'barcodes' for the identification of individual bacterial species. Bacterial identities are simultaneously assigned through monitoring by spectral imaging and classification of microbes in the images using a machine-learning algorithm. HiPR-FISH bypasses the previous financial constraints by simplifying the encoding sequence to cheaply synthesized DNA sequences and requiring only ten different types of fluorophore.

Using an automated program to delineate a multitude of single cells in a dense crowd, Shi and colleagues used HiPR-FISH to locate and determine the species identity of individual bacteria in samples from the mouse gut and in samples of oral microbes in human plaque. These different microbial ecosystems are both examples of bacterial communities that can contain hundreds of distinct species.

This demonstration of a previously impossible level of analysis of complex communities using single-cell level mapping, enables the quantitative study of bacterial spatial organization, such as the determination of the distance between specific microbial species residing in a host. Such high-resolution data are important to answer key questions concerning the behaviour of microbial communities, such as who interacts with whom, and where those interactions take place. Interactions are theoretically possible between microbes in close spatial proximity, so HiPR-FISH opens a new era in the study of microbial ecology by enabling micrometre-scale mapping of the spatial distance between hundreds of microbial species in complex communities.

Shi *et al.* assessed the distance between different bacterial species normally resident in the mouse gut and measured how these distances changed after antibiotic treatment. Such therapy is known to alter the assortment and abundance of bacterial species in the gut[11]. The largest distance changes due to antibiotic treatment observed by Shi and colleagues were between *Oscillibacter* and *Veillonella*, which are microbes that are both individually associated with health benefits in the human gut[12,13]. Whether and how these bacteria interact functionally remains to be uncovered. However, the fourfold increase in spatial distance between these bacteria after antibiotic use raises the possibility that the antibiotic treatment might disrupt an interaction that aids the host. Identifying such interactions and deciphering the underlying mechanisms will boost our understanding of how microbial communities respond to, and recover from, environmental perturbation.

By shedding light on microbial biogeography, this work charts new paths for exploring microbial interactions in complex ecosystems. Exciting next steps to anticipate include the elucidation of mechanisms by which environmental disturbances alter bacterial spatial organization, and how altered organization affects community function. For example, how does antibiotic exposure result in an increase in distance between *Oscillibacter* and *Veillonella*? Is the spatial proximity between specific bacteria important for community recovery after disturbances such as antibiotic exposure?

Finally, expanding on this technology to access the spatial organization of transcriptional responses would enable maps to be generated that reveal the spatial gradients of bacterial species and their functions. These future applications will truly revolutionize our understanding of complex microbial communities and the spatial diversity that is so fundamental for life.

Jen Nguyen and **Carolina Tropini** are at the School of Biomedical Engineering and the Department of Microbiology and Immunology, University of British Columbia, Vancouver V6T-1Z3, Canada. **C.T.** is also at the Humans and the Microbiome Program, Canadian Institute for Advanced Research, Toronto, Canada.
e-mail: carolina.tropini@ubc.ca

1. Stacy, A., McNally, L., Darch, S. E., Brown, S. P. & Whiteley, M. *Nature Rev. Microbiol.* **14**, 93–105 (2016).
2. Tropini, C., Earle, K. A., Huang, K. C. & Sonnenburg, J. L. *Cell Host Microbe* **21**, 433–442 (2017).
3. Falkowski, P. G., Fenchel, T. & Delong, E. F. *Science* **320**, 1034–1039 (2008).
4. Donia, M. S. & Fischbach, M. A. *Science* **349**, 1254766 (2015).
5. Shi, H. *et al. Nature* **588**, 676–681 (2020).
6. Moter, A. & Göbel, U. B. *J. Microbiol. Methods* **41**, 85–112 (2000).
7. Beliveau, B. J. *et al. Proc. Natl Acad. Sci. USA* **115**, E2183–E2192 (2018).
8. Valm, A. M. *et al. Proc. Natl Acad. Sci. USA* **108**, 4152–4157 (2011).
9. Valm, A. M., Oldenbourg, R. & Borisy, G. G. *PLoS ONE* **11**, e0158495 (2016).
10. Moffitt, J. R. *et al. Proc. Natl Acad. Sci. USA* **113**, 14456–14461 (2016).
11. Ng, K. M. *et al. Cell Host Microbe* **26**, 650–665 (2019).
12. Li, J. *et al. Proc. Natl Acad. Sci. USA* **113**, E1306–E1315 (2016).
13. Scheiman, J. *et al. Nature Med.* **25**, 1104–1109 (2019).

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Biogeochemistry

Making the most of wetland restorations

Jacques C. Finlay

Wetlands remove nitrate pollution from water effectively. An analysis shows that this effect is constrained in the United States by the distribution of wetlands, and could be increased by targeting wetland restoration to nitrate sources. **See p.625**

Human activities have increased the amounts of reactive nitrogen compounds — forms of nitrogen that can be used by organisms for growth — in the environment. The resulting overabundance of reactive nitrogen has far-reaching consequences for ecosystems, climate, and human health and well-being[1]. Fertilizers are the largest global source of anthropogenic nitrogen inputs[2], and so intensive efforts have been made to reduce nitrogen transport from agricultural land to ground and surface waters, but with mixed results. On page 625, Cheng *et al.*[3] report that sources of river nitrogen pollution in the United States are often spatially separated from existing wetlands (Fig. 1), which can remove nitrate from water, and show that wetland restoration targeted to nitrate sources would yield substantial benefits for downstream water quality.

The beneficial effects of wetlands on water quality are well documented, and wetlands are widely used both in urban and rural settings to remove pollution arising from human activities[4]. The biogeochemical conditions in wetlands particularly favour the removal of nitrate, which is often the dominant form of nitrogen pollution in water. However, the global area of wetlands has reduced drastically over the past two centuries[5,6], and losses continue despite greater protections being established. The need for wetland restoration is clear, but it is difficult to calculate the potential contributions that restorations could make to nitrate removal for large water catchment areas by scaling up the effects of individual wetlands. This is because water-quality outcomes are highly sensitive to the geographical distribution of wetlands relative to that of nitrogen sources[7–9].

Cheng *et al.* tackle this problem by combining an inventory of US wetland distribution with models of nitrogen transport. Their analysis affirms — with much greater precision than was possible in past studies — that remnant and restored wetlands in agricultural areas have a disproportionately large role in mitigating

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Figure 1 | Restored wetlands in the South San Francisco Bay Area. Cheng *et al.*[3] report that wetland restoration targeted to the regions of the United States that contribute most to nitrate pollution could have a big effect on environmental water quality.

river nitrogen pollution. Without these wetlands, the negative impacts of nitrogen pollution on coastal zones and on many inland waters would be much worse.

The authors then develop scenarios for expanding wetland coverage in the United States, taking into account the large misalignment between current wetland distributions and regions that have high nitrogen levels in water run-off. They estimate that an aggressive strategy that increases wetland area by 10% in agricultural areas that have the highest nitrogen run-off would almost double nitrogen removal by wetlands, compared with current levels. Such a restoration effort would be costly, requiring investments of several billions of US dollars annually to convert a modest amount of productive farmland to wetlands. However, as Cheng *et al.* discuss, current conservation spending is probably similar in magnitude, and so reprioritizing funds to target nitrogen sources more effectively could help to pay some of the costs. The study makes a compelling case for better use of conservation investments to deal with the stubborn problem of nitrogen pollution.

Many other challenges remain to be addressed before large-scale expansion of wetland restoration could begin. A more comprehensive accounting of the benefits, and of other costs, is needed to fully understand the economic implications of restorations along the lines described by the authors. For example, wetlands provide other important ecosystem services such as sequestering atmospheric carbon, supporting biodiversity, and reducing flooding and stream-bank erosion[6]. Thus, the benefits of wetland restoration would extend to other ecosystem services.

However, wetland disservices, such as the release of greenhouse gases[10], could offset some of these benefits. Continued efforts to reduce the transport of agricultural pollutants that cannot be effectively removed by wetlands, such as phosphorus, will also be necessary. Finally, substantial policy and legal uncertainties regarding US federal rules governing water management on private land[11] must be resolved to overcome barriers to conservation efforts. Despite these challenges, Cheng and colleagues' work describes a way forward to achieve long-standing, but elusive, US policy goals.

The authors point out that the increasing availability of data and modelling tools will create further opportunities to use wetland restorations effectively within individual river catchment areas. For example, the benefits of a downstream wetland might be less if an upstream wetland has already reduced the amount of incoming nitrate; Cheng *et al.* could not address this effect in depth in their nationwide study, but detailed studies at smaller scales (see refs 9 and 12, for example) could help to further optimize the placement of restored wetlands to maximize the benefits for nitrate removal and other services[13].

The costs of large-scale wetland restoration are sure to be a challenge given increased economic pressure on US farming from global competition, trade policies and climate shifts. However, the lack of progress in meeting goals for reducing nitrogen pollution, and the likelihood that such pollution will worsen in a warming world, mean that new approaches are needed. Cheng and colleagues' findings make a compelling case for a renewed emphasis on wetland restorations in the catchment-scale management of agricultural land. By identifying current mismatches between sources and sinks of nitrogen pollution in the United States, the authors provide a road map for more effectively unleashing the potential of wetland restoration to help solve water-quality problems.

Jacques C. Finlay is in the Department of Ecology, Evolution and Behavior, University of Minnesota, Saint Paul 55108, Minnesota, USA.
e-mail: jfinlay@umn.edu

1. Organisation of Economic Co-operation and Development. *Human Acceleration of the Nitrogen Cycle* (OECD, 2018).
2. Galloway, J. N. *et al. Bioscience* **53**, 341–356 (2003).
3. Cheng, F. Y. *et al. Nature* **588**, 625–630 (2020).
4. Mitsch, W. & Gosselink, J. *Wetlands* 5th edn, Ch. 19 (Wiley, 2015).
5. Gardner, R. C. & Finlayson, M. *Global Wetland Outlook: State of the World's Wetlands and their Services to People 2018* (Ramsar Convention Secretariat, 2018).
6. Zedler, J. B. & Kercher, S. *Annu. Rev. Environ. Resour.* **30**, 39–74, (2005).
7. Mitsch, W. J. & Gosselink, J. G. *Ecol. Econ.* **35**, 25–33 (2000).
8. Crumpton, W. G. *Water Sci. Technol.* **44**, 559–564 (2001).
9. Hansen, A. T., Dolph, C. L., Foufoula-Georgiou, E. & Finlay, J. C. *Nature Geosci.* **11**, 127 (2018).
10. Burgin, A. J., Lazar, J. G., Groffman, P. M., Gold, A. J. & Kellogg, D. Q. *Ecol. Eng.* **56**, 26–35 (2013).
11. Creed, I. F. *et al. Nature Geosci.* **10**, 809–815 (2017).
12. Czuba, J. A., Hansen, A. T., Foufoula-Giorgiu, E. & Finlay, J. C. *Water Resour. Res.* **54**, 1312–1337 (2018).
13. Zedler, J. B. *Front. Ecol. Environ.* **1**, 65–72 (2003).