



How Nitrogen Is Lost
Bess B. Ward
Science **341**, 352 (2013);
DOI: 10.1126/science.1240314

This copy is for your personal, non-commercial use only.

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

The following resources related to this article are available online at www.sciencemag.org (this information is current as of September 23, 2013):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/content/341/6144/352.full.html>

This article **cites 14 articles**, 3 of which can be accessed free:

<http://www.sciencemag.org/content/341/6144/352.full.html#ref-list-1>

transposed a piece of jejunum between the esophagus and the stomach in the rat, and found the same hypertrophic response and similar improvements in glycemic control. Altogether, Saeidi *et al.* clearly demonstrate that entry of undigested food into the small intestine triggers reprogramming of glucose metabolism to allow for tissue growth, and that the resulting increase in intestinal glucose disposal improves glycemia. Although a direct causal role was not established for improved glucose homeostasis by either preventing the hypertrophic response or reprogramming glucose metabolism, the combined findings strongly suggest such a role, at least for this rat model.

One factor that has been shown to stimulate gut hypertrophy is glucagon-like peptide 2 (GLP-2) (8), but what is it in the undigested food that triggers hypersecretion of GLP-2 and the metabolic reprogramming response? Is it related to the absence of bile acids and pancreatic digestive enzymes in the Roux limb that are particularly important for the absorption of fat? This idea is supported by findings in both rodents and humans that after gastric bypass surgery, there is a decreased preference for fat, likely the result of difficulties with fat absorption (9, 10). Another question concerns the time course of enhanced intestinal glucose use. Doubling or even qua-

drupling of the intestinal mass does not happen overnight. Saeidi *et al.* report that maximal expansion was reached at 1 month. It will be important to monitor gut tissue growth earlier, as hypersecretion of incretins (hormones that augment insulin secretion) and improvements of glycemic control occur as early as 10 days after surgery in humans (11). Assessment of a full time course of the adaptive response and its consequences for glucose use, all while controlling for weight loss, will be revealing.

Are the similar beneficial effects of different types of bariatric surgeries on glycemic control mediated by the same mechanism (12)? For much less intrusive surgical interventions, such as sleeve gastrectomy, gastric emptying mechanisms and normal digestion are preserved to some extent, so there might be less of an adaptive hypertrophic response. To date, gut hypertrophy has not been examined after this much simpler surgery. There also is the question of whether the mechanism proposed by Saeidi *et al.* in the rat is applicable to bariatric surgeries in humans. When control subjects were given the same low amounts of food eaten by surgical patients, the same rapid improvements in glycemic control were observed (13, 14), suggesting that acute calorie restriction rather than gut hypertrophy is important for diabe-

tes remission, at least during the early post-surgical period, before substantial weight loss has occurred.

Will the mechanism identified by Saeidi *et al.* eventually lead to “knifeless” metabolic interventions—“bypassing the bypass,” as the authors put it? The answer hinges on the possibility to enhance glucose disposal in the gut (or in any other organ) by pharmacological and/or nutritional approaches. In the meantime, there’s the old-fashioned approach—exercise.

References

1. H. Ling *et al.*, *Pharmacotherapy* 10.1002/phar.1277 (2013).
2. K. A. Holes-Lewis *et al.*, *Am. J. Med. Sci.* **345**, 284 (2013).
3. D. E. Cummings, J. Overduin, K. E. Foster-Schubert, *J. Clin. Endocrinol. Metab.* **89**, 2608 (2004).
4. C. W. le Roux *et al.*, *Ann. Surg.* **243**, 108 (2006).
5. N. Saeidi *et al.*, *Science* **341**, 406 (2013).
6. M. B. Mumfrey, L. M. Patterson, H. Zheng, H. R. Berthoud, *Neurogastroenterol. Motil.* **25**, e70 (2013).
7. C. F. Hansen *et al.*, *PLoS ONE* **8**, e65696 (2013).
8. D. L. Sigalet *et al.*, *Dig. Dis. Sci.* **51**, 1557 (2006).
9. H. Zheng *et al.*, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R1273 (2009).
10. C. W. le Roux *et al.*, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **301**, R1057 (2011).
11. R. Peterli *et al.*, *Obes. Surg.* **22**, 740 (2012).
12. M. A. Stefater *et al.*, *Endocr. Rev.* **33**, 595 (2012).
13. I. Lingway *et al.*, *Diabetes Care* 10.2337/dc12-2316 (2013).
14. M. A. Lips *et al.*, *Clin. Endocrinol.* 10.1111/cen.12254 (2013).

10.1126/science.1242673

OCEANS

How Nitrogen Is Lost

Bess B. Ward

As in the back garden, productivity in the ocean is often limited by the availability of nutrients, principally nitrogen (N) and phosphorus (P). On a global scale, the rates of nitrogen fixation (input) and nitrogen loss (output) are believed to be roughly equal. Both rates are, however, very uncertain. Nitrogen loss processes occur in subsurface water and sediments and depend on the supply of organic matter, derived from primary production in surface waters. Reports in the last few years have changed our understanding of the controls and pathways responsible for nitrogen loss.

Two main processes are responsible for nitrogen loss: denitrification and anaerobic ammonium oxidation (anammox). These processes rely on fundamentally different

organisms and metabolic pathways. Denitrification is the sequential reduction of nitrate (NO₃) to dinitrogen gas (N₂) via oxidized intermediates, whereas anammox combines ammonium (NH₄) and nitrite (NO₂) to yield N₂. Most denitrifying organisms obtain their carbon (C) for growth from organic matter produced by phytoplankton, whereas anammox bacteria fix their own CO₂ into biomass. Most denitrifiers consume and degrade organic matter, releasing dissolved inorganic nitrogen (including NH₄ and NO₂). Anammox bacteria require a source of NH₄ and NO₂, derived from the breakdown of organic matter by other microbes (see the figure).

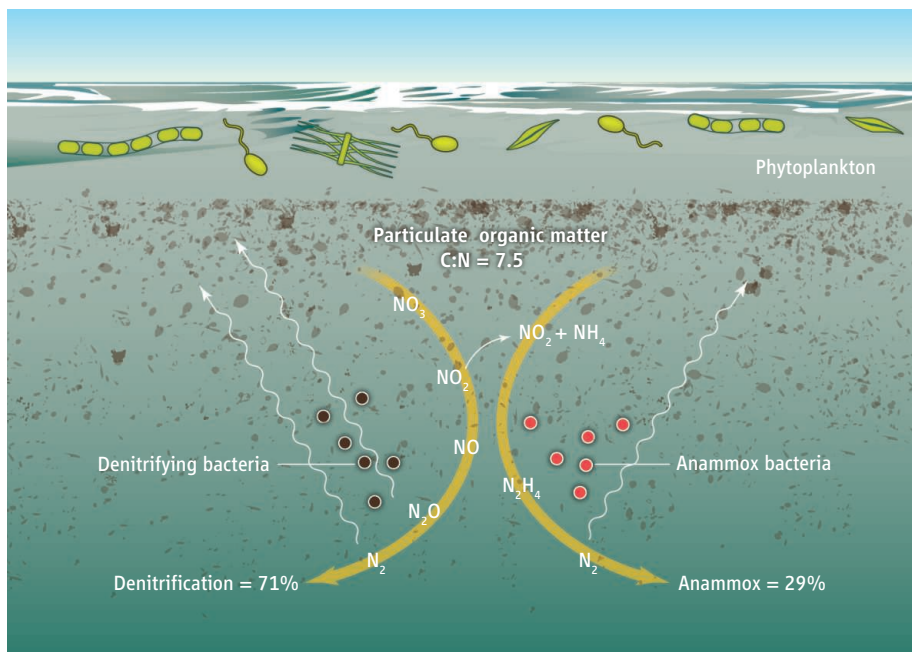
Anammox is a slow process mediated by bacteria with minimum generation times of nearly 2 weeks. Most marine bacteria and their grazers have generation times of a few hours to a few days at most, and it was therefore surprising when only anammox was

The supply and composition of organic matter control the processes by which fixed nitrogen is lost from the ocean.

reported in marine environments, including open-ocean regions containing very low oxygen concentrations [oxygen minimum zones (OMZs)] (1). In these regions, denitrification had been assumed to be the sole process responsible for nitrogen loss.

The source of NH₄ and NO₂ to support anammox in the absence of denitrification was a mystery. Organic matter in the ocean has a well-established average composition based on the primary producers from which it derives. Complete decomposition and mineralization of this material to form CO₂ and N₂ gases can occur by denitrification and anammox in the ratio of 71:29. Any other ratio requires the supply of nitrogen or carbon from an additional source (2). Reports of 100% anammox (3) suggested major inconsistencies either in the experiments or in our understanding of carbon and nitrogen cycling in the ocean.

Department of Geosciences, Princeton University, Princeton, NJ 08544, USA. E-mail: bbw@princeton.edu



Drivers of nitrogen loss. The two bacterial processes leading to nitrogen loss in oxygen minimum zones of the ocean depend on the supply of organic matter from the surface layer. Recent studies explain how the rates and proportions of denitrification and anammox are controlled in ocean waters and sediments.

Subsequent investigations of denitrification and anammox in the major OMZs of the world ocean reported either anammox (1, 4) or denitrification (5, 6), but rarely both. Two hypotheses were suggested to explain the apparent lack of denitrification in some locations. Lam *et al.* (7) suggested three alternative sources for ammonium (nitrite concentrations are normally sufficient in OMZ waters): microaerobic respiration, that is, organic matter degradation at the expense of oxygen; transport of ammonium from sediments or by lateral advection from other water masses; and dissimilatory nitrate reduction to ammonium ($\text{NO}_3 \rightarrow \text{NH}_4$). However, there is no oxygen in OMZ waters most of the time (8), such that even intermittent oxygen supply cannot support the necessary degradation rates for microaerobic respiration. Transport of ammonium from sediments and dissimilatory nitrate reduction may both contribute to the ammonium supply, but not at rates sufficient to support the observed consumption by anammox (9).

According to the second hypothesis, denitrification occurs in OMZs, but its distribution is patchy in space and time as a result of an episodic supply of organic matter (1, 10). It was suspected that the limited scope of the small number of expeditions to date provided only tiny snapshots of the OMZs, thus having missed major episodes of denitrification. In this scenario, denitrification is patchy and dynamic in range, whereas anammox is slow, steady, and consistent, but ultimately depen-

dent upon denitrification for the supply of dissolved inorganic nitrogen (DIN).

Recent findings appear to resolve the controversy in favor of the second hypothesis. In a transect along the coast of South America (the eastern tropical South Pacific OMZ), Dalsgaard *et al.* (11) detected both anammox and denitrification. Anammox occurred at almost every station at low rates, whereas denitrification was less commonly detected but occurred at very high rates at a few stations. When averaged over their data set, the ratio of nitrogen loss as N_2 from denitrification and anammox was 72:28, close to the expected ratio and far from the dominance by anammox previously reported from the same region on the basis of a few measurements (1, 4).

Independently, work in my lab found that the overall loss rate depends on the amount of organic matter added and that the ratio of denitrification:anammox depends on the carbon to nitrogen ratio (C:N) of the available substrate. In both sediments (12) and OMZ waters (13), natural organic material with a C:N ratio very close to the ocean average (~ 7.5) resulted in a denitrification:anammox ratio of 70:30—almost exactly the predicted ratio. Incubations augmented with amino acids or sucrose plus ammonium had higher and lower anammox proportions, respectively, in line with the C:N ratios of these substrates.

In the OMZ, where DIN can only come from the degradation of organic matter in the water column (see the figure), the total rate

of nitrogen loss and the relative contributions of denitrification and anammox must be constrained by the supply of organic matter. The extensive field measurements by Dalsgaard *et al.* (11) show that denitrification and anammox are differently distributed in time and space. Our experiments (12, 13) provide an explanation for those differences in terms of the composition of the episodic organic matter supply. Thus, no major revision of the nitrogen cycle, nor transport of DIN independently of organic matter supply, is required to explain nitrogen loss in OMZs.

Although these recent reports appear to resolve a major controversy, further complications may be hidden under the cover of denitrification. Most denitrifiers consume organic matter, but some obtain the energy for CO_2 fixation from the oxidation of reduced sulfur compounds. Usually, these organisms are considered to be important only in sediments, but recent evidence of sulfide oxidation (14) and of active expression of autotrophic sulfur oxidation genes (15) suggest that a cryptic sulfur cycle is linked to denitrification in the OMZs. Organic matter thus may not be the only control on denitrification rates in these regions.

Denitrification and anammox together, occurring in the OMZs and ocean sediments, effectively consume the organic material sinking out of the surface waters and account for all the oceanic nitrogen loss. The dependence of nitrogen loss rates on organic matter supply implies a tightly coupled oceanic nitrogen cycle, which controls the fertility of surface waters.

References and Notes

1. B. Thamdrup *et al.*, *Limnol. Oceanogr.* **51**, 2145 (2006).
2. W. Koeve, P. Kähler, *Biogeochem.* **7**, 2327 (2010).
3. M. M. Kuypers *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 6478 (2005).
4. M. R. Hamersley *et al.*, *Limnol. Oceanogr.* **52**, 923 (2007).
5. J. C. Nicholls *et al.*, *Limnol. Oceanogr.* **52**, 156 (2007).
6. B. B. Ward *et al.*, *Nature* **461**, 78 (2009).
7. P. Lam *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4752 (2009).
8. B. Thamdrup, T. Dalsgaard, N. P. Revsbech, *Deep Sea Res. Part I Oceanogr. Res. Pap.* **65**, 36 (2012).
9. T. Kalvelage *et al.*, *Nat. Geosci.* **6**, 228 (2013).
10. B. B. Ward *et al.*, *Deep Sea Res. Part I Oceanogr. Res. Pap.* **55**, 1672 (2008).
11. T. Dalsgaard *et al.*, *Limnol. Oceanogr.* **57**, 1331 (2012).
12. A. R. Babbín, B. B. Ward, *Environ. Sci. Technol.* **47**, 4189 (2013).
13. A. R. Babbín *et al.*, American Society for Limnology and Oceanography Aquatic Sciences Meeting, New Orleans, LA, 17 to 22 February 2013, abstract S533-26.
14. D. E. Canfield *et al.*, *Science* **330**, 1375 (2010).
15. F. J. Stewart *et al.*, *Environ. Microbiol.* **14**, 23 (2012).

Acknowledgments: B. Chang, A. Babbín, and A. Jayakumar reviewed an early version of the manuscript.

10.1126/science.1240314