Redox reactions and weak buffering capacity lead to acidification in the Chesapeake Bay

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The combined effects of anthropogenic and biological CO₂ inputs may lead to more rapid acidification in coastal waters compared to the open ocean. It is less clear, however, how redox reactions would contribute to acidification. Here we report estuarine acidification dynamics based on oxygen, hydrogen sulfide (H₂S), pH, dissolved inorganic carbon and total alkalinity data from the Chesapeake Bay, where anthropogenic nutrient inputs have led to eutrophication, hypoxia and anoxia, and low pH. We show that a pH minimum occurs in mid-depths where acids are generated as a result of H₂S oxidation in waters mixed upward from the anoxic depths. Our analyses also suggest a large synergistic effect from river-ocean mixing, global and local atmospheric CO₂ uptake, and CO₂ and acid production from respiration and other redox reactions. Together they lead to a poor acid buffering capacity, severe acidification and increased carbonate mineral dissolution in the USA’s largest estuary.
Anthropogenic carbon dioxide (CO$_2$) has increased more rapidly in the atmosphere since the Industrial Revolution than natural CO$_2$ increase in any period of the last ~800,000 years$^{1-5}$; consequently, it has been known that the uptake of CO$_2$ by the ocean has altered surface seawater acid-based chemistry lowering pH by about 0.1 unit and calcium carbonate saturation state by roughly 0.5. This process, known popularly as ocean acidification (OA) for over a decade, will continue to decrease seawater pH by about 0.3 units by the end of the century$^{3,4}$. It is likely that OA will cause detrimental effects on the health of marine organisms and ecosystems and alter the associated biogeochemical processes$^{5-7}$.

Recent research indicates that eutrophication can exacerbate OA, where respiratory processes contribute a far greater acidification in the coastal oceans relative to the open ocean$^{8-13}$. Coastal eutrophication occurs with increased inputs of nutrients from the application of chemical fertilizers, discharges of human and animal wastes, and atmospheric NO$_x$ inputs from fossil fuel burning, which have fueled large algal blooms in many coastal water bodies, especially those near population centers$^{14}$. It is well known that decomposition of algal organic matter from highly productive surface water leads to the development of seasonally low oxygen (hypoxic) or even zero oxygen (anoxic) bottom waters in many coastal water bodies in the world$^{15,16}$. However, the coupling between redox and acid–base chemistry has not been explored extensively in seasonally anoxic and partially mixed estuaries nor in permanently anoxic deep basins although redox chemistry and pH have been reported before in the latter$^{13,17-22}$. Specifically, it is not known how subsurface water pH dynamics are influenced by anaerobic respiration and the oxidation of reduced chemical species (notably H$_2$S) in seasonally low oxygen (O$_2$) estuaries around the world let alone the interaction of these processes with the anthropogenic CO$_2$ induced OA.

The Chesapeake Bay is the largest estuary in the United States with a well-documented history of eutrophication over the past half century$^{23-25}$. A recent report demonstrates that some regions of the bay have suffered a long-term pH decline related to eutrophication$^{26}$. However, few process studies have examined the CO$_2$ system and pH in the Chesapeake, and those that exist have focused on tributaries in the southern reaches of the estuary$^{27,28}$. To address the coupling between acid–base chemistry and redox chemistry and its contribution to coastal OA, we sampled the water column repeatedly for several days within a deep basin of the main-stem bay in August 2013 and 2014, a time of peak hypoxia and anoxia, and these data were supplemented with an April 2015 (pre-hypoxia) study. In this paper, we report and explain the occurrence of a pH minimum at and above theoxic–anoxic boundary due to H$_2$S oxidation. We further demonstrate how a combination of processes drives down pH and aragonite mineral saturation state, leading to CaCO$_3$ mineral dissolution in subsurface waters. Finally, we present a general geochemical model to explain why large eutrophic estuaries, exemplified by the Chesapeake Bay, are particularly vulnerable to the acidification stresses caused by the increase of anthropogenic atmospheric CO$_2$ and aquatic eutrophication and respiration.

**Results**

Acidification due to eutrophication-induced local CO$_2$ uptake. Partial pressure of carbon dioxide (pCO$_2$) in surface waters of the Chesapeake Bay exceeded 1600 μatm in its upper reach and was below the atmospheric level (~390 μatm) in the mid-bay for all three cruises between spring and summer (Fig. 1a, b, Table 1, Supplementary Fig. 1, and Methods). The low pCO$_2$ was accompanied by high chlorophyll-a, a phytoplankton biomass proxy, and supersaturated dissolved O$_2$ for much of the year in the mid- and lower-bay (Supplementary Figs. 2–5), indicating net biological production fueled by high riverine nutrient loading. The low surface pCO$_2$ should lead to atmospheric CO$_2$ invasion

![Fig. 1 Measured surface water pCO$_2$ overlapped on a site map of the Chesapeake Bay.](image-url)
and may contribute to water column CO₂ accumulation and acidification, particularly given atmospheric concentrations are ∼40% greater than the pre-industrial and bay has a long water residence time of 100 days²⁹,³⁰. While complete water column mixing and destratification occurs occasionally during storms²⁹,³⁰, smaller wind events more frequently mix water and chemical species down to middle depths (Fig. 2a, Supplementary Figs. 6 and 7). Turbulence in the tidally driven bottom boundary layer will then mix the chemical species in the bottom water³¹. We have estimated the air-to-water CO₂ flux and its impact on water column total dissolved inorganic carbon (DIC) and pH over the period of spring to summer (Table 1). To calculate the effect of DIC increase on bottom water pH decrease, we have modified the popularly used CO2SYS program to include H₅S–HS⁻ and NH₃–NH₄⁺ species in the acid–base equilibrium calculations as the bottom water in August contains these reduced chemical species (Methods). The resulting bottom-water pH decreases (0.08–0.13 over the spring-summer period; Table 1) are significant when compared with pH decrease due to CO₂ uptake from the atmosphere in the open ocean (0.11). However the time scales of acidification due to local CO₂ uptake (months) are much shorter than open ocean uptake (decadal to centennial).

We also note that acidification induced by local CO₂ uptake is caused by both increased atmospheric CO₂ and coastal eutrophication. This is in sharp contrast with the CO₂ uptake in the open ocean where atmospheric forcing is comparable to the coastal ocean but biological CO₂ removal and physical mixing are less intense or frequent. Clearly, climate change, anthropogenic

### Table 1 Air-sea CO₂ flux and its impact on bottom-water DIC and pH in the middle Chesapeake Bay

| Month Year | Air pCO₂ (µatm) | Water pCO₂ (µatm) | Wind speed (m s⁻¹) | CO₂ flux (mmol m⁻² d⁻¹) | ΔDIC (µmol kg⁻¹) | ΔpH |
|------------|----------------|------------------|-------------------|--------------------------|-----------------|-----|
| August 2013 | 380.2 ± 11.2 | 309.1 ± 99.3 | 4.4 ± 2.3 | −4.3 ± 0.4 | 21.2 | −0.081 |
| August 2014 | 373.1 ± 9.1 | 251.6 ± 70.9 | 4.2 ± 2.2 | −6.7 ± 0.7 | 33.1 | −0.13 |
| April 2015  | 409.1 ± 8.8 | 341.3 ± 116.6 | 5.8 ± 3.6 | −6.6 ± 0.9 | 32.6 | −    |

The mid-bay region is defined as the area between 37.9 and 39.0° N. Monthly averaged wind data were calculated from National Data Buoy Center station COVM2-8577018 at Cove Point LNG Pier, MD.

Fig. 2 Vertical distributions of measured chemical properties at the focused study site in August 2013. a) Salinity, b) total alkalinity (TA), c) dissolved inorganic carbon (DIC), d) dissolved oxygen, e) pH (25 °C and NBS scale), and f) H₂S concentration. See Fig. 1 for location (station 858). The lines are the lower and upper boundaries between days 1 and 2 and days 3–5, respectively. August 2014 data are presented in Supplementary Fig. 8 for comparison and affirmation of the 2013 observations.
inputs, and natural processes have jointly altered the carbon cycle and stressed aquatic environments in the coastal zone.

The spatial gradients of pCO₂ observed here and inferred net autotrophy are consistent with prior investigations using oxygen-based approaches to measuring primary production and respiration. Kemp et al. concluded that the Chesapeake Bay was net autotrophic overall, but heterotrophic conditions (where respiration exceeded photosynthesis) prevailed in low-salinity regions where we measured supersaturated pCO₂ (Fig. 1a, b, Supplementary Fig. 1) and O₂ was consistently undersaturated (Supplementary Figs. 2b, 3–5). Dissolved O₂ tends to be undersaturated in northern regions of the bay given high respiration rates associated with external loads of organic carbon.

These heterotrophic conditions gave way to a near balanced and an autotrophic metabolism in the mid- and lower-bay, leading to a mean, bay-wide net ecosystem production, which is consistent with strong under saturation of pCO₂ in these seaward regions (Fig. 1a, b and Supplementary Fig. 1). The mid- and lower-bay stations (CB3.3C and south) tended towards O₂ supersaturation during most months of the year, especially during the warmer months (Supplementary Figs. 2b, 4 and 5).

Despite some interannual variability in the seasonal pattern of dissolved O₂ saturation, the years of 2013–2015 indicate similar seasonal patterns. Oxygen-based estimates of metabolism showed consistent surface-layer net O₂ production and bottom-layer net O₂ consumption, the rates of which were highly correlated. Although oxygen-based methods could not be applied under oxygen-depleted conditions, independent measures of sulfate reduction (SR) in sediments, which dominated the benthic metabolism during warm months and led to significant sediment–water sulfide fluxes in the mid-bay, clearly support the accumulation of sulfide observed in August 2013 (Fig. 2f).

Subsurface pH minimum due to oxidation of reduced chemicals. Repeated vertical profiles during both summers revealed a consistent pH minimum below the surface mixed layer at our focused study station, a deep site in the upper part of the mid-bay. Salinity profiles at this site (Fig. 2a) combined with a time series of wind speed indicate a physical mixing event before our first sampling on August 9, 2013. Stratification quickly re-established when wind speed reduced and the wind direction switched from favoring mixing to favoring stratification (Supplementary Fig. 6a, b). Total alkalinity (TA) and DIC were lower in the surface, but became higher in the bottom water (Fig. 2b, c). Dissolved O₂ was at saturation or supersaturation in the surface due to gas exchange and biological production and was not detectable below 10–15 m depths due to respiration (Fig. 2d).

On day 1, the mixed layer depth was still as deep as 15–18 m, but within 2 days, it shoaled to 10 m (Fig. 2a). Following this dynamic change, the O₂ penetration depth changed from about 15 m on day 1 to about 10 m on days 3–5 (Fig. 2d). Simultaneously, water column pH (25 °C and NBS scale) decreased greatly over this period (Fig. 2e). For example, at the depth of 6 m, pH decreased from nearly 8.0 on day 1 to ~ 7.5 on days 3–5. A pH minimum (7.35 ± 0.03) occurred at 11–13 m depth in the low O₂ zone (<10 μmol kg⁻¹), below which pH increased slightly and then became constant at 7.45 ± 0.02. This pH minimum and the associated rapid pH decrease above it within a short period of <2 days have not been previously documented, although large pH changes were observed or expected in many strongly productive or stratified shallow water systems. Such dramatic decreases in both O₂ and pH over just 2 days could likely put the biological system under stress. This pH minimum was also observed in August 2014, though water column O₂ and pH profiles in August 2014 were relatively stable before and during the 5-days cruise, as wind speeds were less strong and less variable than those of the 2013 (Supplementary Fig. 7a, b), the oxycline (where O₂ decreases rapidly) and the pH minimum were even sharper and shallower, and bottom-water pH, DIC and TA were lower in 2014 than 2013 (Supplementary Fig. 8).

We suggest the oxidation of reduced chemicals is responsible for the pH minimum in the low O₂ zone and the rapid pH decrease above it where declining O₂ gradients were steepest (Fig. 2c). The coupling between acid–base and redox chemistry is described by the following formula:

\[
\begin{align*}
\text{H}_2\text{S} + 2\text{O}_2 &= \text{2H}^+ + \text{SO}_4^{2-} \quad (1) \\
\text{NH}_4^+ + 2\text{O}_2 &= \text{2H}^+ + \text{NO}_3^- + \text{H}_2\text{O} \quad (2) \\
\text{Mn}^{2+} + 0.5\text{SO}_2 &= \text{2H}^+ + \text{MnO}_2 \quad (3) \\
\text{Fe}^{2+} + 0.25\text{O}_2 + 1.5\text{H}_2\text{O} &= \text{2H}^+ + \text{FeOOH} \quad (4)
\end{align*}
\]

In August 2013, because of the strong mixing event prior to our cruise, the total concentration of H₂S was only 5 μmol kg⁻¹ at the 20 m depth on day 1, but it rapidly increased to 30–40 μmol kg⁻¹ on days 3–5 when the water column was restratified (Fig. 2f). Oxidation of other reduced chemicals accumulating in the bottom water could also have contributed to the formation of the pH minimum. NH₄⁺ concentration measured near our site was 15–20 μmol kg⁻¹ at 20 m (Supplementary Fig. 9). Also, during days 3–5, bottom water [Mn²⁺] and [Fe²⁺] became as high as 7 and 2 μmol kg⁻¹, respectively. When these reduced species (total concentration ~ 60 μmol kg⁻¹) were mixed upward into oxygenated water, they were oxidized, hydrogen ions were generated. TA was decreased and thus the water became more acidified (see Eqs. (1)–(4)). However, we recognize the oxidation of reduced species are often complex involving many intermediate steps and side products and could have different H⁺ production ratios.

It has been shown that oxidation of H₂S by O₂ is sufficiently slow that H₂S can be brought near to the surface during vigorous mixing events and lead to fish kills in coastal waters. Similarly, ammonia oxidation is not instantaneous. We suggest that the slow oxidation kinetics and rapid mixing facilitate the transport of reduced species and can subsequently result in acidification of the oxygenated near- and sub-surface waters, potentially resulting in a negative impact on aquatic organisms. If, for example, one volume of bottom water of 60 μmol kg⁻¹ of reduced chemicals is mixed with one volume of sufficiently oxygenated water, the resulting mixed water has the total concentration of the reduced chemicals halved to 30 μmol kg⁻¹, and eventually ~ 60 μmol kg⁻¹ of acid (or −ΔTA) would be generated due to the oxidation of the reduced chemical species (Eqs. (1)–(4)). Based on CO2SYS simulations, the predicted pH decrease due to these oxidation reactions can be up to 0.20 pH units in Chesapeake Bay waters, although other mixing ratios and incomplete reactions due to slow kinetics may generate less of a pH decrease (Methods). This pH decrease is substantial and is consistent with our observations (Fig. 2e; also see a model simulation of TA, DIC, O₂, and pH evolving loci below). Note that while the size and location of this pH minimum may vary depending on the strength of the physical mixing and [H₂S] in the bottom water, it occurs whenever bottom-water anoxia exists regardless of whether a prior severe mixing event has occurred as in our 2013 study, because moderate mixing occurs constantly in the bay (Supplementary Fig. 7).
In sediment porewater, a pH minimum was reported at and above the O2 penetration depths as a result of oxidation of reduced chemicals, which diffused upward from deeper, anoxic depths42, 43 and was predicted by sediment diagenetic models14, 44. Such a pH minimum was also seen in low O2 waters of permanently stratified and anoxic deep basins including the Baltic Sea13, 17, the Black Sea18, 19, the Framvaren Fjord21, the Hunnabunn Fjord20, and the Carico Basin22 though no one has pointed out this phenomenon except Yao and Miller21 who commented that “the low pH is difficult to explain”. The pH minimum is an interesting feature that results from the decrease in TA:DIC ratio due to acid production during oxidation of reduced chemicals when encountering free O2 due to vigorous physical mixing. To our knowledge, this is the first time that such a pH minimum has been reported and properly interpreted in the water column. We predict that the pH minimum should occur in all oceanic systems that have seasonally or permanently occurring oxic–anoxic boundaries, including the above mentioned cases as well as in the dead-end canals of Delaware Inland Bays36, Lake Grevelingen (the Netherlands)12, the Saanich Inlet46, and estuaries and bays elsewhere17, 20. We further argue that the pH minimum is likely more dynamic in seasonally anoxic coastal systems than permanently anoxic deep basins, due to the shallower water depth and higher frequency of physical disturbances. Physical disturbances such as winds and tides occur regularly in the Chesapeake Bay (Supplementary Fig. 7a, b) and other seasonally stratified coastal waters12, 36. Therefore, their chemical and biological consequences, in the context of coastal OA and deoxygenation, deserve further attention.

**Geochemical drivers and carbonate dissolution.** To separate biological processes from physical mixing and to explore the biogeochemical control mechanisms in a broader context, we examine TA and DIC vs. salinity relationships at this site together with data from other areas of the bay and the river and offshore endmembers (Fig. 3a). Between the river and ocean endmembers, as expected, TA and DIC increased with salinity. However, at our focused study station, all subsurface and bottom-water samples were located well above the mixing lines, indicating net release of CO2 and accumulation of DIC and TA. In addition, both DIC and TA data collected at the Chesapeake Biological Laboratory (CBL) dock, downstream of our focused study site at the lower end of the mid-bay, were also above the mixing lines. Those from the Virginia Institute of Marine Science (VIMS) dock, farther downstream near the bay mouth, however, showed the least enrichment relative to the conservative mixing lines. We also calculated the acid–base buffer factors from TA, DIC, and nutrients (PO43–, H2S, and NH4+) (Methods). It is clear the bay waters are poorly buffered as indicated by their much lower buffer factors compared to offshore waters here and elsewhere (Fig. 3b; also see next section for definitions and explanations).

TA is usually a good conservative tracer of river–ocean mixing within an estuary because it is not influenced by CO2 addition and removal. Because TA and DIC share a common major component (HCO3–), deviations of DIC from the nearly conservative behavior defined by TA and salinity provide a measure of biological use or release of CO247. Bottom waters in the Chesapeake Bay, however, are conspicuously different from this general geochemical behavior normally encountered in oxygenated or moderately low oxygen environments8, 9, 47. DIC not only show a large enrichment against the conservative mixing line, but TA is also substantially enriched; with the excess DIC and TA reaching 275.3 ± 59.5 and 167.3 ± 54.2 μmol kg−1 respectively (Fig. 3a, Methods).

In any estuary, the most important internal sources of TA and DIC are aerobic respiration (AR), SR, and carbonate dissolution (CD)12, 13, 21, 45 (Fig. 4a, Table 2). Because each of these processes has a distinctly different ΔTA to ΔDIC ratio and involves a different pH change (Table 2), ΔTA:ΔDIC ratio and pH change become diagnostic of the geochemical processes. Based on the mixing line prediction, we can calculate the initial DIC and TA values at salinity (S) = 10 g kg−1 for surface water and S = 20 g kg−1 for bottom water. From the solubility constants we can also determine the initial concentrations of O2 in S = 10 and 20 g kg−1 waters. Assuming the bottom water starts with a fully saturated dissolved O2, we can then derive DIC and TA generations and pH change for each step (Table 2). In this poorly buffered water (Fig. 3b), the complete use of O2 solely for AR would drive bottom-water TA and pH lower than the observed values (Fig. 4a, b). Sulfate reduction and CaCO3 dissolution must then be invoked to explain the observed TA and pH. The effects of SO42– reduction on TA and DIC can be estimated from the observed [H2S] (Table 2) and the rest is made up by CaCO3 dissolution (Methods). We envision that these three processes can occur either sequentially (Table 2) or simultaneously when O2, pH, carbonate mineral saturation state are sufficiently low. While the sequential pathway simulates the general patterns of the TA and DIC relationship (Fig. 4a) and the pH and O2 relationship
(Fig. 4b) reasonably well, it appears that CaCO₃ dissolution must have proceeded and occurred simultaneously with AR and SR as the simultaneous pathway simulates the observation better (Fig. 4a, a b) and as is justified by the very low aragonite carbonate saturation state once more than 50% of O₂ is consumed (Table 2).

From the above simulations, we conclude that up to ~70% of the bottom-water TA production comes from CaCO₃ dissolution, which raises bottom-water pH from expected 7.25 to 7.45 and provides an important buffer mechanism in bottom waters (Methods). It has been reported that eutrophication led to lower pH in the polyhaline (S > 18 g kg⁻¹) part of the bay between 1985 and 2006²⁶. Based on our data and model simulations, we suggest that eutrophication in the bay has led to more O₂ consumption, SO₄²⁻ reduction, pH decrease, and dissolution of CaCO₃ shells and abiotic minerals in subsurface and bottom waters, consequently leading to possibly more TA and DIC export to the coastal ocean.

While shellfish calcification can represent a significant store of CaCO₃ in the Chesapeake Bay²⁶, ⁴⁸, much of the CD present in the current study likely also comes from abiotic precipitation in surface waters, a mechanism noted before in the Loire estuary.⁴⁸ The extent of CD in deep waters estimated here could be supported by independently estimated CaCO₃ production in surface waters from deviations from conservative mixing (Supplementary Fig. 10)⁴⁸, which is consistent with the TA deficit observed here in low-salinity surface waters (Fig. 3a). While the precipitation may largely be driven by seasonal dynamics in primary production enhanced by estuarine eutrophication⁴⁹, importantly, current and future increasing atmospheric CO₂ due to fossil fuel production may lower surface water carbonate saturation state enough to decrease mineral formation and thus delivery below the pycnocline. If so, the bay’s deep water would have a reduced capacity to neutralize metabolically generated CO₂, further enhancing eutrophication driven acidification.

Another important metabolic pathway is denitrification which uses NO₃⁻ as the oxidant for organic matter decomposition⁵⁰, ⁵¹. Note that [NO₃⁻] is generally low in the mid-bay (<1 μmol kg⁻¹). However, denitrification is often coupled to nitrification at the sediment water interface. System-wide integrated denitrification rate has been estimated to be about 70 μmol m⁻² h⁻¹ in the Chesapeake Bay (summer time)³², although other estimations are lower. Taking this value as the upper end, we estimate that denitrification can contribute to a DIC production of up to 17 μmol kg⁻¹ and TA production of up to 16 μmol kg⁻¹ in a 10 m bottom-water column and over a 100-day period. This amount is only up to about 8% of the total TA production in the bottom water observed here. Finally, while organic matter decomposition using metal oxides as oxidants is important intermediate steps for biogeochemical cycles, the contributions to alkalinity production must be lower in the bay as recycled [Mn²⁺] (<7 μmol kg⁻¹) and [Fe²⁺] (<2 μmol kg⁻¹)⁵⁰ are much lower than the observed TA production in the bottom water; a conclusion similar to that derived in the Baltic Sea¹³, ²¹.

**Discussion**

The buffering capacity reflects the marine carbonate system’s ability to resist changes in pH (or pCO₂) when DIC and/or TA are altered by physical and biogeochemical processes and when relevant thermodynamic constants are altered by temperature (T) and salinity (S) changes⁵³–⁵⁸. Mathematically, an aquatic system’s ability to resist pH change can be deconstructed into its sensitivity to changes in T, S, DIC, and TA.

\[
dpH = \left( \frac{\partial pH}{\partial T} \right) dT + \left( \frac{\partial pH}{\partial S} \right) dS + \left( \frac{\partial pH}{\partial DIC} \right) dDIC + \left( \frac{\partial pH}{\partial TA} \right) dTA + \ldots
\]  

(5)

Here the first and second terms represent the effects of change in thermodynamic constants as a function of T and S. The third term reflects the pH change when DIC is added while keeping T, S, and TA constant and the fourth term reflects the pH change when a strong acid (H⁺ or -ΔTA) is added while keeping T, S, and DIC constant. The slopes in the third and fourth terms are directly related to the buffer factors βDIC and βTA defined before¹³, ³⁴ with

\[
\beta_{DIC} = -(2.3 \times \frac{\partial pH}{\partial DIC})^{-1}
\]

(6)
and

\[ \beta_{TA} = -(2.3 \times \Delta pH / \Delta TA)^{-1}. \]

In estuarine conditions, because \( \beta_{DIC} \) and \( \beta_{TA} \) are similar in magnitude (also see Fig. 3b), the overall contribution to acidification or \( \Delta pH \) decrease is largely decided by changes in DIC and TA during physical and biogeochemical processes (e.g., at constant \( T \) and \( S \)) and also by the initial buffering capacity (e.g., at variable \( T \) and \( S \)).

The \( pH \) and \( [O_2] \) relationship in Chesapeake Bay waters differs greatly from that observed in northern Gulf of Mexico (nGOM) waters (shaded line in Fig. 4b). It appears that Chesapeake Bay waters are more vulnerable to both anthropogenic CO2 and biological induced acidifications because they have a lower buffering capacity than that of the offshore waters, in particular, in the nGOM as TA and DIC are lower in the Susquehanna River and US eastern margin waters than those of the Mississippi River and nGOM seawater (Figs. 3b, 4b and 5a). However, our simulations and those of the previous studies suggest that lower buffering capacity itself does not necessarily lead to low \( pH \) (Fig. 5a); rather, it allows a much greater \( pH \) decrease when other sources of CO2 or strong acids are added (Fig. 5b). Similar amounts of AR (Table 2) would lead to a \( pH \) decrease of only 0.4 units in the strongly buffered nGOM waters whereas a larger decrease of nearly 0.8 units would occur in the poorly buffered Chesapeake Bay waters at the present day conditions (at \( S = 34 \) and \( 20 \) g kg\(^{-1} \), respectively, Figs. 4b and 5c).

While the OA signal due to CO2 uptake in the open ocean regions is similar across middle and lower latitudes, the manifestation of this anthropogenic CO2 signal through ocean–river mixing in estuaries is dependent on the river TA and DIC values, which are highly variable among the world’s rivers, and whether CO2 is also introduced via microbial respiration. Due to the very high river TA and DIC and the resulting strong buffering capacity over the entire salinity range in the Mississippi River impacted coastal waters, \( pH \) change due to OA is proportional to the open ocean OA source signal and salinity and decreases toward zero salinity (Fig. 5a). In the Chesapeake Bay where average river TA, DIC, and buffering capacity are low, however, the oceanic OA signal is amplified in the low and middle salinity zone. Here the combination of reduced buffering capacity (with decreasing salinity) and a still sufficiently strong open ocean OA signal generates a minimum buffer zone and thus a Maximum Estuarine Acidiﬁcation Zone (MAEZ) (Fig. 5c). The existence of a MAEZ and its salinity range depend not only on the river TA value, but also the TA:DIC ratio. When CO2 addition from AR increases from 0 to 100 and finally to ~200 \( \mu \)mol kg\(^{-1} \) (or 0 to roughly half or to a full \( O_2 \) consumption depending on the salinity and temperature), the minimum buffer zone shifted from salinity ~4 to ~13 (Fig. 5a) and finally to ~23 (Fig. 5b). Note that local CO2 uptake, carbonate mineral dissolution and SR are not included in this discussion (Fig. 5) and would further modify the estuarine buffering capacity as they would modify the TA to DIC ratio in estuarine waters (Table 2, Figs. 3b, 4a, 5a).

Below we further discuss the effects of anthropogenic CO2 and biological CO2 and acid additions on estuarine pH buffering capacity. The marine carbonate system has a minimum buffering or maximum \( pH \) change point when DIC increases approximately equal to that of TA (or TA:DIC \( \approx 1 \)) where [CO3\(^{2-}\)] = [CO2\(^{3-}\)] + [B(OH)\(^{3-}\)] (if we ignore all other weak acid–base species). At this point, any addition or removal of CO2 or acids will result in a maximum \( pH \) decrease or increase. Because DIC is slightly higher than or nearly equal to TA in rivers and is lower than TA in seawater, there may exist a small crossover of DIC and TA at the very low-salinity zone. A peculiar \( pH \) minimum occurring in the low-salinity zone of estuaries is related to this mixing feature as was noticed a long time ago in both closed and open system simulations. Furthermore, how CO2 is added to the estuarine waters affect how the crossover point will move. First we contend anthropogenic CO2 does not directly add to the high pH river water but is mixed into the estuary via river–ocean mixing. In contrast, respiratory CO2 is nearly equally added to the bottom water based on \( O_2 \) consumption regardless of the mixing index or salinity (except that O2 solubility increases when salinity decreases, but it is a small correction). In Fig. 6, we summarize several scenarios illustrating how the crossover point of the TA and DIC to salinity lines or the point of TA:DIC ratio = 1 moves along the TA-salinity line. Adding anthropogenic CO2 to the seawater endmember would move this crossover point to only a slightly higher salinity. Adding biological CO2 (for example 100 \( \mu \)mol kg\(^{-1} \)) to both the river and ocean endmembers would, however, shift the DIC line to a much higher position (parallel to the original line) creating a crossover point located at a salinity substantially higher than the original one. Finally the combined effect of anthropogenic CO2 and biological CO2 from respiration moves the crossover point to a even higher salinity. These crossover points are consistent with the progressive shift of the minimum buffer factor (\( \beta_{DIC} \)) and the maximum acidification zone (\( -\Delta pH \)) presented in Fig. 5. However there appears a difference in the location (salinity) between the minimum buffer factor (\( \beta_{DIC} \)) and

### Table 2 O2 consumption and DIC and TA production during sequential aerobic respiration, sulfate reduction and carbonate mineral dissolution in bottom waters

| Redox | \( \Delta O_2 \) (\( \mu \)mol kg\(^{-1} \)) | \( \Delta DIC \) (\( \mu \)mol kg\(^{-1} \)) | \( \Delta TA \) (\( \mu \)mol kg\(^{-1} \)) | \( \Delta TA:DIC \) | \( pH \) | \( \Omega_{arag} \) |
|-------|-----------------|-----------------|-----------------|-----------------|--------|-------------|
| AR    | (CH2O)2oss(NH3)16(H5PO4) + 106CO2 + 16HNO3 + H3PO4 + 122H2O | (16+1)/106 = -0.16 | 8055 | 1.56 | 1250 | 0.27 |
| -58 (75%) | +45 | -7.2 | 7859 | 1.05 | 1100 | 0.65 |
| -117(50%) | +90 | -14.4 | 7629 | 0.80 | 1100 | 0.65 |
| -174(25%) | +134 | -21.5 | 7421 | 0.40 | 1100 | 0.65 |
| -231(0%) | +1780 | -28.5 | 7237 | 0.26 | 1100 | 0.65 |
| SR    | (CH2O)2oss(NH3)16(H5PO4) + 5350SO2− + 106HCO3− + 53H2S + 16NH3 + H3PO4 | (106+16+1)/106 = 1.14 | 7700 | 0.25 | 1100 | 0.65 |
| 0 | +70.0 | +80.6 | 7199 | 0.25 | 1100 | 0.65 |
| CD    | CaCO3 + CO2 + H2O → Ca2+ + 2HCO3− | 2/1 | 7368 | 0.38 | 1100 | 0.65 |
| 0 | +57.6 | +115.3 | 7368 | 0.38 | 1100 | 0.65 |
| Total | -231.7 | +305.6 | +167.3 | 7368 | 0.38 | 1100 | 0.65 |

AR, aerobic respiration; CD, carbonate dissolution; DIC, dissolved inorganic carbon; SR, sulfate reduction; TA, total alkalinity. The last columns listed the expected pH and aragonite mineral saturation state (\( \Omega_{arag} \)) values at the end of each step; \( pH \) and \( \Omega_{arag} \) values are also calculated at the initial and mid-points of \( O_2 \) consumption (or % of \( O_2 \) saturation). For simultaneous reactions at low \( pH \) and \( \Omega_{arag} \), see the text. The calculation steps and results are detailed in the Methods. Note for a more stable carbonate mineral, calcite, \( \Delta \mu_{calc} = 1.5 \times \Omega_{arag} \).
the maximum acidification ($\Delta$pH). This is because $\Delta$ pH represents the accumulative pH change between an end point and a beginning point while the buffer factor reflects the pH sensitivity at a specific point if additional DIC is added to the system.

In summary, large estuarine water bodies, exemplified by the Chesapeake Bay, are particularly vulnerable to the anthropogenic CO$_2$ and eutrophication-induced coastal OA. In this paper we emphasize that subsurface H$_2$S oxidation ($\sim$0.2 pH units) and local surface CO$_2$ uptake ($\sim$0.1 pH units) work together with known organic matter respiration and the open-ocean OA source signal to drive substantial acidification and CaCO$_3$ dissolution in estuarine subsurface waters. Currently, acidification due to CO$_2$ input from AR (up to 0.8 pH units) in the bay exceeds that from the atmospheric CO$_2$ increase in the open ocean ($\sim$0.1 pH units in surface waters and the signal is mixed into estuaries proportional to salinity) and local estuarine uptake; but towards the end of this century the latter will approach or exceed the former and the synergy between them will also increase. In addition, future increasing atmospheric CO$_2$ due to fossil fuel production may lower carbonate saturation state enough to decrease mineral formation in surface water and thus delivery below the pycnocline, where we have currently demonstrated that CaCO$_3$ dissolution offsets a significant proportion of the metabolic CO$_2$ effect on acidification. We further recognize that natural and anthropogenic acidification mechanisms most relevant to estuarine acidification are characterized by various time scales. They range from nearly instantaneous for acid–base equilibrium$^{66}$, to minutes for CO$_2$ hydration$^{66}$, and to minutes to hours for H$_2$S oxidation$^{36,41,67}$. In contrast, time scales for physical mixing are on the order of tidal or less, to daily and seasonal$^{30,31,68}$ while local CO$_2$ uptake from the atmosphere and its accumulation in the water column and acidification of the bottom water as well as pelagic and benthic respirations operate over tidal to seasonal scales$^{12,13,35,37,69}$. Although anthropogenic changes in external forcing due to variability in river and ocean endmembers may also have a seasonal component, decadal and centennial variation is more important$^{35,70,71}$. This mosaic of processes with different time and space scales poses a great challenge in our ability to understand and predict coastal OA.

**Methods**

**Site and cruise descriptions.** The Chesapeake Bay is the largest estuary in the US. The August 2013 survey started from the upper estuary near the Susquehanna River mouth (Fig. 1a). The upper and middle Chesapeake Bay were surveyed during 9–14 August 2013 by RV Hugh R. Sharp. The water column survey focused mainly at one site south of the Bay Bridge (38°58.8 N, 76°22 W), where a field study
of redox chemistry and water column inorganic carbon and pH were carried out. We consider this site as the up end of the mid-bay. We repeatedly sampled the water column at high slack tides and low slack tides. During this 3-day survey, an excursion was made south to the middle bay near Solomon’s Island. After the completion of the cruise, we set up two 24-h dockside measurements, one at the dock of the Chesapeake Biological Laboratory (CBL; 38.317317°N, 76.450980°W) on Solomon’s Island near the southern end of our ship-based survey and another further south at a pier of the Virginia Institute of Marine Science (VIMS; 37.2473°N, 76.4994°W) near the bay mouth. A similar study was conducted during 18–24 August 2014 (Supplementary Fig. 1). We also conducted a spring survey (11–16 April 2015) to get an initial condition before the hypoxia season (Fig. 1b).

Sample and analytical methods. Salinity, temperature, and O2 were obtained from the CTD Rosette system. Total sulfide (H2S and HS−) was determined by voltammetry using solid state Au/Hg electrodes. Surface water partial pressure of CO2 (pCO2) was measured, position, temperature, and salinity information were measured underway while the ship was sailing or anchored by pumping surface water from under the ship to the shipboard laboratory using an underway pCO2 system. TA and DIC water samples were taken from Niskin bottles and were preserved and stored in 250 mL borosilicate glass bottles with 100 μL saturated HgCl2 solution. TA and DIC samples were stored at refrigerated temperature (~5 °C) before being measured (within 4 weeks). TA samples were measured by open-cell Gran titration with a precision better than ±0.1% using an Apollo Scitech Seawater Total Alkalinity titration system. DIC samples were analyzed by adding phosphoric acid into sample waters to release CO2, which was measured by an infrared CO2 analyzer (LI-COR 7000) with an overall precision of ±0.1% using an Apollo Scitech DIC Analyzer. Both TA and DIC measurements were quality controlled by Certified Reference Materials from Andrew Dickson of the University of California at San Diego. pH samples were taken by the same Niskin bottle and measured by an Orion Ross glass electrode within 1 h after the water temperature of CO2 (PCO2(eq)) by considering water vapor pressure:

\[ \text{pCO2(eq)} = \text{pCO2(water)} \times \exp \left( 0.032 \times (T_{\text{SS}} - T_{\text{w}}) \right) \]

where SST is sea surface temperature (°C) and TSS is temperature in the equilibrator. Our measured atmospheric xCO2 values were also converted from dry condition to near sea surface wet condition (pCO2(water)) by Eq. (10):

\[ \text{pCO2(water)} = \text{PCO2(water)} \times \exp \left( 0.032 \times (T_{\text{SS}} - T_{\text{w}}) \right) \]

where k represents the gas transfer velocity and Ks is the solubility of CO2. We adopted Ho et al.19 as the gas transfer velocity and an ensemble of gas transfer parameters to evaluate the uncertainty range following the previous practice.7–9 Finally, the coefficient C2 corrects the non-rectangular distribution of wind. A negative air–sea CO2 flux means an uptake of atmospheric CO2 for the water. Over at least a 100-day water residence period (from May to August) and over a water column of 20 m, this CO2 flux can be converted into an increase in DIC of 4.3–6.7 (μmol m−2 d−1) × 100 × 20 m = 2.15–33.5 mmol m−3 or 21.2–33.1 mmol kg−1 (here a density of 1021.09 kg m−3 is used). We used the entire water column rather than the surface mixed layer because the main concern here is how local CO2 uptake, via internal mixing, contributes to acidification of the especially vulnerable bottom waters.

Calculation of pH decrease due to local CO2 uptake. With H2S included, the calculation of pH (in NBS scale and at 25 °C) decrease was performed using the modified CO2SYS program. Another note, AquAEnv, also has such a capacity.15 We used day 4 data with S = 16.8618 kg m−3, T = 25.28 °C, depth = 17 m, DIC = 1933.8 μmol kg−1, total [H2S] = 37.79 μmol kg−1, [NH3+NH4+] = 13.6 μmol kg−1, total [H2SiO4−] = 3.5 μmol kg−1, and pH = 7.476 to calculate a TA = 1934.1 μmol kg−1. Then, we subtracted the summer DIC by II = 21.2–33.1 μmol kg−1 (1912.6–1900.7 μmol kg−1) to calculate a new pH (7.557–7.604). Thus, the pH decrease by an increase in DIC derived from local uptake of atmospheric CO2 is 0.081–0.128 pH unit over the entire period from spring to summer.

pH decrease due to oxidation of reduced chemicals. We used day 4 data with S = 15.145 kg m−3, T = 25.36 °C, depth = 12.54 m, DIC = 1767.7 μmol kg−1, [H2S] = 2 μmol kg−1, and pH = 7.354 to calculate a TA = 1968.9 μmol kg−1. Then, we subtracted a 30–60 μmol kg−1 from TA to calculate a new pH (7.152–7.246). Thus, the pH decrease by a 30–60 μmol kg−1 of TA reduction is 0.108–0.202 pH units (represented by the purple arrows in Fig. 4b). The modified version of CO2SYS was used for all the CO2 and pH calculations. Note, adding a <3 μmol kg−1 of T-PO4 would only lead to 0.005 μmol pH unit decrease in the calculation. Thus, its influence is ignored here.

Modification of the CO2SYS program. The modifications were done on the Excel version 2.1 of the program17, which is available for download from CDIAC (http://cdiac.ornl.gov/ftp/co2sys/). A Matlab version is available from the corresponding author. In addition to the total Phosphate and Silicate, the program now accepts the total NH4 and total H2S in μmol (kg of SW)−1. The contribution of each to the alkalinity is given by:

\[ \text{NH}_3 - \text{Alk} = [\text{NH}_3]+K_{\text{NH}_3}/[\text{H}]+[\text{H}] \]

\[ \text{H}_2\text{S} - \text{Alk} = [\text{H}_2\text{S}]+K_{\text{H}_2\text{S}}/[\text{H}_2\text{S}]+[\text{H}] \]

where K_{NH}_3 and K_{H}_2S are the dissociation constants of ammonium (NH4+) and hydrogen sulfide (H2S).

The dissociation constant for NH4+ was taken from Clegg and Whitfield and is valid for S = 0–40 g kg−1 and t = 2 to 40 °C (note ref. 21 essentially provided the same constant). The constant for H2S was taken from Millero et al. and is valid for S = 0–40 g kg−1 and t = 0–35 °C. When the pressure is not zero, a correction is applied according to Millero. A comparison with AquAEnv under [H2S] <50 (or 300) μmol kg−1 shows a good agreement of calculated pH (in free scale) within 0.0003 (or 0.0026) from known TA and DIC. We have further tested the calculations with waters containing high concentrations of H2S and posted this modified version of the CO2SYS program on the CDIAC website for public access22.

Simulation of bottom-water geochemical pathways. We present here the calculation methods for Table 2 and Fig. 4a, b. For the bottom-water condition (S = 19.87 kg m−3 and T = 25 °C), we have a saturated [O2] = 231.7 μmol kg−1, and, from the mixing line at S = 19.87 kg m−3, TA = 1771.1 μmol kg−1 DIC = 1658.5 μmol kg−1, we have pH = 8.066. If all dissolved O2 is used by heterotrophic bacteria for other processes, the respiration via anaerobic metabolism is (pCO2(water)) by considering temperature changes between the surface water and the equilibrator through the following equation:

\[ \text{pCO2(water)} = \text{PCO2(water)} \times \exp \left( 0.032 \times (T_{\text{SS}} - T_{\text{w}}) \right) \]
total of 195.9 μmol kg\(^{-1}\) (i.e., observed 167.3 μmol kg\(^{-1}\) plus expected –28.5 μmol kg\(^{-1}\)) beyond conservative mixing. We estimate TA increase from SO\(_2\)\(^{2-}\) reduction as 80.6 μmol kg\(^{-1}\) from the total concentration of H\(_2\)S (35 μmol kg\(^{-1}\)) by the following equation:

\[
\Delta \text{TA} = (2x[H_2S] + 16/3x[H_2(S)])
\]

where \(x \geq H_2S\) represents an equal amount of HCO\(_3\)\(^-\) and HS\(^-\) production during SO\(_2\)\(^{2-}\) reduction and 16/3x[H\(_2S\)] represents NH\(_3\) production (and contribution to TA) based on stoichiometry (see Table 2). Then the TA generated from CaCO\(_3\) dissolution must be as high as 115.3 ± 20.0 μmol kg\(^{-1}\) by the difference (195.9 – 80.6) and can be contributed up to 70% of total amount of production.

The amount of DIC production following these steps is 305.6 ± 80.6 μmol kg\(^{-1}\), which is within the uncertainty of the observed value of 275.3 ± 59.5 (Fig. 3). The 10% difference (30 μmol kg\(^{-1}\)) can be explained either by TA increase due to organic matter respiration using nitrate (denitrification)\(^{31,84,85}\) as well as probably organic alkalinity contribution\(^{46}\). If indeed a lower C to O ratio (106/154 = 0.688) given in ref. 84 is used, the produced DIC would be close to the observation.

From the resulting pH and Ω\(_{Arge}\) (Table 2), it is clear when O\(_2\) is partially consumed aragonite mineral becomes undersaturated (starting at 75% O\(_2\) saturation) and CaCO\(_3\) dissolution can proceed together with AR. To simulate the system buffer factors, we directly use AquaEnv which includes fully these reduced chemical species. Finally, in Fig. 3b, Buffer factor calculation. We calculate these buffer factors (Fig. 3) following the analytical formula provided by Egleston et al.\(^{54}\) with a typo corrected\(^{87}\). Specifically, we extract out species concentrations and thermodynamic constants from the previously used CO2SYS program. Conditions used are given below.

### Simulation of pH changes

We present here the calculation methods for Fig. 5a, b. Although each term in Eq. (5) may be derived analytically, in this paper, we obtain the overall pH change, \(\Delta \text{pH}\) for Mississippi/GOM bottom water, at 8.369 from CDIAC (http://cdiac.ornl.gov/ftp/co2sys/). The modiﬁed CO2SYS program on Excel version is available from the corresponding author upon reasonable request and will be deposited at the US National Centers for Environmental Information (https://www.nodc.noaa.gov/oceanacidiﬁcation/).

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Acknowledgements
This work was supported by internal funds from the University of Delaware Provost’s office and the College of Earth, Ocean and Environment Dean’s office to W.-J.C., by program from the National Science Foundation (NSF OCE-1559312) and NASA (NNX14AM35G) to W.-J.C., by grants from NSF (OCE-1155385) and the U.S. National Oceanic and Atmospheric Administration (NOAA) Sea Grant program (NA14AR4170087) to G.W.L., and a NOAA grant to J.T., W.-J.C., M.L., G.G.W. I., J.C., and W.-J.C.
and W.M.K. (NA15NO34780190, publication # 17-001). We thank Xinping Hu for discussion and the Chesapeake Bay Program and the Maryland Department of Natural Resources for the monitoring data. This is UMCES publication number 5369.

Author contributions
W.-J.C. and G.W.L. are responsible for the design of the fieldwork. W.-J.C. is responsible for data analysis and writing of the paper. W.-J.H., M.X., A.J., R.M., J.B., N.H. are responsible for data collection and sample analysis. Y.-Y.X. contributes to buffer factor calculation. M.L. contributes to physical mixing part. All authors have contributed to discussion and revision of the paper.

Additional information
Supplementary Information accompanies this paper at doi:10.1038/s41467-017-00417-7.

Competing interests: The authors declare no competing financial interests.

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