

Rewind Annual Scientific Report

From Ancient Shipwrecks to Modern Science: Discover the Black Sea's role in preserving organic carbon in Rewind's scientific report.

November 2023

Table of Contents

Executive Summary		
1. Objectives	5	
2. Introduction	5	
2.1. Microbial respiration in aquatic systems	5	
2.2. Organic matter preservation in anoxic environments	7	
3. Study sites	8	
3.1. Black Sea	8	
3.2. Lake Kinneret	9	
3.3. Selker Noor	10	
4. Methods	11	
5. Results	16	
5.1. Black Sea	16	
5.2. Lake Kinneret	17	
5.3. Selker Noor	19	
6. Summary and Conclusions	21	
References	23	

Executive Summary

Rewind is mitigating global warming by removing CO₂ from the atmosphere. Rewind's approach is based on a natural process, where CO₂ is captured by plants, transported as plant residue through rivers to the bottom of the sea, and accumulates in deep reservoirs of organic carbon. Rewind mimics and accelerates this process by collecting agriculture & forestry residues and transporting them for storage in the deep Black Sea, where euxinic (anoxic and sulfidic) conditions in the water body favor organic matter preservation rather than decomposition. This report summarizes the findings of several in-situ decomposition rate experiments involving various organic materials. These experiments were conducted within three distinct euxinic water bodies: the Sea of Galilee (Lake Kinneret, Israel), the Selker Noor Fjord (Western Baltic Sea, Germany), and the Black Sea.

The primary objective was to assess the decomposition rates of different organic materials, given their inherent characteristics and the influence of the water chemistry in each experimental site. The experimental methodology entailed the deployment of pre-weighed bags, each filled with a unique type of organic material, to specific depths within the water bodies characterized by either oxic or euxinic conditions. By retrieving the submerged bags of organic material after predetermined intervals and measuring the changes in dry weight over time, the decomposition rate of the organic materials was quantified.

The results obtained demonstrate significant variation in the decomposition rate of each type of organic material, contingent upon both the specific properties of the organic material itself and the prevailing site conditions. Notably, in the Black Sea, decomposition slowed down to a halt after 3 months, and in all three sites, wood exhibited the slowest decomposition rate. Specifically in the Black Sea, the wood decomposition was minuscule (4% after 3 months, followed by no mass loss between 3-11 months). Furthermore, the preservation of wood and wheat was more pronounced in the euxinic conditions compared to the oxic conditions, evident from the experiments conducted in the Selker Noor and Sea of Galilee.

In conclusion, these experiments shed light on the intricate dynamics of organic matter decomposition within euxinic bodies of water, offering valuable insights for the advancement of Rewind's climate mitigation efforts and contributing to our understanding of carbon sequestration strategies.

Due to its low decomposition rate, wood was chosen as the primary organic material that will be used in Rewind's initial pilot scale deployments. In subsequent in-situ and laboratory incubation experiments, Rewind will continue measuring the decomposition rate and environmental interactions of various types of organic material.

I. Objectives

The main objective of this series of experiments was to elucidate the decomposition rate disparities of various organic materials under contrasting conditions of oxic and anoxic environments within distinct euxinic water bodies. Within this scope, we analyzed the decomposition rate of various organic substrates under controlled oxic and anoxic conditions at targeted experimental locales.

2. Introduction

The sequestration of terrestrial biomass in the deep ocean has been suggested as a potential method to combat the rapidly increasing atmospheric CO₂ concentrations (Metzger and Benford, 2001; Strand and Benford, 2009; Keil et al., 2010). Rewind proposes to further this approach by taking advantage of the enhanced preservation abilities of the oxygen-starved Black Sea, ensuring that the sunken terrestrial biomass remains sequestered on the abyssal sea floor.

2.1. Microbial respiration in aquatic systems

Microorganisms gain energy from the decomposition of organic matter coupled with reduced available electron acceptors. Under steady-state conditions, a regular sequence of solute profiles in water is created due to decreasing energy that is being yielded by microorganisms from different electron acceptors (Figure 1). Oxygenic respiration yields the largest amount of energy and is, therefore, the prevailing respiration process when oxygen is available. Other – however less efficient – electron acceptors are nitrate, manganese, and iron (Fe3), where these are available, as well as sulfate. As at the bottom of the Black Sea, neither oxygen nor nitrate exist, and manganese and iron (III) are limited, the main electron acceptor is sulfur in sulfate ions. Sulfate is usually abundant in marine waters (282 mmol/kg in normal seawater, about 140 mmol/kg in Black Sea water) and is reduced to H₂S. When even sulfate gets

exhausted and/or the sulfide concentrations exceed the transport velocity (diffusional and/or by water currents), methanogenesis comes into play where organic carbon is partly oxidized to CO_2 and partly reduced to CH_4 (disproportionation).

Both sulfate reduction and methanogenesis are the prevailing respiration processes in the anoxic part of the Black Sea. The methane that diffuses upward can be oxidized by sulfate in the sulfate-methane-transition-zone (SMTZ). This sulfate-driven anaerobic oxidation of methane (AOM) prevents most of the methane from being released to the water column and the atmosphere in marine sediments (Chistoserdova et al., 2005).



Figure 1. Schematic representation of the depth distribution of sedimentary redox-driven diagenetic zones. Electron acceptors and respiration processes by which reactants are consumed are indicated on the left. Idealized pore water profiles of reactants (O₂, NO⁻₂, and NO⁻₃) and products (NO⁻₃, Mn²⁺, Fe²⁺, H₂S, and CH₄) and associated chemical zones are shown on the right (Roberts et al., 2018).

2.2. Organic matter preservation in anoxic environments

Decomposition of organic matter in a marine environment usually occurs in one of two ways: either through oxic processes, in which oxygen acts as an electron acceptor, or via anoxic pathways in which sulfate is the primary electron acceptor (Lee, 1992). Globally, oxic decomposition processes predominantly control biomass contribution to the carbon cycle, with anoxic decomposition consuming only ~10% as much as oxic decomposition (Henrichs and Reeburgh, 1987). Under oxic conditions, organic matter decomposition rates remain high due to the high oxidative potential and weak sensitivity towards the depletion of energy-rich organic compounds. However, in anoxic environments deprived of energy-rich-organics and powerful electron acceptors like oxygen, the decomposition rate becomes therein). In the process of organic matter decomposition, the decomposition of the labile carbon will occur first, at a similar rate in both anoxic and oxic conditions. Once the most labile organic compounds have been consumed, the rate of organic matter decomposition in anoxic settings decreases much more rapidly than when oxygen is present (Westrich and Berner, 1984; Arndt et al., 2013).

The preservation of organic matter in the anoxic Black Sea is evident in the preservation of ancient woody shipwrecks. Ancient vessels have been discovered along the seafloors of the Black Sea and provide further support for the ability of anoxic conditions to preserve organic matter. In 2000, a major deepwater expedition was conducted by the Institute for Exploration in the Black Sea (Ballard et al., 2001; Brennan et al., 2013). Three shipwrecks were discovered in the oxic layers of the Black Sea, and one additional wreck was found deeper, at a depth of 324 m in anoxic waters. In comparison to the three ships in the shallower surface waters, "The ship found within the anoxic layer was intact, in a high state of preservation, and dated to the Byzantine period of 450 A.D." (Ballard et al., 2001).

3. Study sites

3.1. Black Sea

The Black Sea (Figure 2) is a unique marine environment, representing the largest landlocked basin in the world. The shelf and a steep continental slope are dissected by canyons (Ryan et al., 2003; Gunduz et al., 2020). The Black Sea is over 2,200 meters deep at its deepest part. It has a deep abyssal plain that encompasses over 60% of its area. The Black Sea is connected to the Marmara and Aegean Seas through the narrow and shallow Bosporus and Dardanelles Straits, respectively. The freshwater inputs from several large rivers results in the formation of a shallow low salinity layer overlying the salty waters below and results in stable stratification of the water column. The Black Sea is vertically stratified, and most of its volume is completely anoxic. The upper oxic layer encompasses only the uppermost 150-200m (i.e. less than 15% of the water volume). The main effect of such stratification is that the low salinity surface layer (0~150m) is oxygenated, influenced by the atmosphere, and presents a

pronounced seasonal variability Grégoire and Soetaert, 2010; Capet et al., 2012; Stanev et al., 2019; Stanev and Chtirkova, 2021). In contrast, the dense deeper layer (>150m) is rich in sulfides (Murray et al., 2007), also known as euxinic. This density gradient (seawater is denser and thus heavier than freshwater) impedes mixing and is the origin of the oxic-anoxic interface (Buesseler et al., 1994).



Figure 2. The Black Sea, the location of the decomposition experiment (black star).

Rewind

3.2. Lake Kinneret

Lake Kinneret (Figure 3) is a monomictic lake, a lake that mixes from top to bottom during one mixing period each year, located in the north of Israel, with maximum and average depths of 42 and 24 m, respectively. Spring is characterized by the development of phytoplankton blooms in the epilimnion, supported by the nutrient contribution from its catchment area. During this time, the continuous heating of the surface water initiates thermal stratification and the formation of a thermocline (Zohary et al., 2012). In May, as the bloom declines, the hypolimnion becomes enriched with particulate organic carbon, which is utilized by heterotrophic microorganisms, thus gradually depleting oxygen and nitrate until sulfate becomes the dominant electron acceptor (Eckert and Conrad, 2007).



Figure 3. Lake Kinneret and location of the decomposition experiment (black star).

3.3. Selker Noor

The Selker Noor (Figure 4), situated in northern Germany, is a lacustrine body covering a surface area of 0.558 km². Notably, the Selker Noor is subject to substantial nutrient influx originating from surrounding agricultural activities, instigating heightened phytoplankton productivity and fostering organically enriched anoxic sediments. The water column of the Selker Noor displays stratification, with exceptions during periods of wind-induced mixing, provoking rapid dissipation of dissolved oxygen within deeper layers. Consequently, the lower strata of the Selker Noor's water column persistently remain in an anoxic state.



Figure 4. Selker Noor and locations of the decomposition experiment (black stars).

4. Methods

The investigation entailed a series of experiments to assess the decomposition rate of organic matter in the anoxic aquatic environments of Lake Kinneret, the Black Sea, and the Selker Noor. To achieve this, mesh bags filled with different types of organic materials were submerged at varying depths in the respective anoxic layers (see Figures 5-7), with corresponding control samples deployed at oxic depths at Lake Kinneret and Selker Noor.

Oxygen and sulfide concentrations were measured to ensure oxic/anoxic conditions in the water column. At Lake Kinneret and in the Selker Noor, oxygen levels were measured at every visit, while in the Black Sea, a vertical profile (dissolved oxygen, sulfides) was examined only once.

Samples (mesh bags) were retrieved from each of these 3 water bodies at specific intervals. At the experimental sites, the organic materials tested within the bags were similar to facilitate cross-comparisons across the experiments. Table 1 provides comprehensive details concerning the types of organic materials used in each experiment, the depths at which the experiments were conducted, and the extraction intervals.

Experimental site	Organic material	Extraction intervals	Oxic depth	Anoxic depth
Black Sea	Oak, corn, vine, and wheat	1, 3, and 11 months	-	250-270 m
Lake Kinneret	Pine chips, leek, and wheat	1, 2.5, 4, 5, and 6.5 months	8 m	32 m
Selker Noor	Pine chips, leek, and wheat	1, 2, and 3 months	1 m	5 m

Table 1.

The organic materials used in each decomposition experiment, the extraction intervals, and the deployment depths.



Figure 5. Left- Bags filled with organic matter prior to their deployment in the Black Sea, off-shore Georgia. Right- wheat before deployment in the Black Sea.





Figure 6.

6.a Boxes and organic material prior to deposition

6.b Boxes filled with organic matter following their retrieval after 6.5 months in the water. The clean set of boxes on the left was placed in the anoxic layer (32 m depth), while the fouled set of boxes was set in the oxic layer (8 m depth).



Figure 7. Different types of organic matter used in the decomposition experiment in the Selker Noor. Left to right: Leek, wheat, wood chips.

The experimental apparatus (rigs) used in the Black Sea was designed differently to accommodate for deposition and retrieval at 250 m depth. It consisted of a rope connected to a sinker via an acoustic release device, at one end and a float at the other end. The bags filled with organic material were tied to the rope one after the other (Figure 8). The rigs were retrieved by sending an acoustic signal to the acoustic release device, causing the device to disconnect the rope from the sinker, allowing the sample to float to the surface.



Figure 8. Illustration of Black Sea rigs and floatation mechanism. Based on original image by Sub Sea Sonic.

To quantify the rate of organic matter decomposition at each experimental site, the organic materials within the bags were subjected to thorough drying in an oven at a controlled temperature of 60° C (Figure 9) until stable weight was achieved. The remaining fraction of organic material in each bag was then calculated by comparing the dry weight of the bag to the assumed initial weight before deployment. The assumed initial weight was estimated by drying several representative samples of each organic matter type before deposition.



Figure 9. Drying organic materials in the oven at 60° C following their retrieval from the sea

5. Results

5.1. Black Sea

The decomposition experiment in the Black Sea was conducted offshore from Georgia. In this experiment, bags filled with oak, corn, wheat, and vine were deployed to 250m depth, where euxinia occurs (Figure 10). The bags were retrieved after 1, 3, and 11 months. The results of the decomposition experiment (Figure 11) show that oak preserves best (~4% mass loss), followed by vine branches, corn stems, and wheat straw (14%, 46%, and 64% mass loss, respectively). The mass loss rate was the fastest in the first months, followed by a more moderate mass loss rate between 1-3 months. The initial mass loss could be due to the dissolution of soluble compounds (sugars, etc.) and/or decomposition of labile organic matter. For all organic materials besides corn, no mass loss occurred between 3-11 months, which suggests that decomposition, if it occurs at all, is extremely slow.







Figure 11. Black Sea decomposition experiment results. The experiment was conducted in anoxic conditions.

5.2. Lake Kinneret

The decomposition rate experiment in Lake Kinneret was conducted in the center of the lake (Figure 3), at "Station A", as labeled by the Kinneret Research Institution. In this experiment, boxes filled with Pine chips, wheat straw, and leek were deployed to depths of 8 m and 32 m (oxic and anoxic conditions, respectively; Figure 12).

The bags were retrieved after 1, 2.5, 4, 5, and 6.5 months. The results of the decomposition experiment (Figure 13) show that wood preserves best in anoxic conditions (10% mass loss after 6.5 months), followed by wheat (80% mass loss after 6.5 months), and leek (100% mass loss 4.5 months).

The comprehensive loss of mass observed for leek may be attributed to two primary mechanisms: the expeditious decomposition of leek material and its subsequent disintegration into small particles, influenced by chemical and/or biological processes. Subsequently, these particles may undergo elution from the mesh bag. For wheat and leek, the mass loss rate



decreased with time, as the majority of the mass loss occurred in the first two months, followed by a more moderate mass loss rate.

In addition, Figure 13 shows that the decomposition rate of wood (slowest decomposition) was similar in both the oxic and anoxic conditions, though anoxic decomposition was slightly slower. The decomposition of leek (fastest decomposition) was complete after 2.5 months in oxic conditions and after 4 months in anoxic conditions.

The comparative mass loss of wheat in oxic and anoxic conditions was the most disparate; in anoxic conditions, the remaining biomass of wheat was 26%, while in oxic conditions the remaining biomass was <1% (Figure 13).



Figure 12. Lake Kinneret oxygen concentration in the water column during the experiment. Bag deployment depths are marked with a black star.



Figure 13. Lake Kinneret decomposition experiment results. The experiment was conducted in oxic and anoxic conditions.

5.3. Selker Noor

The decomposition rate experiment in Selker Noor was conducted in the center (deep) and northern part (shallow) of the fjord (Figure 4). In this experiment, bags filled with Pine chips, wheat straw, and leek were deployed to 1 and 4 m depth (oxic and anoxic conditions, respectively; Figure 14). The bags were retrieved after 1, 2, and 3 months. The results of the decomposition experiment (Figure 15) show that wood was preserved best in anoxic conditions (4% mass loss after 3 months), followed by wheat and leek (56% and 94% mass loss after 3 months, respectively). For wheat and leek, the mass loss rate decreased with time, as the robust mass loss occurred in the first month, followed by a more moderate mass loss. In addition, Figure 15 shows that the mass loss rate of both wood and wheat was slower in the anoxic conditions compared to the oxic conditions.





Figure 14. Selker Noor oxygen concentration in the water column during the experimental period. Experimental depths are marked with a black star.



Figure 15. the Selker Noor decomposition experiment results. The experiment was conducted in oxic and anoxic conditions.

6. Summary and Conclusions

This report presents the results of anoxic decomposition rates measurements concerning various organic materials through a series of well-structured decomposition experiments conducted in the anoxic waters of the Black Sea, Lake Kinneret, and the Selker Noor. Moreover, in Lake Kinneret and Selker Noor, a control experiment was carried out to ascertain the decomposition rates of organic materials under oxic conditions, facilitating a comprehensive comparison with their anoxic decomposition rates.

The experimental findings distinctly reveal that diverse types of organic materials exhibited dissimilar decomposition rates. Notably, wood, enriched with lignin content (22% lignin in the oak wood used in the Black Sea decomposition experiment) exhibited the highest preservation potential across all three sites (97%, 90%, and 96% preservation in the anoxic water of the Black Sea, Sea of Galilee, and Selker Noor, respectively). Vine branches, which contain intermediate values of lignin (17% lignin in the vine branches used in the Black Sea decomposition experiment), exhibited intermediate preservation potential (86% preservation). On the other hand, "soft" plant materials, which are poor in lignin, such as leeks, corn (7% lignin; Zawawi et al. 2013), and wheat (11-26% lignin; Zhang et al. 2022), displayed more rapid decomposition rates.

The decomposition experiments in the Sea of Galilee and Selker Noor, where similar organic materials were deployed in both oxic and anoxic conditions, showed that the overall decomposition rate was generally accelerated under oxic conditions for all examined organic materials. The organic materials used in those decomposition experiments showed a similar preservation order, as the highest preservation was observed for pine, followed by wheat and leek. In these experiments, the differences in biomass loss between the oxic and anoxic condition rates between oxic and anoxic conditions compared to wheat, which demonstrated a high disparity in

Rewind

Annual Scientific Report

decomposition rates (30% higher preservation in anoxic conditions). Overall, the decomposition rate on all examined organic materials was slower in the Sleker Noor compared to the decomposition rate in the Sea of Galilee. The slower decomposition in the Selker Noor is probably due to lower temperatures of the water column (12-25°C and 16-29 °C in the Selker Noor and Sea of Galilee, respectively), as microbes are more active as the temperature increases , up to a certain point.

While the described series of experiments showed the minor decomposition of wood in anoxic environments only for a period shorter than one year, millennial-scale evidence for wood preservation in anoxic conditions is the well-preserved woody shipwrecks, dated to the 5th Century AD, and found buried in anoxic sediments (Liphschitz, 2012). Organic matter deployment to anoxic aquatic systems, and particularly to the Black sea, could mitigate global warming by sequestering CO₂ to a period of potentially thousands of years.

To further advance our understanding of wood decomposition in anoxic conditions, our research aims to undertake in-situ decomposition rate experiments in the deep regions of the Black Sea (>1000 m). Moreover, we intend to closely monitor the impact of wood decomposition on the chemical properties of the surrounding water, shedding light on the broader environmental consequences of this process. Through these concerted efforts, we aspire to contribute comprehensively to the scientific understanding of carbon sequestration and ecological dynamics within the Black Sea ecosystem.

References

Arndt, S., Jørgensen, B. B., LaRowe, D. E., Middelburg, J. J., Pancost, R. D., & Regnier, P. (2013). Quantifying the degradation of organic matter in marine sediments: A review and synthesis. Earth-science reviews, 123, 53-86.

Ballard, R. D., Hiebert, F. T., Coleman, D. F., Ward, C., Smith, J. S., Willis, K., & Torre, F. (2001). Deepwater archaeology of the Black Sea: the 2000 season at Sinop, Turkey. American journal of archaeology, 105(4), 607-623.

Brennan, M. L., Davis, D., Roman, C., Buynevich, I., Catsambis, A., Kofahl, M., & Duman, M. (2013). Ocean dynamics and anthropogenic impacts along the southern Black Sea shelf examined through the preservation of pre-modern shipwrecks. Continental Shelf Research, 53, 89-101.

Buesseler, K. O., Livingston, H. D., Ivanov, L., & Romanov, A. (1994). Stability of the oxic-anoxic interface in the Black Sea. Deep Sea Research Part I: Oceanographic Research Papers, 41(2), 283-296.

Capet, A., Barth, A., Beckers, J. M., & Marilaure, G. (2012). Interannual variability of Black Sea's hydrodynamics and connection to atmospheric patterns. Deep Sea Research Part II: Topical Studies in Oceanography, 77, 128-142

Chistoserdova, L., Vorholt, J. A., & Lidstrom, M. E. (2005). A genomic view of methane oxidation by aerobic bacteria and anaerobic archaea. Genome biology, 6(2), 1-6.

Eckert, W., & Conrad, R. (2007). Sulfide and methane evolution in the hypolimnion of a subtropical lake: a three-year study. Biogeochemistry, 82, 67-76.

Froelich, P., Klinkhammer, G. P., Bender, M. A. A., Luedtke, N. A., Heath, G. R., Cullen, D., & Maynard, V. (1979). Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochimica et cosmochimica acta, 43(7), 1075-1090.

Grégoire, M., & Soetaert, K. (2010). Carbon, nitrogen, oxygen and sulfide budgets in the Black Sea: A biogeochemical model of the whole water column coupling the oxic and anoxic parts. Ecological Modelling, 221(19), 2287-2301.



Gunduz, M., Özsoy, E., & Hordoir, R. (2020). A model of Black Sea circulation with strait exchange (2008–2018). Geoscientific Model Development, 13(1), 121-138.

Henrichs, S. M., & Reeburgh, W. S. (1987). Anaerobic mineralization of marine sediment organic matter: rates and the role of anaerobic processes in the oceanic carbon economy. Geomicrobiology Journal, 5(3-4), 191-237.

Lee, C. (1992). Controls on organic carbon preservation: The use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems. Geochimica et Cosmochimica Acta, 56(8), 3323-3335.

Liphschitz, N. (2012). Dendroarchaeology of shipwrecks in Israel. Bocconea, 24, 95-104.

Ryan, W. B., Major, C. O., Lericolais, G., & Goldstein, S. L. (2003). Catastrophic flooding of the Black Sea. Annual Review of Earth and Planetary Sciences, 31(1), 525-554.

Stanev, E. V., & Chtirkova, B. (2021). Interannual change in mode waters: Case of the Black Sea. Journal of Geophysical Research: Oceans, 126(2), e2020JC016429.

Stanev, E. V., Peneva, E., & Chtirkova, B. (2019). Climate change and regional ocean water mass disappearance: Case of the Black Sea. Journal of Geophysical Research: Oceans, 124(7), 4803-4819.

Stewart, K., Kassakian, S., Krynytzky, M., DiJulio, D., & Murray, J. W. (2007). Oxic, suboxic, and anoxic conditions in the Black Sea. The Black Sea flood question: Changes in coastline, climate, and human settlement, 1-21

Westrich, J. T., & Berner, R. A. (1984). The role of sedimentary organic matter in bacterial sulfate reduction: The G model tested 1. Limnology and oceanography, 29(2), 236-249.

Zawawi, D., Mohd, Z., Angzzas, S., Halizah, A., & Ashuvila, M. A. (2013). Analysis the chemical composition and fiber morphology structure of corn stalk. Australian Journal of Basic and Applied Sciences, 7(9), 401-405.

Zhang, L., Larsson, A., Moldin, A., & Edlund, U. (2022). Comparison of lignin distribution, structure, and morphology in wheat straw and wood. Industrial Crops and Products, 187, 115432.

Zohary, T., Nishri, A., & Sukenik, A. (2012). Present–absent: a chronicle of the dinoflagellate Peridinium gatunense from Lake Kinneret. Phytoplankton responses to human impacts at different scales, 161-174.