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Early Anthropogenic Transformation of the Danube-Black Sea System

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Liviu Giosan¹, Marco J. L. Coolen², Jed O. Kaplan³, Stefan Constantinescu⁴, Florin Filip⁴, Mariana Filipova-Marinova⁵, Albert J. Kettner⁶ & Nick Thom⁷

¹Geology & Geophysics, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA, ²Marine Chemistry & Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA, ³ARVE Group, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland, ⁴Department of Geography, University of Bucharest, Bucharest, 70709, Romania, ⁵Museum of Natural History, 9000 Varna, Bulgaria, ⁶Institute of Arctic and Alpine Research, University of Colorado, Boulder, CO 80309-0450, USA, ⁷Faculty of Engineering, University of Nottingham, Nottingham, NG7 2RD, UK.

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Correspondence and
requests for materials
should be addressed to
L.G. (lgiosan@whoi.
edu) or M.J.L.C.
(mcoolen@whoi.edu)

Over the last century humans have altered the export of fluvial materials leading to significant changes in morphology, chemistry, and biology of the coastal ocean. Here we present sedimentary, paleoenvironmental and paleogenetic evidence to show that the Black Sea, a nearly enclosed marine basin, was affected by land use long before the changes of the Industrial Era. Although watershed hydroclimate was spatially and temporally variable over the last ~3000 years, surface salinity dropped systematically in the Black Sea. Sediment loads delivered by Danube River, the main tributary of the Black Sea, significantly increased as land use intensified in the last two millennia, which led to a rapid expansion of its delta. Lastly, proliferation of diatoms and dinoflagellates over the last five to six centuries, when intensive deforestation occurred in Eastern Europe, points to an anthropogenic pulse of river-borne nutrients that radically transformed the food web structure in the Black Sea.

The development of human civilization is the most significant environmental event of the last 10,000 years⁴. The global impact of human activities has come into focus as fossil fuel-based industrialization has accelerated over the last century, but the expansion of agriculture may have affected the entire Earth System as early as 8000 years ago⁵. In the ocean, anthropogenic impacts include increasing temperatures, sea level rise, eutrophication and acidification⁶. Evidence for long term, pre-industrial anthropogenic modifications of the oceans remains sparse^{1,7,8}, although humans became active agents of continental denudation thousands of years ago⁹. However, the ability to define baselines and separate natural variability from human-induced changes is essential for understanding biogeochemical cycles and managing marine ecosystems, which ultimately depends on the detection and attribution of long term environmental trends⁶.

Regional seas¹⁰ provide the best opportunity to examine early anthropogenic changes in the marine environment. Their amplified reactivity to continental processes is the result of the limited water exchange with the open ocean and the relatively large fluvial contribution to their overall water budget. We selected the Black Sea to examine if pre-industrial land use was recorded by sedimentation patterns and sedimentary biogeochemistry (Fig. 1A). The landlocked Black Sea is a sensitive recorder of continental signals as demonstrated by drastic and repeated alteration of its ecosystem over the past half century^{2,11}. Although evaporation exceeds precipitation, the freshwater budget of the Black Sea is positive due to river runoff¹². Low salinity water is exported at the surface toward the Mediterranean through the narrow and shallow Straits of Bosphorus; denser water from the Sea of Marmara flows into the Black Sea as an undercurrent. River runoff drives much of the seasonal and interannual variability in salinity and sea level, which in turn affects the water exchange through the Bosphorus¹³. The Danube River, draining ~30% of central and eastern Europe (Fig. 1A), provides over 60% of the entire runoff reaching the Black Sea¹². Because fluvial nutrients feed the surface mixed layer that accounts for only 13% of the entire basin volume, there is a strong coupling between Danube fluxes and the Black Sea's biogeochemistry and ecology^{2,11}. A vigorous cyclonic mean surface circulation with superimposed mesoscale eddies^{13,14} redistributes nutrients in the surface mixed layer at multiannual time scales¹³, whereas nutrient exchanges with deeper suboxic and anoxic layers are secondary in importance¹³.

After the 1960s, the Black Sea was transformed within a decade into the largest eutrophic water body in the world, by discharge of industrial, domestic and agricultural waste coming primarily from the Danube watershed¹¹. In addition, construction of dams within the Danube watershed substantially reduced dissolved silicate

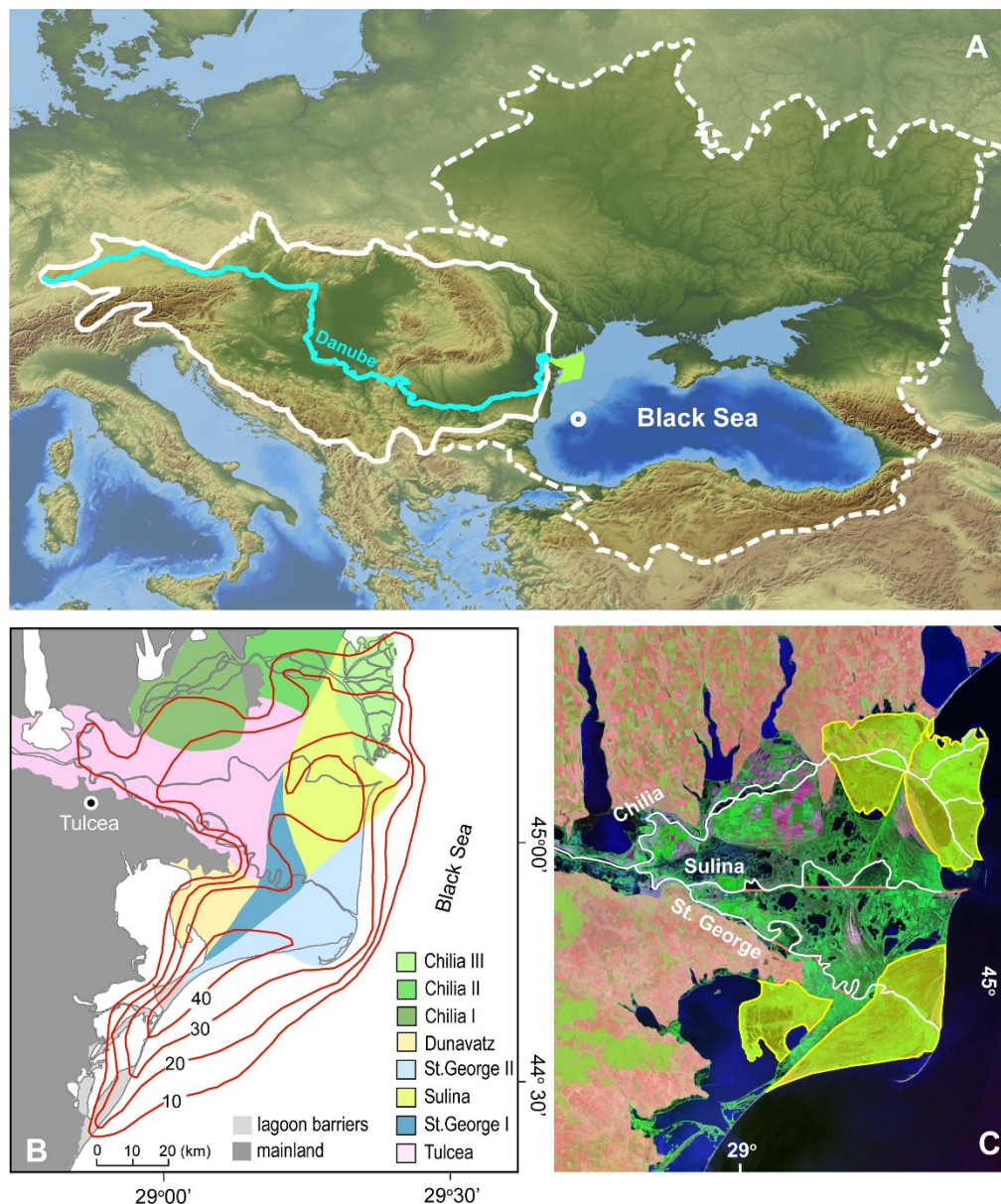


Figure 1 | Location of Danube-Black Sea system and the evolution phases of Danube delta. (A) Black Sea and its watershed (contoured with dashed white line), Danube's watershed (contoured with white line); Danube and Danube delta (blue line and green fill respectively). Location of core GGC18/MC19 is indicated. (B) Geologic map of the Danube delta showing the extent of the present delta plain, the thickness of its deposits (red isopachs in meters) as well as the mapped extent of individual lobes¹⁵ (see also SI): Tulcea (pre-5500 yrs BP to Present); St. George I (~5500–3600 yrs BP); Sulina (~3600–2000 yrs BP); St. George II and Dunavatz, (both ~2000 yrs BP to present); Chilia I (older than 1000 yrs BP); Chilia II (~1000–300 yrs BP); and Chilia III (~300 yrs BP to present). (C) Satellite image showing the expansion of Danube delta over the last 2000 years (land built during this period highlighted with a yellow mask).

loads to the Black Sea² hindering siliceous phytoplankton such as diatoms in favor of algal bloom-forming flagellates and coccolithophores. Breakdown of communist economies in Eastern Europe after 1990 led to a partial recovery of Black Sea ecosystems. The eutrophication period, characterized by drastic changes of the food web structure and collapse of fisheries, is illustrative of the sensitivity of the basin. Similarly, it has been postulated that the Danube-Black Sea system was influenced by changes in pre-industrial land use that modulated material fluxes to the Black Sea¹⁴.

Results

Danube River was one of the main advance routes for Neolithic agriculture from the Fertile Crescent into Europe and land use changes occurred in its basin as early as 7,500 yrs BP. To reconstruct

the history of continental denudation in the Danube basin that accompanied these changes, we generated sequential sediment budgets for the Danube delta. The modern delta covers ca. 4080 km² and has been built in several phases as bayhead, lacustrine and open-coast delta lobes (Fig. 1B)¹⁵. The base of the modern delta is marked by the maximum flooding surface above the previous early Holocene delta that was built during the lake phase of the Black Sea and drowned after the lake reconnected to the ocean at ca. 9000 yrs BP (see SI). Using modern relief as the upper surface, we estimated that ~215 GT of sediments have been stored in the delta above the maximum flooding surface (Fig. 1B). This value corresponds to ~26 MT yr⁻¹ and is considerably lower than Danube's sediment load of ~70 MT yr⁻¹ estimated prior to 1960's, before the most intensive dam construction phase along its course and tributaries¹⁶. However,

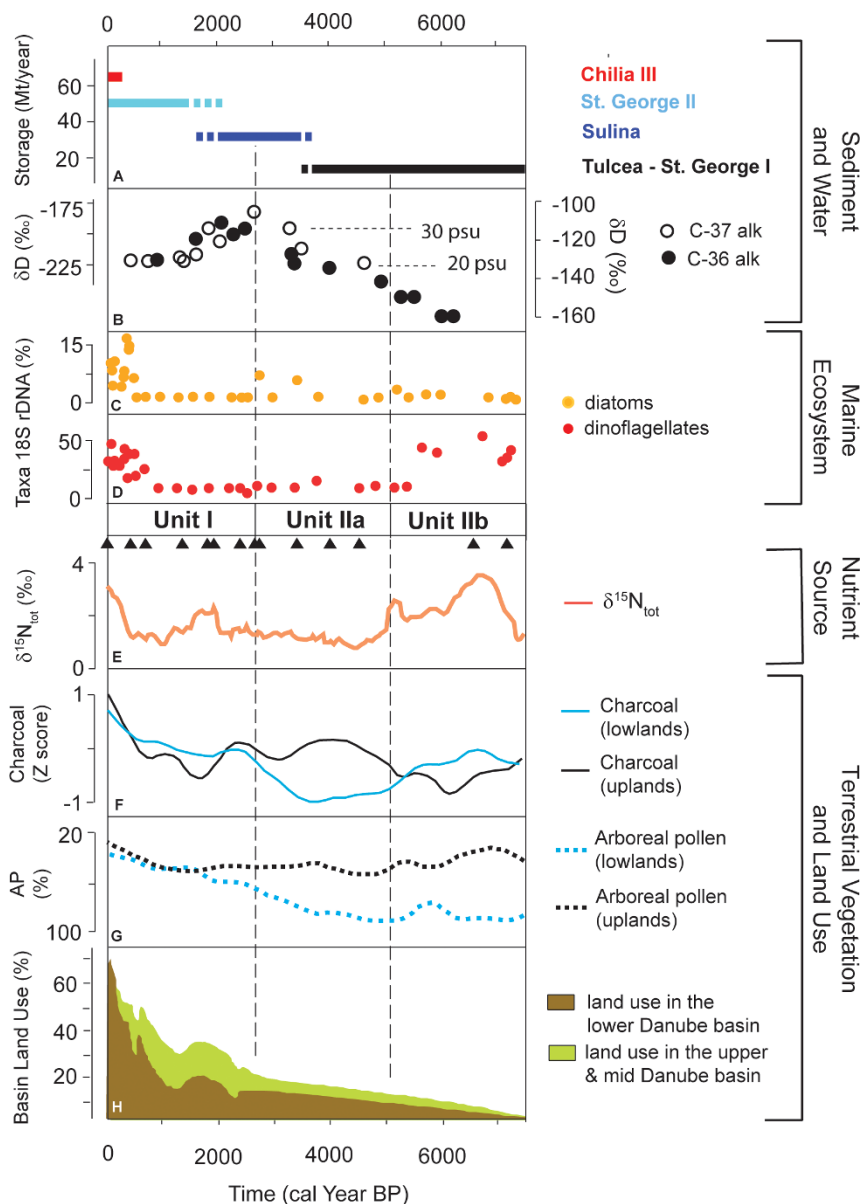


Figure 2 | Evolution of Danube-Black Sea system over the last 7,600 years (see SI for details on methodology and data). Average storage rates for successive evolution phases of the delta (A) are compared to paleoenvironmental proxies measured in core GGC18/MC19: alkenone δD composition as paleo-salinity proxies with salinity levels of ~ 30 and 20 psu calibrated for C-37 alkenones²¹ (B); 18S rDNA (as % of total reads) for diatoms (C) and dinoflagellates (D), principal phytoplankton taxa in the Black Sea, as indicators of food web structure. Lithostratigraphy is indicated by classical names of Black Sea units; black triangles show position of available radiocarbon dates (SI). The Holocene record of nitrogen fixation³⁶ suggests increased continental input of N when $\delta^{15}N$ is high (E). Holocene records of charcoal (F) and arboreal pollen (G) from Romanian low- and uplands³² track land use changes. Modeled deforestation rate that accounts for population history and technological advances^{40,41} is shown as percent of agricultural land in the Danube watershed in the lower basin (eastern Europe) and upper and middle basin (central Europe) (H).

consistent with the higher sediment load estimate, the Danube delta, expanded faster in its later history, which suggests a link to the intensification of land use in the watershed as the population increased and land use technology advanced¹⁷.

Radiocarbon and optically stimulated luminescence (SI) show that $\sim 40\%$ of the delta plain area was constructed over the last two millennia (Fig. 1C). To quantify this late Holocene acceleration, we assessed the sediment storage corresponding to discrete developmental phases showing that average storage rate increased over five times from the early to late Holocene (Fig. 2A). Higher fluvial sediment discharge is the most likely cause for these increasing deltaic storage rates as the delta front advanced on the shelf. We can exclude enhanced trapping by the delta in its later development

phases because the degree of exposure to wave reworking intensified toward the late Holocene as delta lobes advanced outside the sheltered Danube Bay into progressively deeper waters¹⁵. Equally so, diminished trapping along the alluvial valley and tributaries upstream of the delta can be discounted because these settings are still underfilled, providing ample accommodation space for sediments (SI). Our storage rate reconstruction shows a maximum in the last ~ 300 years during the Chilia III lobe construction, but storage rates may have increased earlier as the development of the St. George II lobe took place largely over the last millennium¹⁵ (Fig. 2A; SI). Late Holocene increases in delta plain expansion or sediment storage attributed to changes in land use have been similarly proposed for other European deltas such as the Po^{18–20} and the Rhone²¹.



Were the flux changes that led to the accelerated expansion of Danube delta in the late Holocene significant enough to affect the entire Black Sea? To address this question we used relevant proxies of past physical and biogeochemical conditions in the surface mixed layer (i.e., sea surface water salinity and abundance of major phytoplankton taxa) over the last ~9000 years (SI). Samples for analyses were collected in the western Black Sea (42°46.569'N; 28°40.647'E; 971 m water depth) on the continental slope south of the Danube mouths²². The Holocene sequence shows a typical Black Sea stratigraphy with oxic sediments at the base that were deposited in the low salinity “Black Sea” lake (Unit III), followed by an anoxic sapropel deposited after the ocean reconnection beginning at ~7,550 yrs BP (Unit II) (Fig. 2). At the top, Unit I consists of a laminated coccolith ooze deposited after ~2,560 yrs BP under conditions favorable for blooms of coccolith-bearing *Emiliania huxleyi* (Eh). This abrupt transition from sapropel to coccolith ooze suggests that an environmental threshold must have been crossed in the late Holocene²³.

Previous paleogenetic studies at our site suggested that the occurrence of coccoliths²² as well as diversification in Eh viral population²⁴ during the late Holocene may have been connected to changes in the hydrologic and nutrient regime of the Black Sea. Sea surface temperature, previously reconstructed on long-chain C₃₇ alkenones²², shows a gradual cooling from ~19°C to 15°C from ~5000 toward 500 yrs BP. The δD composition of the same components (SI), measured for the present study (Fig. 2B), indicates a gradual increase in sea surface salinity in Unit IIa after ~5000 yrs BP toward a maximum of >30 psu at ~3000 yrs BP, followed by a decrease toward modern salinities close to 20 psu that characterized the last millennium (Fig. 2B). We also measured Eh-specific C₃₆ alkenone δD composition (SI), which reinforces this sea surface salinity history and also suggests values fresher than ~20 psu between the base of Unit II and 5250 yrs BP where C₃₇ alkenones are not reliable due to a variable haptophyte assemblage²².

An independent reconstruction in the eastern Black Sea²³ also found a late Holocene decrease in surface waters salinity providing support for a general freshening of the basin during this time. Our modeled salinity history of the Black Sea (see SI) suggests that a higher fluvial influx alone would require an unrealistic increase by a factor of three to account for the late Holocene freshening. However, although direct precipitation over the Black Sea probably decreased in the late Holocene²⁵, our model suggests that reduced evaporation during the gradual cooling over the same time period²² may have also contributed significantly to the freshening (SI). In addition, an augmented freshwater budget of the Black Sea due to increased fluvial discharge and reduced evaporation could have reinforced the freshening via hydrodynamic feedbacks. For example, the Mediterranean inflow through the Bosphorus Strait is restricted when the freshwater balance is positive in the Black Sea¹³. Similarly, the mixed layer freshening inhibits mixing across the pycnocline¹² limiting the exchange of salt with the deep waters of the Black Sea. Although we cannot quantify their contributions, an increase in fluvial discharge combined with decreased evaporation appears to be the probable cause for the late Holocene freshening of the Black Sea.

Precipitation reconstructions in the Black Sea drainage basin exhibit a considerable degree of regional variability as well as large century- to millennial-scale fluctuations^{25,26}, which are not evident in the gradual decrease in Black Sea's salinity. The modeled increase in Danube's discharge¹⁶ between the coldest and wettest phase of the late Holocene – the Little Ice Age (LIA) – and the warmest and driest interval corresponding to latter half of 20th Century amounts to only ~10% of the average discharge. Similarly, modeled sediment fluxes for the Danube¹⁶ are also just marginally lower (~5%) during the LIA compared to the second half of 20th Century. This apparent lack of sensitivity implies that the transition from the warm and wet mid

Holocene to the cold and dry late Holocene cannot explain any significant increases in water and sediment delivered to the Black Sea. Intensification of colluvial²⁷, fluvial^{27,28}, deltaic^{19–21} and shelf sedimentation¹⁸ in different climate settings in Europe points instead to land cover change as the main driver of continental scale sediment fluxes. However, increased climate variability in the late Holocene probably contributed to this intense denudation as the erosion sensitivity of landscapes may have increased due to land use changes²⁹. Modern observations in large river basins^{7,30} also support the idea that a contemporaneous increase in freshwater delivery to the Black Sea probably accompanied the increase in sediment discharge, both caused by the massive human-controlled changes in land use during the late Holocene.

Historical records³¹ and land cover changes reconstructed using a scenario that accounts for both population history as well as technological advances suggest that the total deforested area in the lower Danube basin rapidly doubled after ~1,000 AD (Fig. 2H & 3). The land in use at the beginning of this rise was around 30–35% (SI). In contrast, Danube's upper and middle course were already deforested at a comparable level at the peak of the Roman Empire, 2000 years ago (Fig. 2H; SI). On the territory of modern Romania, which contributes close to 60% of the total Danube sediment load¹⁶, pollen studies suggest that tree cover diminished gradually after 3000 years ago in the lowlands (<300 m altitude), but more abruptly in the uplands in the last 1000 years³² (Fig. 2G). Biomass burning increased rapidly in the latter period, especially at altitudes higher than 800 m, but also in the lowlands³², supporting historical accounts of pasture and agricultural land expansion³¹ (Fig. 2F).

Changes in Danube's freshwater and sediment discharges should be reflected by changes in Black Sea's phytoplankton assemblages as nutrient fluxes may have also changed following anthropogenic disturbances such as forest clearance and agriculture^{2,33,34}. Exceptional preservation of organic matter in the Black Sea under anoxic deposition with no bioturbation allows us to explore these changes by identifying and quantifying past plankton taxa based on their fossil sedimentary DNA (Fig. 2 and SI). We find that major phytoplankton taxa in the Black Sea changed drastically over the last millennium. The rise in diatoms after ~500 yrs BP suggests an increase of dissolved silicate input into the Black Sea surface waters (Fig. 2C). Consistent with an increase in stratification, the chemocline was shallower during the late Holocene freshening³⁵. Based on modern circulation¹², we assume that the nutrients are well mixed in the surface layer at scales longer than multiannual but we cannot exclude Si contributions from deep mixing during winter storms especially when the chemocline was shallow. However, such deep nutrient contributions documented to occur during the past 3000 years³⁵ must have been minimal because they did not cause a concurrent rise in diatoms before 500 yrs BP. Instead, the ecological change in the last 500 years likely reflects an increased fluvial influx of dissolved silicate³⁴ as a result of rapid deforestation in the lower Danube basin (Fig. 2 and 3; ref. 32 and SI). Dinoflagellate DNA also rose drastically after ~650 years ago (Fig. 2D), which is consistent with increased nutrient availability following anthropogenic disturbances such as forest clearance and agriculture^{2,33}. Low nitrogen fixation in the Black Sea³⁶ during that period supports an augmented fluvial N influx that coincides within chronological uncertainties with the rise in dinoflagellates (Fig. 2E). The earlier dinoflagellate DNA abundance peak during deposition of Unit IIb (~7000 to 5000 years ago) probably reflects increased nutrient contributions from below the chemocline^{35,36}, although changes in phytoplankton communities linked to the gradual salinization of the basin or changes in the nutrient load of rivers draining into the Black Sea over that interval cannot be excluded. In contrast to the marine ecological changes following the rapid and extensive land use changes in the lower basin over that last ~500 years, the lack of an ecological response in the Black Sea to earlier deforestation in the upper and middle Danube basin points to

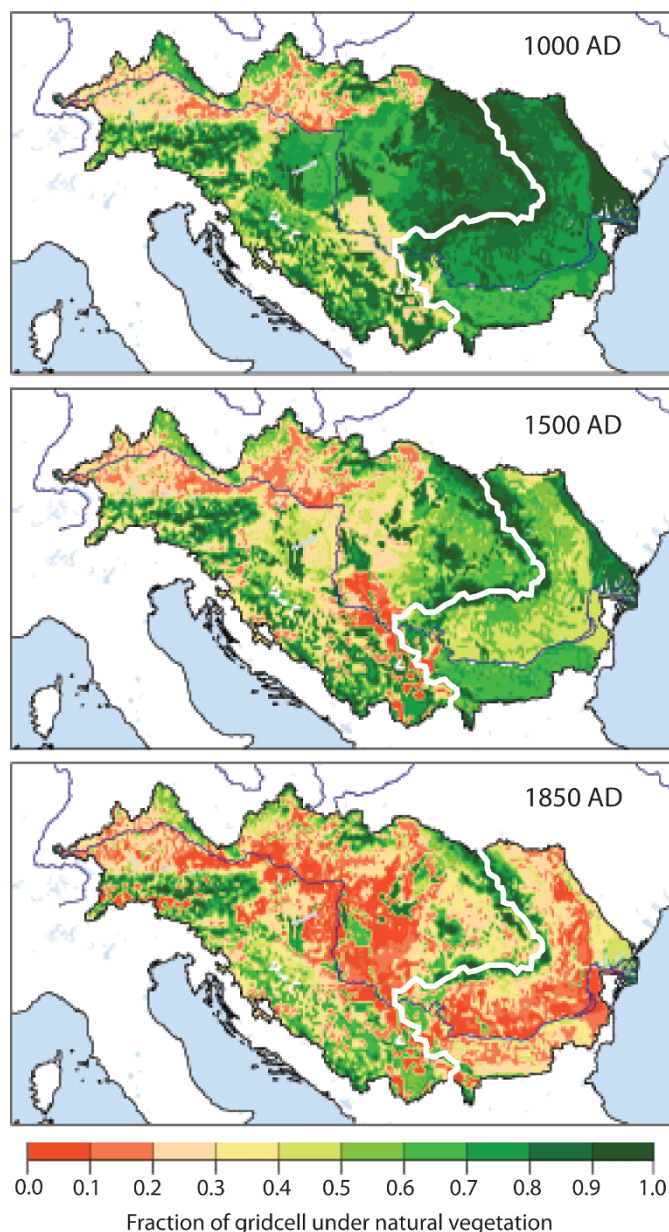


Figure 3 | Modeled land use changes in the watershed of Danube River over the last millennium (white line separates upper and middle basin from the lower basin; see SI for details on modeling). While the upper and middle basin was already heavily deforested at ~1000 A.D., rapid deforestation occurred in the lower basin after 1500 A.D. both prior as well as after the advent of the Industrial Revolution in the region after ~1850 A.D.

possible onland buffering³³ of distant and protracted land cover changes.

Discussion

Our combined sedimentary, paleoenvironmental and paleogenetic evidence suggests that land use changes transformed the Danube delta and the Black Sea well before the advent of the Industrial Revolution. Reconsidering the long term natural vs. human-induced influences should thus provide unique perspectives for management and conservation of coastal and marine resources. For the Black Sea, it remains to be assessed how changes detected in the ecosystem structure have affected biodiversity, productivity and biogeochemical cycling, but an unexpected positive legacy of this human impact

history is the Danube delta itself. The foundation of its exceptional biodiversity³⁷ is the great variety of environments including fresh-water lakes, marshes, dunes, brackish lagoons and estuarine settings that were constructed in large part during the rapid delta growth phase in the late Holocene. At the global scale, coastal settings^{28,38} such as deltas, estuaries and marshes may have been similarly, if diachronously, altered by increased denudation^{3,9}. Although the effects of ancient land use were probably more severe in marginal seas than in open ocean settings, the global role of these seas as large scale filters for material fluxes to the ocean may have been significant and remains to be assessed¹⁰.

Methods

Volumes of sediment accumulated in the Danube delta over the last ~9000 years and successive delta evolution phases¹⁵ were computed between the upper surface consisting of the modern delta plain topography and bathymetry down to ~40 m water depth and a lower surface defined by the pre-Holocene delta plain³⁹. Mass estimates use a dry density of 1.5 g cm⁻³ for water saturated mixed sand and mud with 40% porosity (see SI for details). We extracted historical estimates of land use in the Danube basin from the KK10 standard scenario of Holocene anthropogenic land cover change^{17,40,41} (SI). Changes in Black Sea salinity were modeled using a simplified time-stepping simulation of the two-way flow through the Bosphorus⁴² (see SI). The Manning equation for open-channel flow was used to describe the outflow and the Poiseuille equation for pipe flow was adopted for the inflow.

Sedimentary alkenone δD , a proxy for salinity in the Black Sea²³, was measured using an Agilent 6890 GC equipped with a DB-5MS column (60 m length; 250 μ m i.d.; 0.25 μ m film thickness; J&W, Folsom, CA, USA) coupled to a DeltaPlus XL (Bremen, Germany) isotope ratio mass spectrometer via a pyrolysis interface operated at 1440 °C (see SI). Total genomic DNA from 44 selected sediment intervals used in other (paleo)genetic studies (e.g., reference 24), served as template for PCR amplification of environmental eukaryotic 18S rRNA gene fragments (V1 to V3 region) and subsequent 454 GS FLX Titanium pyrosequencing using facilities of Engencore (Columbia, SC) and conditions as described in detail in the SI. Each 1/8 section of the 454 picotiterplate contained 15 pooled barcoded PCR products. Dereplication and taxonomic analysis of remaining high quality sequence reads (500–6000 sequence reads per sample, 360 bp in length) was done using the PyroTagger pipeline (<http://pyrotagger.jgi-psf.org/>).

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Author contributions

The project was initiated by L.G. and M.C. and designed by L.G., M.C., J.O.C. and A.K. J.O.C. and N.T. contributed the land use and hydrological modeling components respectively. Field and lab data were collected by L.G., M.C., S.C., F.F., and M.F.-M. Danube delta data were analyzed and interpreted by S.C., L.G. and F.F. L.G. wrote the paper with contributions from all authors.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests: The authors declare no competing financial interests.

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