leads to soil compaction through trampling, which increases soil bulk density (Nolte et al., 2013b). This soil compaction has significant impact on its physicochemical properties such as lowering the hydraulic conductivity, and thereby the marshes ability to drain the water that is occasionally flooding the salt marshes (Harvey and Nuttle, 1995). This reduced drainage increases waterlogging and decreases soil aeration, which in turn lowers the redox potential of the soil, and affects the exchange and turnover of nutrients, organic matter and gasses in the soil (Keshta, 2017; Mueller et al., 2017; Schrama et al., 2013). The connectivity
of macropores play a crucial role for the drainage of salt marshes (Harvey and Nuttle, 1995), yet, the effect of livestock grazing on the soil macropores and their connectivity has not been quantified.

The three-dimensional structure of the soil including the percentage of macropores and their connectivity can be assessed in detail using Computed Tomography (CT) scanning. This technology targets a broad range of key parameters relevant for soil science including soil density (Petrovic et al., 1982), grain-size distribution (Homberg et al., 2009; Munkholm et al., 2012; Taina et al., 2008), macropore structures and porosity (Anderson et al., 1990; Homberg et al., 2012; Rozenbaum et al., 2012), and micromorphology (Taina et al., 2008). Several CT-devices including medical-CT (Luo et al., 2010; Mooney et al., 2012), micro-CT (Homberg et al., 2012) and synchrotron-CT (Khan et al., 2012; Rozenbaum et al., 2012; Zong et al., 2015) could be used to analyze these parameters. Water pathways carrying nutrients through the soil pores (micro and macropores) enhance the understanding of hydrological subsurface in salt marsh (Hu et al., 2018) and that has greater impact on ecosystem service and functions. Within salt marsh research, the application of CT is rather new, but was recently applied for porosity measurements and pore network characterizations to compare natural and restored salt marshes (Dale et al., 2019; Spencer et al., 2017; Van Putte et al., 2019), as well as root and rhizome visualization (Dauve et al., 2011; Spencer et al., 2017; Wigand et al., 2016).

To understand how livestock grazing affects soil parameters in salt marshes, and how it impacts drainage of marsh soils, we conducted CT on soil cores from two long-term (about 30 years) grazing experiments in the Wadden Sea. The experiments took place in two individual marshes, each having a grazed and an ungrazed area allowing for direct comparison. The three-dimensional structure of key soil parameters, such as density, macroporosity and pore connectivity, was visualized using CT scanning. Furthermore, CT result findings were interpreted in relation to soil drainage (i.e. travel of the water through the soil). We hypothesize (1) grazing to increase soil density and decrease soil connectivity and macroporosity. As a consequence of the grazing effect on soil parameters, we hypothesize (2) grazed marshes have slower drainage rate.

2. Material and methods

2.1. Study sites

The study was conducted on the mainland coast in the Schleswig-Holstein Wadden Sea National Park in the North of Germany (Fig. 1A). The area is characterized by a long history of human interventions, such as the construction of ditched sedimentation fields with brushwood groynes to enhance sedimentation (Mueller et al., 2019a) and high stocking density grazing (Esselink et al., 2009). The establishment of the National Park in
1985 led to a reduction of sheep grazing. To understand how this change in management would affect the ecosystem, long-term grazing experiments were installed in the late 1980s (Kiehl et al., 1996). Two of these sites, namely the Dieksanderkoog (DSK) and Sönke-Nissen-Koog (SNK) are used in this study (Fig. 1B). Both sites include a sheep-grazed salt marsh area, which is separated from the ungrazed control treatment by a deep creek (Nolte et al., 2013b) that cannot be crossed by the animals (Fig. 1 C & D). The grazing pressure is unevenly distributed within the grazing treatment (Bakker et al., 2020), as the sheep prefer to stay close to the seawall, where freshwater is provided. In this study, possible effects of abandonment of grazing in the ungrazed control treatment is studied in the top 15-25 cm of the soil (soil horizon separation as showed in Fig. 2), representing soil layers established after 1985 (Nolte et al., 2013b). Deeper soil layers represent the pre-1985 situation, when the control treatment area was also grazed.

DSK (53°80’23” N, 8°53’8” E) is situated at the mouth of the Elbe Estuary and is characterized by a tidal range of 3 m. Elevation above mean high tide (MHT) of the studied area ranges between 0.29 m and 0.78 m. DSK is a very wide marsh with a distance of approximately 2300 m between the seaward marsh edge and the seawall. The mean accretion rate for the past 30 years based on $^{137}$Cs dated cores was found to be between 0.61 cm yr$^{-1}$ and 1.04 cm yr$^{-1}$ (Nolte et al., 2013b). Due to this high sediment supply, the marsh has been expanding over the past decades. SNK (54°38’4” N, 8°50’2” E) is located 75 km north of the DSK. The tidal range at this site is slightly higher with 3.4 m and elevation above MHT ranges between 0.41 to 0.48 m. The distance between the seaward marsh edge and the seawall is approximately 1000 m. Accretion rates are lower than at DSK and range from 0.54 cm yr$^{-1}$ to 0.89 cm yr$^{-1}$ (Nolte et al., 2013b). After the cessation of grazing, the vegetation community in the ungrazed control of both sites changed from short-growing *Puccinellia maritima* (SNK) or *Festuca rubra* (DSK) to the tall and biomass rich late successional *Elymus athericus*. The grazed treatment in SNK is still dominated by *Puccinellia maritima*, while at DSK, *Festuca rubra* and *Elymus athericus* are found in the grazed treatment (Kiehl et al., 2001; Nolte et al., 2013a).
2.2. Soil core sampling and collection

In October 2017, soil cores were collected at both sites using sharp-edge PVC-pipes with a diameter of 18 cm and gently inserted in the marsh soil to a depth of 50 cm – with gentle hammering applied as appropriate.
Soil was excavated around the pipe in order to retrieve the core with slight soil disturbance and compaction (less than 1 cm difference was observed between soil level inside and outside of the core). Four soil cores were collected from each site with two cores from the grazed and ungrazed treatments, respectively (Fig. 1 C & D). Within each treatment, one of the two cores was collected closer to the seawall (‘landward’) and the other was collected at a greater distance to the seawall (‘seaward’) in order to elucidate spatial differences within treatments. This results in a total number of eight soil cores, which were wrapped with plastic to prevent drying and water loss, and immediately brought to the laboratory for further analysis. In this study, we used the accretion rate data provided in (Nolte et al., 2013b) for assessing the soil depth corresponding to the start of grazing experiment in both DSK and SNK.

2.3. CT scanning

The cores were kept in the PVC pipes and scanned using a Toshiba Aquilion 64™ Computed tomography (CT) scanner at the hospital Klinikum Bremen-Mitte, with an X-ray source voltage of 120 kV and a current of 600 mA. The CT scans have a resolution of 0.351 mm in x- and y-direction and 0.5 mm resolution in z-direction. The z-direction represents the depth along the core axes, while x and y are perpendicular to the core axes (reconstruction interval: 0.3 mm). Images were reconstructed using Toshiba's patented helical cone beam reconstruction technique. The obtained CT data were processed using the Amira ZIB edition software version 2017.39 (Stalling et al., 2005; http://amira.zib.de). The PVC pipes, together with about 2 mm of the core rims, were removed from the CT data in order to discard marginal artefacts resulting from the coring process. The scans were visualized in 3D, separating three key soil components by threshold segmentation: 1) sediment (≥20HU; HU: Hounsfield units; a quantitative scale for radio-density”), 2) water/root-filled pores (≥-980 HU and <20 HU) and 3) air-filled pores (<-980 HU). Subsequently, the following soil parameters were identified and/or calculated for each of the soil cores: air-filled and water/root-filled pores, pore connectivity index, and soil macroporosity and density (estimated based on the X-ray attenuation of the soil) (Anderson et al., 1990).

2.4. Processing of CT-scan images

Image analyses allowed for quantification of key parameters in the soil representing depth profiles averaging over the 234 cm² core area. Pore and soil volumes were identified and quantified using the Material Statistics-module (volume per slice) and used for calculation of soil macroporosity (only pores > 10 mm³ were visualized and presented). The extension of individual pores in the soil were identified applying the ‘Connected Components’-module, which identifies connected pores in 3D. The identified pores were subsequently parameterized with the Shape Analysis module to visualize all pores, while only pores >10 mm³ are presented to
reduce image overwhelming. Number of disconnected pores divided by total pore volume within each slice were used as a semiquantitative proxy for soil permeability (degree of soil pore connectivity ‘hereafter: pore connectivity index’). In this case, soils with high connectivity index had fewer distinct pores, because most of the pores were connected, which was represented by a low value in the connectivity index. In contrast, soils with low connectivity had more distinct pores because pores were disconnected, which were represented by high value in the connectivity index. The X-ray attenuation (HU) was used as a proxy for wet soil bulk density. To avoid the impact of the core margins, all the segmented soil was reduced by 3 voxels and calculated its mean value in X-ray attenuation in every xy-oriented CT-Slice, followed by using the mean value determined with the Statistics per slice per label algorithm of the Material Statistics-module of the segmented soil. Voxels with a value higher than 1000 (HU) were considered outliers and discarded from the analyses. The soil macroporosity was visualized by applying the ‘AverageValueInNeighborhood’-module using a cube volume of about 1 cm\(^3\) (31 \times 31 \times 31 isotropic voxel) on segmentation data in which all pores were represented by the value 1 and soil by the value 0. For the visualization of soil density, the ‘Average Value In Neighborhood’-module was applied on the segmented soil - using a cube volume of about 1 cm\(^3\).

2.5. Soil analyses

After CT scanning, soil cores were opened along the side and soil samples were taken at five centimeters intervals using standard soil sample rings (\(\varnothing=53\) mm, depth = 5 cm ; Eijkelkamp). The rings were pushed into the side of the core to 7 cm depth to avoid sampling the core edges. Samples were stored in plastic bags at 4\(^\circ\)C and were processed within few days from sampling. Soil samples were dried at 105\(^\circ\)C to a constant weight and weighed to quantify soil bulk density (g cm\(^{-3}\)) and soil moisture content (%) based on weight loss. For soil particle size analyses, the soil cores were opened by sawing the PVC tube in halves and revealing soil core from inside. Half of the core was carefully removed to enable visual inspection of stratigraphy where layers appear in different shades of grey; one is lighter and one is darker. Hence, we refer to these as “light” and “dark” layers. Distinct light and dark layers were observed and samples (5 g) were collected from ten of these. In order to test for difference in soil composition between layers, soil samples were collected from the dark and light layers in the soil cores and analyzed for grain-size distribution. Particle-size measurements (McGregor et al., 2009) were performed in the Particle-Size Laboratory at MARUM, University of Bremen with a Beckman Coulter Laser Diffraction Particle Size Analyzer LS 13320 (soil samples preparation with detailed methods are available in the supplementary material).

2.6. Hydrology
To assess the effect of grazing on water drainage, change of water level relative to soil surface was measured in the landward position of SNK. Here, three pressure sensors (type ‘Micro-Diver’, Eijkelkamp) were deployed in three monitoring wells covering both the grazed and ungrazed treatment as well as a tidal creek (Fig. 1C), which was used to record the reference water levels. The monitoring wells for ungrazed and grazed were located approximately 48 m from nearest tidal creek (Fig. 1C). The wells consisted of perforated PVC-tubes surrounded by fabric to prevent clay and silt from collecting in the well. Pressure sensors inside the wells were installed below the marsh surface level (45-50 cm from marsh soil surface). An additional pressure sensor was installed close by on the mainland to record air pressure for compensation (type ‘Baro-Diver’, Eijkelkamp).

Measurements were recorded at five minute intervals in autumn 2016, while data presented (Fig. 8) are corresponding to two events inundating the entire marsh platform of SNK were recorded on 08.08.2016 and 28.09.2016, respectively.

2.7. Statistical analyses

Soil samples were grouped into two categories: top soil (0-20 cm) and bottom soil (deeper than 20 cm). Over soil depth, repeated measure ANOVA was used to test the main effects of treatment (grazed vs ungrazed) and position (landward vs seaward) on the soil bulk density and moisture content for the top and bottom soils separately (Supplement 1). Data were found to be normally distributed with homogeneous variances and data used for presentation are mean ± SE unless otherwise noted. Tukey’s post-hoc test was used to examine the least significant difference between the means. All statistical analyses were performed using SAS 9.4.

3. Results

3.1. Visualization of soils parameters using CT scans

Central CT-slice of the core (Fig. 2: column 1) showed clear stratification in soil layers (dark vs light layers). In the SNK landward grazed core, a clear horizon separation for all parameters (Fig. 2: columns 2-5) was found at 15 cm. In contrast, in SNK landward ungrazed core, a clear horizon separation was found at 35 cm, demonstrating difference in soil pores, macroporosity, and density between the grazed and ungrazed treatments. In the SNK seaward grazed core, the horizons were less clear, but a horizon separation was detected at 20 cm, most pronounced for density (Fig. 2: column 5). In the SNK seaward ungrazed core, connectivity index (Fig. 2: column 3) and density (Fig. 2: column 5) showed a horizon separation at 38 cm. Comparing SNK grazed and ungrazed treatments in general, it is noteworthy that connectivity index tended to be higher in the ungrazed treatment, and air-filled pores were almost exclusively found in the ungrazed treatment. Moreover, air-filled...
pores were more frequently present at seaward compared to landward salt marsh. In the DSK landward grazed
core, there was a horizon separation for all parameters (Fig. 2: column 2-5) at 8 cm. By contrast, in the DSK
landward ungrazed core, a clear horizon separation was found at 13 cm, demonstrating difference between the
grazed and ungrazed treatment. In the DSK seaward grazed core, a clear horizon for all soil parameters (Fig. 2:
column 2-5) was found at 4 cm. By contrast, in the DSK-seaward ungrazed core, a clear horizon separation was
found at 12 cm. Comparing DSK grazed and ungrazed treatments in general, it is noteworthy that the ungrazed
treatment had higher pore connectivity index and macroporosity than the grazed treatments. Moreover, air-filled
pores tended to have higher occurrence in the ungrazed core soil profile.

Fig. 2. Three-dimensional (3D) visualization of key soil parameters obtained from CT scan images processed
with AMIRA software. The following parameters were visualized: column 1: 2D central CT slice of soil core, 2:
3.2. Water/root-filled pores

In SNK, the depth profiles for water/root-filled pores differed between grazed and ungrazed treatments, showing a higher volume of water/root-filled pores in the ungrazed treatment (Fig. 3 A). High pore-water volume was characteristic for the topsoil. Water/root pore volume was particularly high in the top soil of the ungrazed treatment. At greater depth (below 20 cm), the profiles were overlapping and representing the situation in the past, when both treatments were equally grazed. There were no pronounced differences between the landward and seaward positions in pore volume. In DSK, water/root pore volume was higher in the top 0-10 cm. In the seaward position, a high volume was detected down to 15 cm in the ungrazed treatment, whereas high volume was detected down to 5 cm in the grazed treatment. In the landward position, no pronounced difference in water/root-filled pore volume was found between the grazed and ungrazed treatments. However, it was higher in the topsoil of DSK than in SNK, and at depth (<15 cm) the water/root-filled pore volume was lower in DSK than in SNK.
Fig. 3. Depth profiles of water/root-filled pores volume (mm$^3$) derived from CT-scans. Horizontal punctuated and solid lines represent the depth corresponding to the start of grazing experiment in the grazed and ungrazed treatment, respectively.

3.3. Air-filled pores

In SNK, the depth profiles for air-filled pores volume differed between the grazed and ungrazed treatment (Fig. 4). Air-filled pores were almost exclusively present in the ungrazed treatment, where air-filled pores were detected down to 15 cm, coinciding with the time when grazing was terminated in the ungrazed treatment. In the grazed treatment, a small amount of air-filled pores was observed in the top 4 cm, it was, however, smaller than in the ungrazed treatment. No marked differences between the landward and seaward positions were found in air-filled pores. In DSK, air-filled pores were observed in both the grazed and ungrazed treatment. However, in the grazed treatment air-filled pores were restricted to the top-5 cm, whereas air-filled pores were found down to
15 cm in the ungrazed treatment. No clear differences between the landward and seaward positions were detected for air-filled pores at DSK.

![Graphs showing depth profiles of air-filled pores volume (mm$^3$)](image)

**Fig. 4.** Depth profiles of air-filled pores volume (mm$^3$) derived from CT-scans. Horizontal punctuated and solid lines represent the depth corresponding to the start of grazing experiment in the grazed and ungrazed treatment, respectively.

### 3.4. Pore connectivity index as a proxy for pore permeability

Pore permeability is semi-quantitatively measured and represented as connectivity index (Fig. 5) stating the number of individual pores relative to the total pore volume. Here, low values represent high connectivity with a well-connected network of soil pores. In SNK, the ungrazed treatment showed a higher connectivity (low values) in the top 35 cm of the cores than grazed treatment. In contrast, below 35 cm, connectivity of grazed and ungrazed treatments was similar. In DSK, the depth profiles for pore connectivity differed between the grazed
and ungrazed treatments in the top 20 cm of the cores showing higher pore connectivity in the ungrazed than
grazed treatments. In contrast, below 20 cm, connectivity index of grazed and ungrazed treatments was similar.

![Figure 5](image.png)

**Fig. 5.** Depth profiles of connectivity index used as a proxy for pore permeability derived from CT-scans. Horizontal punctuated and solid lines represent the depth corresponding to the start of grazing experiment in the grazed and ungrazed treatment, respectively.

### 3.5. Soil macroporosity

In SNK, the depth profiles for macroporosity differed between the grazed and ungrazed treatments showing a higher macroporosity in the topsoil (top 20 cm) of the ungrazed treatment (Fig. 6). At greater depth (below 20 cm), the profiles for grazed and ungrazed treatments were more similar representing the situation prior to 1985, where both treatment areas were evenly grazed. There were no pronounced differences between the landward and seaward positions in macroporosity. In DSK, higher soil macroporosity was observed in the
top soil only (10-15 cm soil depth) in both landward and seaward position, while bottom soil (deeper than 20 cm) showed very low soil macroporosity (lower than 5%). In DSK landward, no marked differences between soil macroporosity in grazed and ungrazed marshes, while DSK seaward ungrazed marsh has higher soil macroporosity than grazed marsh noticeable in the top 15 cm only.

**Fig. 6.** Depth profiles of soil macroporosity (Vol. %) derived from CT-scans. Horizontal punctuated and solid lines represent the depth corresponding to the start of grazing experiment in the grazed and ungrazed treatment, respectively.

3.6. Soil density based on X-ray attenuation

In SNK landward and seaward positions (Fig. 7A and 7B) and at the top 8 cm only, the ungrazed treatment had a higher soil density than the grazed treatment since SNK site receives more sediment and has different vegetation composition, however, at 10-20 cm soil depth, soil in the grazed treatment was more dense than in
the ungrazed treatment which similar to results presented in supplement 1 where top soil, in general, is more
dense in grazed than ungrazed marsh. At the seaward DSK position (Fig. 7D), we found the grazed treatment to
have a higher soil density at depth 5-15 cm, while at the landward position (Fig. 7C), differences in soil density
between grazed and ungrazed treatments were not obvious.

Fig. 7. Depth profiles of the soil density derived from CT-scans; x-ray attenuation (HU) is used as a proxy for
soil density. Horizontal punctuated and solid lines represent the depth corresponding to the start of grazing
experiment in the grazed and ungrazed treatment, respectively.

3.7. Soil physical parameters

Grazing had a significant effect on top soil bulk density (supplement 1, F = 196.04, P = 0.045) where
grazed treatments had higher soil bulk densities than ungrazed treatments (0.92±0.09 and 0.79±0.10 g cm⁻³ for
landward positions, respectively, and 0.99±0.11 and 0.84±0.12 g cm⁻³ for seaward positions, respectively,
supplement 1A). The core stratigraphies showed clearly visible dark and light layers (Supplement 2A), which
correspond with the layers observed in the CT images (Fig 2. Column 1-5). For instance, at seaward grazed saltmarsh at SNK, light layer appear at soil depth of 10 cm (column 1 at Fig. 2.) which correspond to lower soil density (Fig. 2 column 5) at the same depth. Light layer has higher sand content than dark layer and these alternations in the sediment conditions are event-driven, primarily by storms bringing in and depositing larger portions of coarse grained suspended material, than during normal tidal interactions. A larger proportion of fine sands was found in the light layers than in the darker layers. The dark layers had a larger proportion of smaller grained silts and clays. The alternation in grain-size distribution supports that the stratification is event-driven.

Layering is found in both the grazed and ungrazed marshes showing that stratification was unaffected by grazing.

3.8. Hydrology

In our study, the two time events presented at Fig. 8 are during spring tide (8 August, 2016 corresponds to the 5th day of lunar cycle & 28 September, 2016 corresponds to the 27th of the lunar cycle). During these two events, both marsh surfaces were flooded with water and hence, we are comparing them after the flooding took place. Water level relative to soil surface is presented in Fig. 8. On 08.08.2016 prior to tidal flooding, the water table was at -23 cm in the ungrazed and at -9 cm in the grazed treatment. After high tide, water drained slightly faster from the ungrazed treatment, falling below the soil surface level within about 1.5 hours. After 6 hours, the water table had reached a relatively stable level of -15 cm in the ungrazed treatment, whereas the water table was still above the soil surface in the grazed treatment. On 28.09.2016 prior to the tidal flooding, the water table was at -43 cm in both the grazed and ungrazed treatments. After high tide, water drained slightly faster from the ungrazed treatment, falling below the soil surface level within 1 hour. After 6 hours, the water table had reached a level of -17 cm in the ungrazed treatment, whereas the water table remained above the soil surface at 1 cm in the grazed treatment for a longer time. In general, the hydrological observations showed a higher water drainage rate in the ungrazed treatment compared to the grazed treatment.
4. Discussion

In our study, the use of the CT scan technique for saltmarsh cores enabled the analyses of soil parameters including soil pore permeability and macroporosity in three-dimensional way. In line with our first hypothesis, the results indicate that livestock grazing in salt marshes decrease soil macroporosity (Fig. 2: column 4) and increase top soil density (supplement 1). As a result of less permeability represented by less connected soil pores, water drained slower under livestock grazing as expected based on our second hypothesis.

4.1. Application of CT-scanning in salt marshes

CT-scanning allows for visualization and quantification of key soil parameters in salt marshes. The 3D visualization facilitates a visual comparison of individual cores, which allow of observation of the spatial variation in soil parameters. Recently, characterization and quantification of soil physical parameters in a non-destructive approach has gained high focus from saltmarsh researchers using CT scanning for obtaining high image resolution (mm to µm). A major issue in applying CT to salt marsh, or soils in general, is the sample size and the respective voxel size of the obtained CT scan. Besides its methodological limitations, CT imaging offers a powerful tool to improve our understanding of soil textures and components in three dimensions, and allow consequently a better evaluation of soil behavior and processes (Taina et al., 2008).

In this study, CT clearly demonstrated a spatial variation in soil horizons (Fig. 2) due to change in grazing practices, and marked layered structure of key soil parameters (Fig. 2 and supplement 2), due to event
driven deposition of material. In this study average values were calculated over a large cross-sectional area covering 243 cm$^2$ of soil core capturing and accounting for the natural variation in the marsh soil. This is a clear advantage over more destructive methods depending on core slicing and/or subsampling, which disturb the soil structure. In this study, the non-descriptive nature of the CT-scanning rendered an analysis of the pore connectivity (Fig. 5) showing higher connectivity in ungrazed soils, and thereby a better drainage rate, which was confirmed by a subsequent drainage study (Fig. 8). A major advantage of medical-CT is the operator-independent image acquisition. In contrast to micro and synchrotron CT-devices, all medical-CT devices are calibrated to Hounsfield-units, which provide a principal comparability of the obtained data from various devices (Cnudde and Boone, 2013). However, differences might still occur due to differences in resolution and reconstruction software. During data processing, the most critical operation is the segmentation, usually based on thresholding, of the various components of interest. However, as long as similar objects of similar size are measured with the same device, resolution, and applied threshold, the errors are constant and allow their comparison. Another limitation for CT scanning is the partial volume effect which must be taken into account (Cnudde and Boone, 2013), which signifies that a voxel value is the mean of the x-ray attenuation over the complete voxel volume. Only a combined approach using referenced subsamples of various size from a soil sample being measured with the respective CT devices would allow to capture a soil in its entire complexity.

4.2. Effects of grazing on physical soil parameters

Grazing led to lower soil permeability, lower macroporosities, and higher top soil densities (Column 3, 4, and 5 in Fig. 2 and supplement 1A). This is consistent with the outcome of previous grazing experiments in the Wadden Sea that have demonstrated a significant impact of grazing on soil parameters (Elschot et al., 2013; Nolte et al., 2013b) and redox chemistry (Bakker et al., 2020; Mueller et al., 2017). Many of these studies argue that trampling leads to soil compaction, which reduces water drainage and thus the availability of oxygen in the soil. Soil redox potential often used as indication for the oxygen availability in the soil with lower values as an indication for prolonged period of waterlogging (Mitsch and Gosselink, 2007), which is a result for soil compaction and blocking the water pathway through soil pores. In our study, trampling by sheep led to denser and more compacted soils (Supplement 1), which is similar to findings from other temperate and tropical marshes (Haines-Young and Potschin, 2010; Tanentzap and Coomes, 2012). CT scanning in our study allowed for a specific focus on pore connectivity in the soil, which facilitates the vertical flow of water and thereby water drainage of the salt marsh. The results clearly show that permeability (Fig. 5) and macroporosity (Fig. 6) is lower with grazing, particularly, in the top profile of the soil cores. As a result of mechanical stresses that
have been added to the soil surface, grazing can alter the physical and chemical nature of the soil, and that may negatively impact the ecosystems services and functions provided by saltmarshes including blue C storage (Davidson et al., 2017; Mueller et al., 2019a), however some studies reported that the impact of grazing on blue C storage is minimal on a broader-scale (Harvey et al., 2019).

4.3. Differences in physical soil parameters between landward and seaward locations in salt marshes

Our results showed that grazing had a stronger impact on landward compared to seaward positions. Grazing practices had an impact on soil compaction (Supplement 1) and soil horizons formation as noticed in SNK salt marshes located at landward position (Fig 2), where the grazed marshes showed higher soil density at the upper soil profile (0-15 cm) compared with the ungrazed marshes. This pattern can be explained by the grazing behavior of livestock. For cattle, behavioral studies in salt marshes have shown a grazing intensity gradient, with a decreasing local grazing intensity with increasing distance to the freshwater source located close to the seawall (Esselink et al., 2002; Nolte et al., 2013b). A similar behavior in sheep is seen as the cause for an observed gradient in vegetation height at SNK (Bakker et al., 2020). Consequently, the landward marsh is more exposed to grazing from livestock animals, leading to more trampling and soil compaction, and this is the course of the lower macro-porosity and connectivity shown at the landward marsh (Column 4, and 5 in Fig. 2).

4.4. Effect of grazing on salt marsh drainage

The marsh hydrology measurements demonstrated that water drains slower in the grazed salt marsh (Fig. 8). This observation is supported by our CT-scans showing lower water/root-filled pore volumes (Fig. 3) and lower soil pore permeability in the grazed marsh (Fig. 5), leading to lower drainage rates. This is further explained by the observations of air-filled pores (Fig. 4), which almost exclusively were found in the ungrazed marsh. This provides evidence that sheep grazing lowers drainage of the salt marsh and cause longer periods of waterlogging. Our findings of higher bulk density in top soil (Supplement 1) and lower water/root-filled pore volume in the grazed marsh (Fig. 3) suggests that compaction of the soil, due to trampling by the sheep, is responsible for the altered hydrological conditions. These observations are supported by previous research in salt marshes reporting a positive correlation between soil macroporosity and their ability to let water travel through (Van Putte et al., 2019), and compaction of soil as a result of trampling leading to low drainage rate and higher surface runoff (Gifford and Hawkins, 1979).

4.5. Implications and outlook
The results of our study may have implications for ecosystem functions and services delivered by salt marshes in general and in particular at the Wadden Sea. As we found grazing having pronounced impacts on soil physical structure and on drainage rate, our results are relevant for future management strategies of salt marshes, particularly in regard to carbon sequestration, which is one of the most important ecosystem services of salt marshes (Kirwan and Megonigal, 2013; McLeod et al., 2011). The low drainage rate of the grazed marsh allows the marsh soil to stay waterlogged for a longer period of time. This plays a significant role in marsh lowering oxygen availability and redox potential, altering the microbial community and organic matter turnover and thereby increasing their carbon sequestration capacity. While our study supports the evidence that grazing may enhance carbon sequestration rate in grazed saltmarsh by altering the soil redox after prolonged water logging, it is however still unclear if continuous aboveground biomass removal and lower plant productivity as a result of grazing will counteract the lower carbon turnover rate which is a crucial part for assessing net carbon sequestration rates.

### 5. Conclusion

Salt marsh grazing by sheep decreased macroporosity and pore connectivity due to compaction lowering the pore space, which also increased the top soil density. Marsh hydrology was impacted by grazing resulting in slower water drainage after inundations, resulting from low drainage after tidal inundation caused by lower pore volume and connectivity keeping the soil waterlogged for a longer period of time. Our current results demonstrated that livestock grazing in salt marsh at SNK and DSK had an impact on soil parameters leading to lower pore connectivity and macroporosity. These grazing implications have greater direct and indirect impact on ecosystem services and functions provided by salt marsh at the Wadden Sea including carbon sequestration and excess nutrients removal.

### Acknowledgements

We would like to thank Dr. Martin Stock and the Administration of the Wadden Sea National Park Schleswig-Holstein for allowing us to access the sites and take samples. This research was partly funded by the Bauer-Hollmann Foundation in the framework of the project INTERFACE. The authors would like to acknowledge the Society of Wetland Scientists (SWS) and Department of Environmental Science and Technology at the University of Maryland College Park - USA for their funding support for the Wetland Ambassador Fellowship awarded to the first author during which he conducted the research at Hamburg University – Germany. JT acknowledges funding by the DFG Research Center/Cluster of Excellence “MARUM
– The Ocean in the Earth System”. Klinikum Bremen-Mitte and Prof. Dr. Arne-Jörn Lemke and Christian Timann are thanked for providing their facilities and supporting the performed computed tomography measurements. For KKJ, the research was funded in part by The Helmholtz Climate Initiative (HI-CAM). HI-CAM is funded by the Helmholtz Association’s Initiative and Networking Fund. The authors are responsible for the content of this publication. The authors would like to acknowledge the reviewers whose comments helped to improve the quality of the manuscript.

References


Keshta, A.E.S.S., 2017. Hydrology, Soil Redox, and Pore-Water Iron Regulate Carbon Cycling in Natural and Restored Tidal Freshwater Wetlands in the Chesapeake Bay, Maryland, USA.


Highlights

- Investigating soil cores using minimally non-destructive X-ray CT analysis allows identification of soil parameters in undisturbed soil zones
- Grazed salt marshes have lower soil macroporosity and higher density than ungrazed salt marshes
- Ungrazed salt marshes have higher drainage rate than grazed saltmarshes
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: