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Warming stimulates sediment denitrification at the expense of anaerobic ammonium oxidation

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Temperature is one of the fundamental environmental variables governing microbially mediated denitrification and anaerobic ammonium oxidation (anammox) in sediments. The GHG nitrous oxide (N_2O) is produced during denitrification, but not by anammox, and knowledge of how these pathways respond to global warming remains limited. Here, we show that warming directly stimulates denitrification-derived N_2O production and that the warming response for N_2O production is slightly higher than the response for denitrification in subtropical sediments. Moreover, denitrification had a higher optimal temperature than anammox. Integrating our data into a global compilation indicates that denitrifiers are more thermotolerant, whereas anammox bacteria are relatively psychrotolerant. Crucially, recent summer temperatures in low-latitude sediments have exceeded the optimal temperature of anammox, implying that further warming may suppress anammox and direct more of the nitrogen flow towards denitrification and associated N_2O production, leading to a positive climate feedback at low latitudes.

The anthropogenic production of reactive nitrogen (Nr) has increased tenfold over the past century and is expected to continue to increase to keep pace with the growing world population[1]. The impact of anthropogenic Nr on ecosystems and biogeochemical cycles has become the second most serious environmental issue at the global scale[2–4]. In parallel, climate change is predicted to be a major cause of species extinction and biodiversity degradation in the coming century[5,6], and species that occupy narrow thermal niches are likely to be most vulnerable to global warming[7]. Since almost all nitrogen-associated processes are microbially driven, rising temperature will inevitably promote or suppress specific microbial activities[8,9], and thus affect global biogeochemical cycles with climate feedbacks[10].

The land–ocean interface receives excess Nr and serves as a hotspot for Nr removal. This occurs through denitrification in anoxic environments[11] and the relatively newly discovered anaerobic ammonium oxidation (anammox) process[12], both of which reduce Nr to biologically unavailable dinitrogen (N_2). Both denitrification and anammox are widely observed in anoxic habitats such as paddy soils[13], salt marshes[14], mud flats[15] and aquatic sediments[16]. In contrast to denitrification, anammox does not involve release of N_2O, which is about 300 times more potent than CO_2 in terms of greenhouse warming potential. Thus, anammox is recognized as a more climate-friendly Nr-removal pathway relative to denitrification. These two processes and their partitioning largely determine the size of the global Nr sink and N_2O source. Previous studies have shown that anammox could be crucial in some deep-sea sediments[17,18], although Nr removal by benthic denitrification generally exceeds removal by anammox[19–21] in such environments. Devol concluded that the fraction of total N_2 production by anammox (ra%) ranged from 0–79% in marine sediments and typically constitutes approximately 10–40% of the total Nr removal[16]. The variance in ra% has been attributed to a number of environmental factors such as temperature, the quantity and quality of organic matter, and nitrate concentration[19,22–24]. Among these potential environmental variables, temperature is one of the most fundamental factors governing microbial metabolism of denitrification and anammox, but the temperature effect has not been sufficiently resolved. Global ra% and temperature data[25] show that ra% has an inverse relationship with the temperature at which samples were collected or incubated. Despite decades of study, the factors that control the partitioning between sedimentary denitrification and anammox remain underexplored[16], specifically, the knowledge of how the two Nr-removal processes respond to future warming remains limited due to lack of field measurements[19,26–29]. Understanding the environmental factors of Nr-removal pathways and associated N_2O production, particularly temperature, will be essential for Earth system models[11] to accurately quantify the Nr-removal flux and to predict future biogeochemical cycles and associated climate feedbacks driven by reactive nitrogen.

A few studies have examined the temperature dependence of sedimentary denitrification and anammox in aquatic ecosystems, mostly focused on sediments in continental shelves and estuaries in temperate or polar regions[19,26–29], but there is a lack of reports from subtropical regions, especially for sediments deposited in shallow freshwater creeks, estuaries, tidal flats, mangroves and aquaculture ponds. These coastal systems receive anthropogenic Nr and will directly experience future temperature rise. In this study, we applied the ^{15}N-isotope pairing technique to sediments collected from various coastal aquatic systems in southern China, a typical subtropical region (Supplementary Fig. 1), to identify how temperature change may affect the potential rates of Nr-removal pathways, the denitrification-driven N_2O production rate, and the partitioning between denitrification and anammox in subtropical sediments. By adding our experimental data for subtropical regions to existing data for various ecosystems, we evaluate the latitudinal differences in thermal sensitivity of microbial communities involved in Nr removal to global warming in relation to N_2 and N_2O production rates at an ecosystem level.

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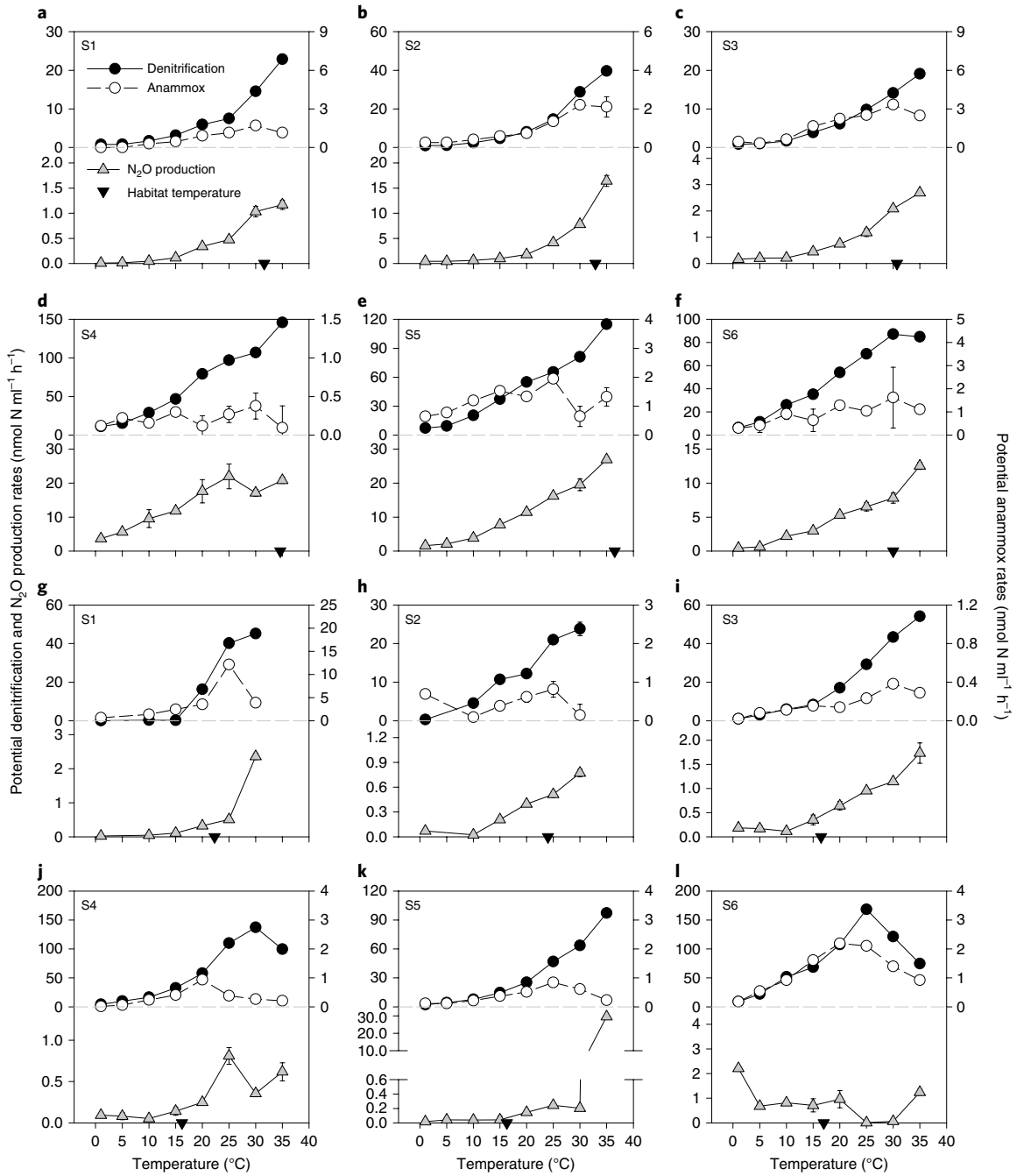

Fig. 1 | Temperature responses of sedimentary denitrification, anammox and related N_2O production potential rates. a–l, Temperature manipulation experiments for samples collected during summer (**a**–**f**) and winter (**g**–**l**) at different sampling sites. Sampling points are indicated in the top left of each graph. Error bars denote the s.d. of triplicates (most are smaller than the symbols).

Temperature response of Nr-removal processes and N_2O production

We observed consistently distinct temperature response patterns of denitrification and anammox (Fig. 1). From these thermal performance patterns, we obtained different optimum temperature (T_{opt}) values for both denitrification and anammox for various habitats and seasons. In the brackish-water ecosystems (estuary, aquaculture pond and tidal flat; see Supplementary Table 1), denitrification potential continuously increased with increasing incubation temperature up to 35 °C in both seasons (Fig. 1a–e,g–k), except for site S4 in winter, where T_{opt} for denitrification was approximately 30 °C (Fig. 1j). This suggests that the T_{opt} for denitrification in our study system should be higher than 35 °C in summer and higher than 30 °C in winter. T_{opt} values for anammox were determined to be 25–30 °C in summer and 20–30 °C in winter (Fig. 1). In the freshwater ecosystem (site S6 in both seasons), T_{opt} values for denitrification and anammox were found to be 30 °C in summer (Fig. 1f), whereas in winter, the T_{opt} values for denitrification and anammox were 25 °C and 20 °C, respectively (Fig. 1l). Overall, T_{opt} for denitrification was consistently higher than for the corresponding anammox in both seasons (P values in Supplementary Table 3); leaving aside the seasonal variation in T_{opt} for denitrification due to the uncertainty, the T_{opt} for anammox was significantly higher in summer than in winter ($P = 0.017$; Supplementary Table 4). In addition, the T_{opt} values

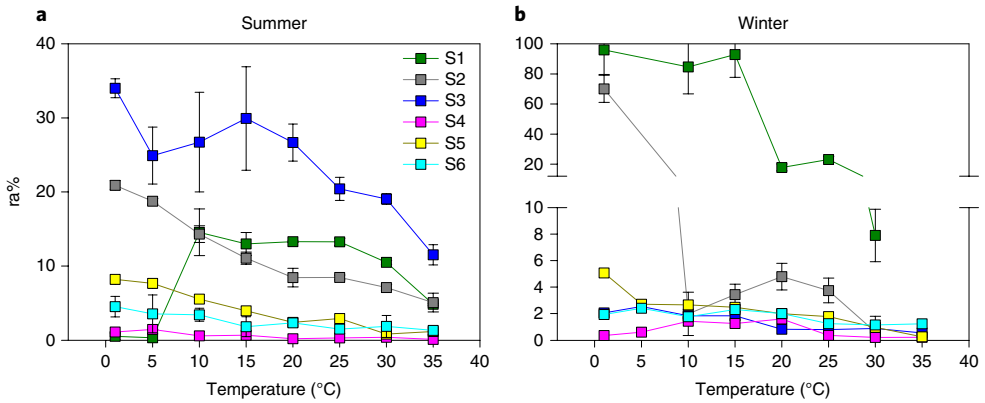

Fig. 2 | Temperature responses of the relative contribution of anammox to total gas (N_2 + N_2O) production (ra%). a,**b**, Temperature responses at different sampling sites in summer (**a**) and winter (**b**). Error bars represent the s.d. of triplicates.

for both Nr-removal processes in brackish-water ecosystems were higher than those in freshwater ecosystems regardless of season. Remarkably, the habitat temperature was higher than the observed T_{opt} for the anammox community during summer in the subtropical regions (Fig. 1a–f).

Sedimentary production of N_2O, an intermediate during denitrification, was observed to increase with warming in both summer and winter samples. The response of N_2O production to temperature change showed a consistent pattern, with potential rates increasing exponentially as the incubation temperature increased, regardless of sites and seasons (Fig. 1).

Temperature effects on relative contribution of anammox

The relative contribution of anammox to total Nr removal (ra%) showed a large spatial variation among stations, with increasing contributions at lower incubation temperature for all except two cases (S1 in summer and S4 in winter; Fig. 2). These two exceptions exhibited relatively high ra% at moderate temperatures, with low ra% at both high and low temperature extremes (Fig. 2). Additionally, four ra% data points in winter exceeded 40% due to the extremely low $^{30}N_2$ production at low temperature (see gas production rates of S1 and S2 in winter in Supplementary Fig. 8), thus leading to an exceptionally high contribution of anammox to total Nr removal (Fig. 2b). The average ra% at the habitat temperature across our study sites was approximately 7% in summer and 5% in winter.

Q_{10} values for Nr-removal processes and N_2O production

To investigate whether the warming effect on the intermediate product (N_2O) and the end product (N_2) is invariable during denitrification, the Q_{10} values for N_2 and N_2O production were calculated separately (Supplementary Figs. 2 and 3). The Q_{10} of denitrification-related N_2 production was in the range 1.9–2.7 (2.2 ± 0.3, mean ± s.d.) in summer and 2.0–3.5 (2.6 ± 0.6) in winter (Fig. 3); the denitrification-derived N_2O production had slightly higher Q_{10} in both summer (2.9 ± 0.9) and winter (3.1 ± 0.6, with exclusion of two extreme measurements at sites S4 and S6). The higher Q_{10} values for the denitrification-derived N_2O production imply that warming might direct more nitrogen flow towards N_2O during nitrate removal. For denitrification as a whole, the Q_{10} was in the range 1.9–2.7 (2.3 ± 0.4) and 2.2–3.5 (2.9 ± 0.5) in summer and winter, respectively (Fig. 3). For anammox, the Q_{10} was in the range 1.3–2.5 (1.9 ± 0.5) in summer and 2.1–4.8 (2.4 ± 0.2) in winter (excluding site S4 in winter) (Fig. 3). No significant spatial and seasonal differences were observed for Q_{10} values of denitrification-derived N_2O and N_2 production (see *P* values in Supplementary Tables 5 and 6). Combining the Q_{10} values from both seasons (Fig. 3), the Q_{10} values for denitrification-derived N_2O were slightly higher than those for denitrification-derived N_2 ($P = 0.042$; Supplementary Table 6); moreover, the Q_{10} values for denitrification were consistently higher than those for anammox ($P = 0.021$; Supplementary Table 7).

The temperature dependence of denitrification-associated N_2O production in aquatic sediments is rarely reported. Our results in subtropical coastal sediments show that warming directly stimulates N_2O production by increasing sedimentary denitrification (Fig. 1). There are very few estimates in coastal sediments available for comparison of the Q_{10} values of sedimentary N_2O production. Our results (1.2–7.1) in subtropical aquatic sediments were within the range of the few available studies, which reported a Q_{10} of 1.8 in lake sediments[30] and 3.7–12 in grassland or soils[31,32]. For the Q_{10} of denitrification, our estimates (1.9–3.5) were slightly lower than two estimates (3.8 and 5.0) for the subtropical region, but were in line with the measurements in a few studies from temperate (1.7–2.5) and polar (1.6–2.3) regions (Fig. 4a). Q_{10} values for anammox in our study (1.3–4.9) were similar to those from the limited anammox studies from temperate (1.6–3.1) and polar (1.4–2.7) regions (Supplementary Table 8). Accordingly, the Q_{10} values of denitrification-derived N_2O production, denitrification and anammox in this study are comparable with values reported in the literature, supporting the suggestion that our results may be applicable to natural environments more broadly at a global scale, even though the slurry incubation methodology provides potential rates. Both denitrification and anammox are responsive to temperature change in aquatic ecosystems. Although other site-specific and season-specific factors, such as content of organic compounds, substrate concentration and community structure, may have roles in the variability of Q_{10}, the results in Fig. 3 indicate statistically different warming response patterns among denitrification-derived N_2O production, denitrification and anammox. This coherent pattern implies that warming may favour denitrification more than anammox, and consequently, direct more of the nitrogen flow towards denitrification, in which the nitrogen bifurcation to N_2O is even higher, leading to a positive climate feedback.

To obtain a more comprehensive picture of the temperature response of Nr-removal pathways at the global scale, we assembled a cross-system comparison among studies from other regions, including pure culture bacteria[33], polar continental shelf[28,29], polar intertidal flats[27], polar fjords[22], temperate inner continental shelf[26], temperate intertidal flats[27], temperate estuary[26], subtropical tidal zones[27], rivers[34], streams[35], anoxic groundwater aquifer[36], riparian soil[37], mountain lakes[38] and denitrification bed[39]. Both denitrification and anammox showed wide ranges of T_{opt} values (Fig. 4b and

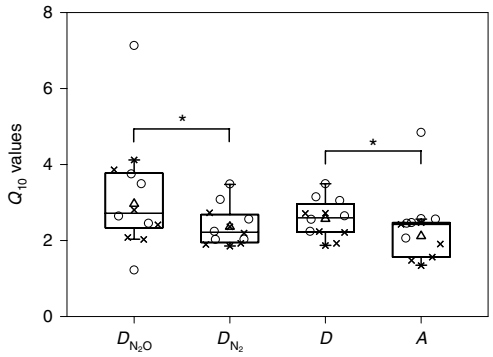

Fig. 3 | Variation of Q_{10} values for denitrification-related gas production and Nr-removal processes from both seasons. Q_{10} values for denitrification-related gas production and Nr-removal processes for both seasons are shown. D_{N_2O}, potential rate of denitrification-derived N_2O production; D_{N_2}, potential rate of denitrification-derived N_2 production; D, potential rate of denitrification; A, potential rate of anammox. The mid lines and triangles in the boxes indicate the median and the mean of Q_{10} values, respectively, from two seasons; the lower and upper lines of the boxes are the first and third quartiles; the lower and upper whiskers represent the minimum and maximum values, respectively. The black crosses and circles denote the data from summer and winter, respectively. Statistical significance is tested between D_{N_2O} and D_{N_2}, and between D and A; $^*P < 0.05$. See specific Q_{10} values for different processes in Supplementary Figs. 2 and 3.

Supplementary Table 8). Combined with our results from the subtropical region, the enriched database reveals how community-level Nr-removal processes vary with temperature change latitudinally (Fig. 4b). The high T_{opt} values of more than 35 °C in summer and more than 30 °C in winter for denitrification found in our brackish-water ecosystems were consistent with those from a subtropical tidal zone in the Gulf of Mexico[22], where denitrification T_{opt} values were observed to be 35–37 °C (sites 4 and 8 in Fig. 4b). Our T_{opt} values were significantly higher than those found in the temperate and polar sediments (21.0–31.1 °C; Fig. 4b) except for two sites reported by Canion et al.[27] and Brin et al.[26] (sites 16 and 24 in Fig. 4b), which had comparable T_{opt} to those in our study. For anammox, the observed T_{opt} values (20–30 °C in both seasons) in our study were similar to the T_{opt} of 22–33 °C found in temperate sediments (Fig. 4b) and to the T_{opt} of 25–30 °C in a culture of marine anammox bacteria (Supplementary Table 8). However, our T_{opt} were higher than the anammox optimum of 12 °C and 15 °C found in continental shelf sediments from polar regions by Dalsgaard and Thamdrup[29] and Rysgaard et al.[28], respectively, where habitat temperatures were markedly lower than those in our study (sites 29 and 34 in Fig. 4b). Relative to the anammox optimum of 37 °C found in wastewater treatment systems[40,41], almost all measurements from field observations were much lower.

T_{opt} has been widely used to study the temperature tolerance of microbes in laboratory studies[22,42]. In Fig. 4b, the denitrification T_{opt} values were generally higher than 25 °C, which agrees well with the mesophilic response to temperature for denitrifying communities, as reported in most laboratory studies[22,43]. Additionally, we find that except for the three data points in ref. [26], T_{opt} values for denitrification were consistently higher than those for anammox regardless of season or region (Fig. 4b), suggesting that denitrifiers have a higher thermal tolerance than anammox bacteria. Notably, T_{opt} values increase from polar to low-latitude regions for both denitrification and anammox, corresponding to the latitudinal pattern of habitat temperature (Fig. 4b). In addition, the temperature differences between T_{opt} and habitat temperature significantly decrease towards low latitudes for both Nr-removal processes. Moreover, the projected temperature after warming by 2100 in the representative concentration pathway (RCP) emission scenario 8.5 is close to the denitrification T_{opt} and even exceeds the upper limit of anammox T_{opt} in the subtropical summer (Fig. 4b). Such a pattern suggests that habitat temperature will remain in a tolerable range for denitrification and anammox in mid-high latitudes, whereas in low latitudes, a small temperature increase may have large effects on the growth of anammox bacteria, although low latitudes in the Northern Hemisphere are likely to experience less warming in the future relative to the mid-high latitudes[44], and thus result in a shift in the community activity of Nr-removal microbes. On the basis of our observations, we hypothesize that anammox at low latitudes is vulnerable to global warming, with the consequence that the proportional contribution of anammox to total Nr removal declines. Considering the ability of microbes to adapt to different temperatures and the large timescale at which climate change occurs, however, anammox communities may shift in composition towards microbial species that are more adapted to warming temperatures.

In this study, we address the direct impact of temperature on sedimentary Nr-removal processes and associated N_2O production. However, environmental regulators often co-vary across global ecosystems, and the variation in T_{opt} values for denitrification and anammox might be due to other environmental factors, such as microbial assemblage, organic carbon availability or nitrate and oxygen concentration[27,38,45–47]. The latitudinal variation pattern of T_{opt} in the two tested pathways (Fig. 4b) indicates that habitat temperature has a significant role in regulating thermal dependence, even though the T_{opt} for anammox may respond in a different way to that for denitrification in relation to habitat temperature due to varying thermal tolerance between these two microbial communities. In our case, T_{opt} values for denitrification and anammox in the freshwater ecosystem were lower than those in brackish-water ecosystems in both seasons (Fig. 1); such a difference might be caused by the different compositions of microbial assemblages[45,47]. Future studies combining isotopic and molecular techniques are needed to clarify the relative influence of physicochemical and biological factors[27]. Additionally, substrate availability is a known regulator of the temperature performance of denitrifiers[27,38,46]. Substrate-dependent temperature tolerance can also be found in freshwater phytoplankton species[48]. In general, warming tends to stimulate organic carbon- and nitrogen-transformation processes[49]. GeoChip hybridization-based analysis revealed that the abundance of genes involved in organic carbon decomposition and denitrification increased significantly in response to warming[50,51]. Warming can facilitate the degradation of recalcitrant carbon to labile and dissolved organic carbon, and this additional supply of electron donors can subsequently stimulate heterotrophic denitrifiers[50,52]. However, the specific effect of warming-derived carbon decomposition on denitrification and anammox requires further investigation. The studies mentioned here indicate that various environmental factors may synergistically influence the temperature response of microbial species; nevertheless, our analysis clearly shows that denitrification and anammox communities respond sensitively but dissimilarly to warming at the ecosystem level.

According to the fifth assessment report of the IPCC, the projected warming at the global scale by the end of the 21st century relative to 1986–2005 is likely to be between 0.3 °C and 1.7 °C and between 2.6 °C to 4.8 °C in scenarios of low GHG emissions (RCP 2.6) and high GHG emissions (RCP 8.5) scenarios, respectively[44]. Intertidal sediments are expected to directly experience such warming due to the increasing air and water temperatures; thus, the sedimentary denitrification and associated N_2O production potential may be intensified with increasing temperature, as shown by the immediate response of sedimentary Nr removal and N_2O production in our study. Encouraged by the globally consistent pattern of experimentally derived Q_{10} for denitrification (Fig. 4a), and given

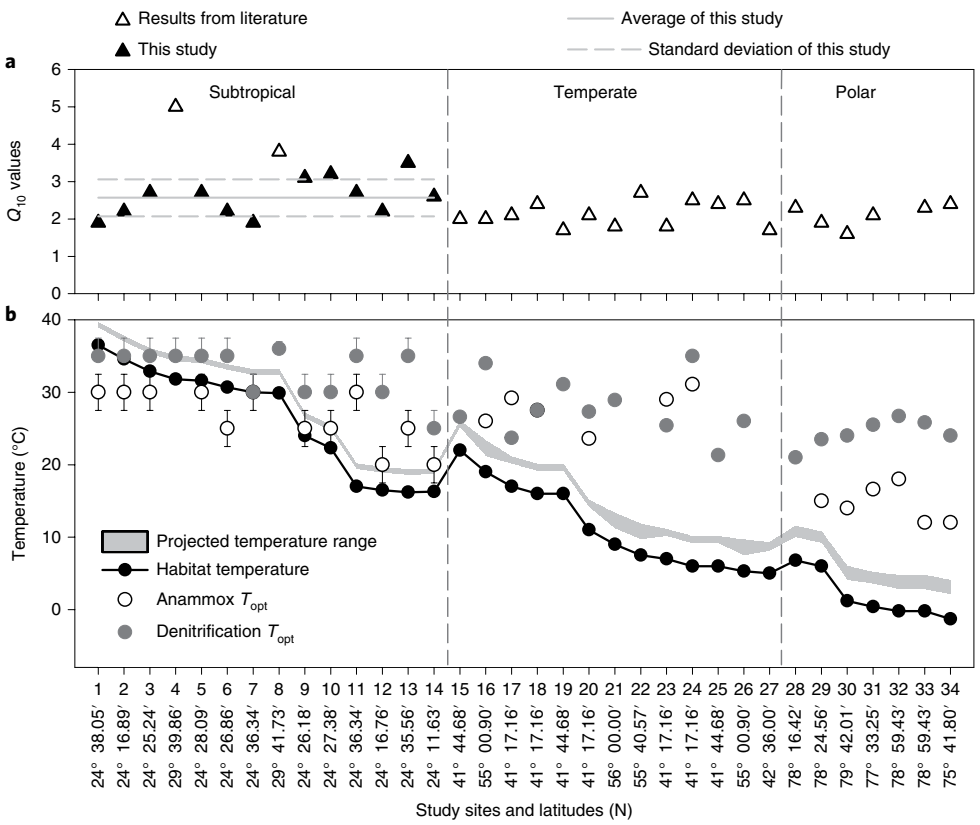

Fig. 4 | Compiled literature values for denitrification and anammox in subtropical, temperate and polar sediments globally. a,**b**, The Q_{10} values for denitrification (**a**) and habitat temperatures, projected temperature range and T_{opt} values for denitrification and anammox (**b**) are shown at different latitudes. The projected temperature range is the sum of habitat temperature and projected warming range by 2100 in RCP scenario 8.5. The numbers indicate sites and latitudes from different studies, as shown in Supplementary Table 8. Note that the error bars for T_{opt} in our study were taken to be ±2.5 °C and show only positive error bars for denitrification when T_{opt} is higher than 35 °C. The specific values are listed in Supplementary Table 8.

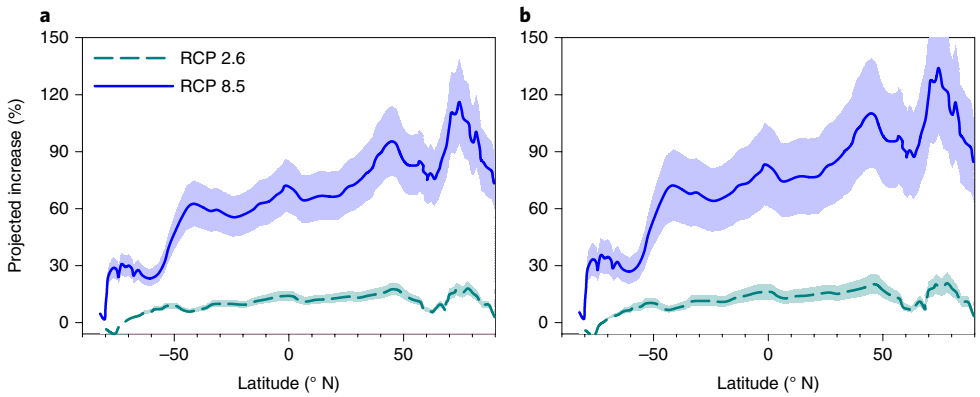

Fig. 5 | The projected increase of sedimentary denitrification and associated N_2O production in coastal environments. a,**b**, The increase of denitrification (**a**) and N_2O production (**b**), as caused by the potential warming across latitudes under RCP 2.6 and RCP 8.5 scenarios. In both plots, the lines show the mean and the shaded areas show the s.d.

the lack of estimates of Q_{10} for coastal sedimentary N_2O production in mid-high latitudes, we applied our results from low latitudes to predict future global Nr removal and associated N_2O production under warming climate conditions, assuming that all environmental parameters other than temperature are invariable. However, these predictions are subject to large uncertainties because of the underlying assumptions and the differential effect of warming on multiple factors governing carbon and nitrogen cycling in marine systems. Considering only the changing temperature and applying the average Q_{10} value of 2.6±0.5 for denitrification and 2.9±0.8 for N_2O production, the projected increase suggests that by 2100, sedimentary Nr removal in marine environments will increase significantly by up to 18% under the RCP 2.6 scenario and 116% under the RCP 8.5 scenario, whereas N_2O production will increase by up to 21% under the RCP 2.6 scenario and 134% under the RCP 8.5 scenario, with the highest increases in the northern high-latitude ecosystems

(Fig. 5). We refrain from presenting a global projection for anammox, because such an approach may not apply because of its lower thermo-tolerance (that is, conditions are likely to exceed T_{opt} values in low-latitude regions). Thus, we believe that while future warming may promote both Nr-removal processes at mid-high latitudes, particularly in the Northern Hemisphere, at low latitudes, warming may suppress anammox (the more climate-friendly community involving no GHG production), with the overall result that denitrification and N_2O production directly stimulated by warming becomes of higher relative importance, thus leading to enhanced warming feedback to the global climate system.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41558-020-0723-2.

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References

1. Battye, W., Aneja, V. P. & Schlesinger, W. H. Is nitrogen the next carbon? *Earth's Future* **5**, 894–904 (2017).
2. Rockstrom, J. A safe operating space for humanity. *Nature* **461**, 472–475 (2009).
3. Cox, P. M., Betts, R. A., Jones, C. D., Spall, S. A. & Totterdell, I. J. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. *Nature* **408**, 184–187 (2000).
4. Galloway, J. N. et al. The nitrogen cascade. *Bioscience* **53**, 341–356 (2003).
5. Field, C. B. & Barros, V. R. Added value from IPCC approval sessions. *Science* **350**, 36 (2015).
6. Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. & Courchamp, F. Impacts of climate change on the future of biodiversity. *Ecol. Lett.* **15**, 365–377 (2012).
7. Magozzi, S. & Calosi, P. Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Glob. Change Biol.* **21**, 181–194 (2015).
8. Allison, S. D. & Treseder, K. K. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Glob. Change Biol.* **14**, 2898–2909 (2008).
9. Gudasz, C. et al. Temperature-controlled organic carbon mineralization in lake sediments. *Nature* **466**, 478–481 (2010).
10. Duan, S. W. & Kaushal, S. S. Warming increases carbon and nutrient fluxes from sediments in streams across land use. *Biogeosciences* **10**, 1193–1207 (2013).
11. Seitzinger, S. et al. Denitrification across landscapes and waterscapes: a synthesis. *Ecol. Appl.* **16**, 2064–2090 (2006).
12. Kuypers, M. M. M., Marchant, H. K. & Kartal, B. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **16**, 263–276 (2018).
13. Zhu, G. et al. Anaerobic ammonia oxidation in a fertilized paddy soil. *ISME J.* **5**, 1905–1912 (2011).
14. Peng, X. et al. Long-term fertilization alters the relative importance of nitrate reduction pathways in salt marsh sediments. *J. Geophys. Res. Biogeosci.* **121**, 2082–2095 (2016).
15. Koriyama, M., Koga, A., Seguchi, M. & Ishitani, T. Factors controlling denitrification of mudflat sediments in Ariake Bay, Japan. *Environ. Monit. Assess.* **188**, 1–14 (2016).
16. Devol, A. H. Denitrification, anammox, and N_2 production in marine sediments. *Annu. Rev. Mar. Sci.* **7**, 403–423 (2015).
17. Engström, P., Penton, C. R. & Devola, A. H. Anaerobic ammonium oxidation in deep-sea sediments off the Washington margin. *Limnol. Oceanogr.* **54**, 1643–1652 (2009).
18. Trimmer, M. & Nicholls, J. C. Production of nitrogen gas via anammox and denitrification in intact sediment cores along a continental shelf to slope transect in the North Atlantic. *Limnol. Oceanogr.* **54**, 577–589 (2009).
19. Brin, L. D., Giblin, A. E. & Rich, J. J. Environmental controls of anammox and denitrification in southern New England estuarine and shelf sediments. *Limnol. Oceanogr.* **59**, 851–860 (2014).
20. Crowe, S. A., Canfield, D. E., Mucci, A., Sundby, B. & Maranger, R. Anammox, denitrification and fixed-nitrogen removal in sediments from the Lower St. Lawrence Estuary. *Biogeosciences* **9**, 4309–4321 (2012).
21. Tan, E., Hsu, T.-C., Huang, X., Lin, H.-J. & Kao, S.-J. Nitrogen transformations and removal efficiency enhancement of a constructed wetland in subtropical Taiwan. *Sci. Total Environ.* **601–602**, 1378–1388 (2017).
22. Canion, A. et al. Temperature response of denitrification and anammox reveals the adaptation of microbial communities to in situ temperatures in permeable marine sediments that span 50° in latitude. *Biogeosciences* **11**, 309–320 (2014).
23. Rich, J. J., Dale, O. R., Song, B. & Ward, B. B. Anaerobic ammonium oxidation (anammox) in Chesapeake Bay sediments. *Microb. Ecol.* **55**, 311–320 (2008).
24. Teixeira, C., Magalhaes, C., Joye, S. B. & Bordalo, A. A. Potential rates and environmental controls of anaerobic ammonium oxidation in estuarine sediments. *Aquat. Microb. Ecol.* **66**, 23–32 (2012).
25. McTigue, N. D., Gardner, W. S., Dunton, K. H. & Hardison, A. K. Biotic and abiotic controls on co-occurring nitrogen cycling processes in shallow Arctic shelf sediments. *Nat. Commun.* **7**, 13145 (2016).
26. Brin, L. D., Giblin, A. E. & Rich, J. J. Similar temperature responses suggest future climate warming will not alter partitioning between denitrification and anammox in temperate marine sediments. *Glob. Change Biol.* **23**, 331–340 (2017).
27. Canion, A. et al. Temperature response of denitrification and anaerobic ammonium oxidation rates and microbial community structure in Arctic fjord sediments. *Environ. Microbiol.* **16**, 3331–3344 (2014).
28. Dalsgaard, T. & Thamdrup, B. Factors controlling anaerobic ammonium oxidation with nitrite in marine sediments. *Appl. Environ. Microbiol.* **68**, 3802–3808 (2002).
29. Rysgaard, S., Glud, R. N., Risgaard-Petersen, N. & Dalsgaard, T. Denitrification and anammox activity in Arctic marine sediments. *Limnol. Oceanogr.* **49**, 1493–1502 (2004).
30. Myrstener, M., Jonsson, A. & Bergstrom, A. K. The effects of temperature and resource availability on denitrification and relative N_2O production in boreal lake sediments. *J. Environ. Sci.* **47**, 82–90 (2016).
31. Smith, K. A., Thomson, P. E., Clayton, H., Mctaggart, I. P. & Conen, F. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. *Atmos. Environ.* **32**, 3301–3309 (1998).
32. Parkin, T. B. & Kaspar, T. C. Nitrous oxide emissions from corn–soybean systems in the midwest. *J. Environ. Qual.* **35**, 1496 (2006).
33. Kawagoshi, Y., Fujisaki, K., Tomoshige, Y., Yamashiro, K. & Wei, Q. Temperature effect on nitrogen removal performance and bacterial community in culture of marine anammox bacteria derived from sea-based waste disposal site. *J. Biosci. Bioeng.* **113**, 515–520 (2012).
34. Pfenning, K. S. & McMahon, P. B. Effect of nitrate, organic carbon, and temperature on potential denitrification rates in nitrate-rich riverbed sediments. *J. Hydrol.* **187**, 283–295 (1996).
35. Boulêtreau, S., Salvo, E. & Lyautey, E. Temperature dependence of denitrification in phototrophic river biofilms. *Sci. Total Environ.* **416**, 323–328 (2012).
36. Jørgensen, C. J., Ole Stig, J., Bo, E. & Jens, A. Microbial oxidation of pyrite coupled to nitrate reduction in anoxic groundwater sediment. *Environ. Sci. Technol.* **43**, 4851–4857 (2009).
37. Ambus, P. Control of denitrification enzyme activity in a streamside soil. *FEMS Microbiol. Lett.* **102**, 225–234 (1993).
38. Palacin-Lizarbe, C., Camarero, L. & Catalan, J. Denitrification temperature dependence in remote, cold and N-poor lake sediments. *Water Resour. Res.* **54**, 1161–1173 (2018).
39. Warneke, S., Schipper, L. A., Bruesewitz, D. A., Mcdonald, I. & Cameron, S. Rates, controls and potential adverse effects of nitrate removal in a denitrification bed. *Ecol. Eng.* **37**, 511–522 (2011).
40. Kuenen, J. G. Extraordinary anaerobic ammonium-oxidizing bacteria. *ASM News* **67**, 456–462 (2001).
41. Strous, M., Kuenen, J. G. & Jetten, M. S. Key physiology of anaerobic ammonium oxidation. *Appl. Environ. Microbiol.* **65**, 3248–3250 (1999).
42. Morita, R. Y. Psychrophilic bacteria. *Bacteriol. Rev.* **39**, 144–167 (1975).
43. Isaksen, M. F. & Jorgensen, B. B. Adaptation of psychrophilic and psychrotrophic sulfate-reducing bacteria to permanently cold marine environments. *Appl. Environ. Microbiol.* **62**, 408–414 (1996).
44. Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L. & Sunday, J. M. Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* **569**, 108–111 (2019).
45. King, D. & Nedwell, D. B. Changes in the nitrate-reducing community of an anaerobic saltmarsh sediment in response to seasonal selection by temperature. *Microbiology* **130**, 2935–2941 (1984).
46. Veraart, A. J., de Klein, J. J. & Marten, S. Warming can boost denitrification disproportionately due to altered oxygen dynamics. *PLoS ONE* **6**, e18508 (2011).
47. Hallin, S. et al. Soil functional operating range linked to microbial biodiversity and community composition using denitrifiers as model guild. *PLoS ONE* **7**, e51962 (2012).
48. Bestion, E., Schaum, C. E. & Yvon-Durocher, G. Nutrient limitation constrains thermal tolerance in freshwater phytoplankton. *Limnol. Oceanogr. Lett.* **3**, 436–443 (2018).

49. Greaver, T. L. et al. Key ecological responses to nitrogen are altered by climate change. *Nat. Clim. Change* **6**, 836–843 (2016).
50. Xue, K. et al. Tundra soil carbon is vulnerable to rapid microbial decomposition under climate warming. *Nat. Clim. Change* **6**, 595–600 (2016).
51. Zhou, J. et al. Microbial mediation of carbon-cycle feedbacks to climate warming. *Nat. Clim. Change* **2**, 106–110 (2011).
52. Tscherko, D., Kandeler, E. & Jones, T. H. Effect of temperature on below-ground N-dynamics in a weedy model ecosystem at ambient and elevated atmospheric CO_2 levels. *Soil Biol. Biochem.* **33**, 491–501 (2001).

Methods

Seasonal sampling and pretreatment. The study area is in the Fujian Province in southern China (Supplementary Fig. 1), which experiences a typical subtropical climate. The monthly average air temperature ranges from 10 °C to 32 °C, while the highest temperature can reach 37 °C. The average annual rainfall is ~1,200 mm[53], of which ~75% occurs in the wet season from April to October[54]. The excessive use of fertilizers in the Jiulong River watershed has led to intensive Nr pollution in downstream regions; Chen et al.[55] quantified the annual Nr input to the Jiulong River watershed to be ~129 kg N ha^{-1} yr^{-1}, resulting in high ammonium and nitrate concentrations in the downstream estuary[56]. Our samples were collected from diverse habitats in Jiulong River estuary and its nearby region, where nitrogen is highly saturated (Supplementary Fig. 1), including the brackish-water ecosystems (estuary, aquaculture pond and tidal flat) and the freshwater ecosystem (freshwater creek).

We conducted campaigns in both summer (20 July 2018) and winter (26 November 2017 and 18 December 2017). Six sites were sampled in each campaign (Supplementary Fig. 1), including estuarine regions, tidal flats, an aquaculture pond and a freshwater creek (Supplementary Fig. 1 and Supplementary Table 1). Both surface sediments and its overlying water were collected. The surface 2 cm of sediments were collected using a grab sampler and immediately transferred into a hermetic bag, whereas the overlying water was collected by using a 2 l brown bottle. During sampling, the temperature of sediments and the salinity in overlying water were measured on-site using a thermometer and WTW analyser, respectively. After collection, the sediments and water samples were stored in an ice chest in the field and transported back to laboratory within 4 h for chemical analyses and incubation experiments.

In the laboratory, the overlying water was filtered using a 0.22 μm membrane, and the filtrate was then prepared for later temperature-gradient incubation experiments and nutrients analysis, including ammonium (NH_4^+) and nitrite plus nitrate (NO_x^-). Meanwhile, the sediments collected from each site were divided into two parts; one part was stored at −20 °C for later analysis of physiochemical characteristics of sediments, including the porosity and contents of organic carbon (OC) and organic nitrogen (ON). The remaining sediments were applied to conduct temperature-gradient incubation experiments.

Temperature-gradient incubation assays. The temperature dependence of denitrification and anammox were investigated by applying temperature-gradient incubation experiments. The experiments were conducted in thermostat incubators with a temperature control accuracy of ±1 °C. Temperature was set between 1 and 35 °C with 5 °C intervals.

Potential rates of denitrification and anammox were measured using slurry incubations, a simplified version of the technique described by Thamdrup and Dalsgaard[57]. In brief, the surface sediments were mixed and vigorously stirred with the filtered water in a ratio of 1:4 (v/v) to make homogenized slurries. The slurries were then purged with helium to ensure anoxic conditions. Dissolved oxygen concentration in slurries was monitored by using an oxygen sensor (OX 50, Unisense) with continuous helium flushing until the oxygen was undetectable[58]. Subsequently, 3 ml of anoxic slurries were transferred into gas-tight borosilicate vials (12 ml, Labco Exetainer), and the vials were then sealed with butyl rubber septa and screw caps. The headspace in the vials was again purged with helium. A total of 15 vials for each temperature treatment were prepared at each sampling site. After the preparation, all the vials were pre-incubated for ~24 h at room temperature to eliminate residual oxygen and background NO_x^-. To acclimate the microbes to the designed temperature, another 24 h pre-incubation was performed at this temperature before ^{15}N-tracer addition.

After complete pre-incubations, three of the 15 vials in each experimental set were used for residual NO_x^- concentration analysis. In the remaining 12 vials, the slurries were spiked with $^{15}NO_3^-$ (Sigma-Aldrich, 98% ^{15}N atoms) to a final concentration of 100 μmol ^{15}N l^{-1}, and were then thoroughly mixed. In each temperature set, 3 of the vials were immediately fixed with 100 μl of saturated $ZnCl_2$ solution after the ^{15}N-tracer addition and assigned as initial zero-time samples. The remaining nine vials were then incubated at the designed temperature in the dark. During the incubation, the samples were collected after about 0.5, 1 and 2 h. Three replicates were sacrificed for each time interval by adding 100 μl of saturated $ZnCl_2$ solution. After the incubation, all of the fixed samples were kept upside down at room temperature in the dark before ^{15}N-labelled N_2 and N_2O measurement. Detailed time-course results for summer and winter can be seen in Supplementary Figs. 6 and 7, respectively, which show the data quality of our rate measurements.

T_{opt} values for denitrification and anammox were estimated from the thermal sensitivity curves, and assigned an uncertainty of 2.5 °C to reflect the 5 °C spacing of the incubation temperatures. Denitrification rates did not always show a clear downturn with increasing temperature, and for those experiments, T_{opt} values were set to the top incubation value of 35 °C and are thus minimum estimates. This means the difference in thermal sensitivity of the two processes may be even larger than shown here.

Chemical analysis. Sediment porosity was measured from the weight loss of a known amount of wet sediments dried at 60 °C to a constant value. The dry sediments were then acidified with 1 N HCl to remove inorganic carbon and freeze dried, and the OC and ON contents in sediments were determined with a FLASH 2000 elemental analyzer[59]. The concentration of NO_x^- was analysed by vanadium (III) reduction with chemiluminescence detector[60]. The NH_4^+ concentration was measured by the indophenol blue spectrophotometric method[61] with a detection limit of 0.5 μmol l^{-1}. The environmental characteristics of sampling sites are provided in the Supplementary Information. The ^{15}N-labelled gas products in fixed samples, including $^{29}N_2$, $^{30}N_2$, $^{45}N_2O$ and $^{46}N_2O$, were quantified by using a Thermo Finnigan Delta V Plus isotope-ratio mass spectrometer equipped with a Gas-Bench II and a PoraPlot Q gas chromatography column (see details in ref. [62]).

Rate calculation and temperature-sensitivity evaluation. We applied the quantification technique as described[62] for potential rate calculation. The potential rates of denitrification, anammox and associated N_2O production were quantified according to the $^{29}N_2$, $^{30}N_2$, $^{45}N_2O$ and $^{46}N_2O$ production rates by using the following equations:

$$D_{N_2O} = (P_{45} + 2P_{46}) \times (r_{14} + 1) = 2P_{46} \times (r_{14} + 1)^2 \quad (1)$$

$$D = 2 \times P_{30} \times (r_{14} + 1)^2 + (P_{45} + 2P_{46}) \times (r_{14} + 1) \quad (2)$$

$$A = (P_{29} - 2 \times r_{14} \times P_{30}) \times (2 \times r_{14} + 1) \quad (3)$$

$$D_{N_2} = D - D_{N_2O} \quad (4)$$

where D_{N_2O}, D, A and D_{N_2} represent the potential rates of N_2O production, denitrification, anammox and denitrification-derived N_2, respectively. P_{29}, P_{30}, P_{45} and P_{46} denote the $^{29}N_2$, $^{30}N_2$, $^{45}N_2O$ and $^{46}N_2O$ production rates, respectively, which were determined from the time-series incubation experiments (see Supplementary Figs. 7 and 8); and r_{14} is the ratio between $^{14}NO_3^-$ and $^{15}NO_3^-$ undergoing nitrate reduction, which is derived from the following equation:

$$r_{14} = \frac{C_{14} + 0.02 \times C_{15}}{0.98 \times C_{15}} \quad (5)$$

where C_{14} is the residual NO_x^- concentration after the secondary pre-incubation. C_{15} is the added ^{15}N-labelled tracer during the incubation, here referred to $^{15}NO_3^-$ (100 μmol l^{-1}). The numbers 0.02 and 0.98 represent the proportion of ^{14}N and ^{15}N in the added $^{15}NO_3^-$, respectively.

The Q_{10} value, which reflects the biological rate in response to a temperature increase of 10 °C, was calculated to evaluate the temperature sensitivity of denitrification, anammox and N_2O production[61]. The relation between biogeochemical rates and temperature (below the optimum temperature) was simply expressed as an exponential function:

$$R(T) = R(T_0) \times e^{kT} \quad (6)$$

where k is the temperature coefficient. $R(T)$ and $R(t_0)$ represent the denitrification, anammox or N_2O production rate measured at temperature T and T_0, respectively. Q_{10} was then calculated using the following equation, and the standard error in Q_{10} was estimated from the regression line:

$$Q_{10} = e^{10k} \quad (7)$$

In addition, because the temperature sensitivity of microbes can be described in terms of activation energy (E_a), and many related studies have used this approach, we provide E_a results and discussions in the Supplementary Information. The responses of biological denitrification and anammox activity to temperature change were modelled using the integrated form of the Arrhenius equation[63,64] to evaluate the thermal sensitivity of Nr-removal microorganisms.

$$\ln[R(T)] = -E'_a\left(\frac{1}{kT} - \frac{1}{kT_c}\right) + \ln[R(T_c)] \quad (8)$$

$$E_a = E'_a \times 96.485 \quad (9)$$

where T is the absolute temperature (below optimum temperature, unit in Kelvin) and T_c is the standardized temperature (here T_c = 15 °C = 288.15 K, adopted from Perkins et al.[64]). $R(T)$ and $R(T_c)$ represent the potential rates of denitrification or anammox at temperature T and T_c, respectively; k is the Boltzmann constant (8.62×10^{-5} eV K^{-1}) and $\ln[R(T_c)]$ is the Arrhenius constant in the traditional equation. The E'_a and E_a represent the apparent activation energy with units of eV and KJ mol^{-1}, respectively. The value of 96.485 is a constant since 1 eV is equal to 96.485 KJ mol^{-1}. The standard error in E_a was estimated from the regression line.

Prediction of sedimentary denitrification and N_2O production increase. Based on the Q_{10} values for sedimentary denitrification and the associated N_2O production, we modelled the increase of Nr removal and N_2O production (ΔR)

as a result of global warming by the end of the 21st century according to the following equation,

$$\Delta R(\%) = 100 \times \frac{\Delta T \times Q_{10}}{10} \quad (10)$$

where ΔT is the projected warming (as reported by Pinsky et al.[44]), which represents the global warming by the end of the 21st century relative to 1986–2005 under RCP 2.6 and RCP 8.5 scenarios. Note that the average Q_{10} value of 2.6 ± 0.5 for denitrification and 2.9 ± 0.8 for N_2O production from two seasons in our study was applied in this equation. The standard deviation of ΔR is calculated using the error propagation.

Statistical analyses. Exponential and linear regressions in this study were conducted using SigmaPlot (v.12.5). The effects of season and process on the Q_{10} and optimum temperature values were examined using two-way ANOVA; the difference of optimum temperature values for anammox between summer and winter was tested using one-way ANOVA. The spatial difference of different parameters were analysed separately using one-sample *t*-test. All statistical analyses were conducted using SPSS (IBM, v.19) at a 0.05 significance level, the *n*, *F*, *t*, degree of freedom (df) and *P* values are provided in Supplementary Tables 3–7.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The data that support the findings of this study can be requested from the corresponding author upon request.

References

53. Cao, W., Huang, Z., Zhai, W., Li, Y. & Hong, H. Isotopic evidence on multiple sources of nitrogen in the northern Jiulong River, Southeast China. *Estuar. Coast. Shelf Sci.* **163**, 37–43 (2015).
54. Hong, Q., Cai, P., Shi, X., Li, Q. & Wang, G. Solute transport into the Jiulong River estuary via pore water exchange and submarine groundwater discharge: new insights from $^{224}Ra/^{228}Th$ disequilibrium. *Geochim. Cosmochim. Acta* **198**, 338–359 (2016).
55. Chen, N., Hong, H., Zhang, L. & Cao, W. Nitrogen sources and exports in an agricultural watershed in Southeast China. *Biogeochemistry* **87**, 169–179 (2008).
56. Yan, X. et al. Distribution, fluxes and decadal changes of nutrients in the Jiulong River Estuary, Southwest Taiwan Strait. *Chin. Sci. Bull.* **57**, 2307–2318 (2012).
57. Thamdrup, B. & Dalsgaard, T. Production of N_2 through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Appl. Environ. Microbiol.* **68**, 1312–1318 (2002).
58. Tan, E. et al. Organic matter decomposition sustains sedimentary nitrogen loss in the Pearl River Estuary, China. *Sci. Total Environ.* **648**, 508–517 (2019).
59. Kao, S. J., Liu, K. K., Hsu, S. C., Chang, Y. P. & Dai, M. H. North Pacific-wide spreading of isotopically heavy nitrogen during the last deglaciation: evidence from the western Pacific. *Biogeosciences* **5**, 1641–1650 (2008).
60. Braman, R. S. & Hendrix, S. A. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium (III) reduction with chemiluminescence detection. *Anal. Chem.* **61**, 2715–2718 (1989).
61. Zheng, Z. Z. et al. Effects of temperature and particles on nitrification in a eutrophic coastal bay in southern China. *J. Geophys. Res.* **122**, 2325–2337 (2017).
62. Hsu, T. C. & Kao, S. J. Simultaneous measurement of sedimentary N_2 and N_2O production and a modified ^{15}N isotope pairing technique. *Biogeosciences* **10**, 7847–7862 (2013).
63. Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M. & West, G. B. Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789 (2004).
64. Perkins, D. M. et al. Consistent temperature dependence of respiration across ecosystems contrasting in thermal history. *Glob. Change Biol.* **18**, 1300–1311 (2012).

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Author contributions

E.T. and S.-j.K. conceived the study and designed the experiment; E.T., W.Z., M.D. and L.T. performed the experiment and measured the samples; E.T., M.D., T.-C.H., L.T. and S.-j.K. analysed the data; E.T., Z.Z., X.Y., T.-C.H., J.J.M., T.W.T. and S.-j.K. contributed to the discussion of the results and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Software and code

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Data collection	We collected our data from the published literatures. No software was used.
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Study description	This study mainly focused on the effect of temperature on sedimentary nitrogen removal rates and associated nitrous oxide production in subtropical regions. This experiment includes 8 temperature sets from 1 to 35°C with a 5°C interval at each sampling site and three replicates for each temperature set.
Research sample	The sediment samples were collected from different subtropical ecosystems with different salinity and habitat. Only the natural nitrogen removal organisms were included in the study. We also compiled the existing datasets which conducted in temperate and polar regions at the global scale.
Sampling strategy	The surface 2 cm of sediments were collected using a grab and immediately transferred into a hermetic bag, whereas the overlying water was collected by using a 2 L brown bottle.
Data collection	The rate-related data was measured using a Thermo Finnigan Delta V Plus isotope ratio mass spectrometer.
Timing and spatial scale	We conducted campaigns in both summer (20 July 2018) and winter (26 November 2017 and 18 December 2017). Six sites were sampled in each campaign, including estuarine regions, tidal flats, an aquaculture pond and a freshwater creek. All of the sampling sites were included in an area with a range of latitude from 24.3-24.7°N and a range of longitude from 117.7-118.5°E.
Data exclusions	No data was excluded from the analysis in this study.
Reproducibility	All attempts to repeat the experiment were successful.
Randomization	The sediments collected from one site were mixed with the overlying water and then the slurry was added into 15 vials, these vials were allocated into three groups randomly.
Blinding	We collected the historical data on the temperature dependence of nitrogen removal processes in temperate and polar regions, so blinding was not possible.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	The temperature during field sampling was 16-24°C and 30-37°C in winter and summer, respectively, and no rainfall was experienced.
Location	The samples were collected from Jiulong River Estuary and the adjacent coastal bays, a typical subtropical region in South China, with water depth of 0-11.2 m, latitude of 24.3-24.7°N and longitude of 117.7-118.5°E.
Access and import/export	The sediments and water samples were stored in an ice chest in the field and transported back to laboratory within 4 h for chemical analyses and incubation experiments. Our permits were obtained from Xiamen University.
Disturbance	Nearly no disturbance was caused by the study since only ~500 mL sediments and water were collected from each site.

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Laboratory animals	This study did not involve any laboratory animals.
Wild animals	We collected sediment samples from aquatic ecosystems, involving different bacteria under natural conditions.
Field-collected samples	The field samples were collected from natural ecosystems, including estuary, tidal flat and mangroves, with temperature of 16-24°C and 30-37°C in winter and summer, respectively.
Ethics oversight	No ethical approval or guidance was required since our study just include natural bacteria.

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