

# Bacteria added to farm soil curb nitrogen emissions

Guang He & Frank E. Löffler

Innovative solutions are needed to decrease greenhouse-gas emissions. Field trials show that supplementing farm soil with a bacterium that consumes the greenhouse gas nitrous oxide can substantially lower harmful emissions. See p.421

As the consequences of climate change become increasingly clear<sup>1,2</sup>, the global research community is being challenged to deliver innovative solutions that lower emissions of climate-warming gases. On page 421, Hiis *et al.*<sup>3</sup> report one such solution to decrease emissions of nitrous oxide ( $\text{N}_2\text{O}$ , also known as laughing gas), which are associated with the application of nitrogen-containing fertilizer.

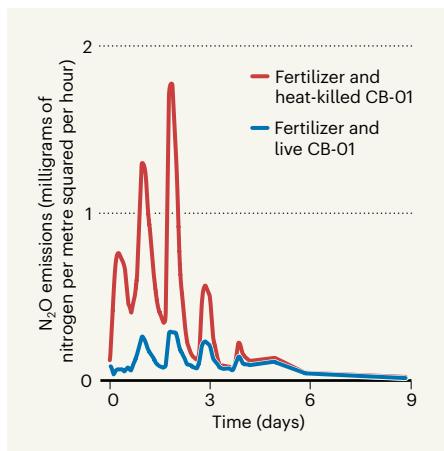
$\text{N}_2\text{O}$  is an important climate-active gas that has a warming potential nearly 300 times greater than that of carbon dioxide (assessed using the metric of a 100-year time horizon)<sup>1</sup>. In addition to trapping heat in the atmosphere,  $\text{N}_2\text{O}$  exacerbates warming by depleting ozone in the stratosphere, and cumulative  $\text{N}_2\text{O}$  emissions between 1851 and 2021 contributed 0.06 °C to the increase in global mean surface temperature<sup>2</sup>.  $\text{N}_2\text{O}$  concentrations reached a level of 332 parts per billion by volume in 2019, an increase of about 23% over the past 250 years, with the highest increases occurring in the past 50 years<sup>1</sup>. This level of  $\text{N}_2\text{O}$  in Earth's atmosphere has not been reached for at least 800,000 years<sup>1</sup>.

At the root of increasing  $\text{N}_2\text{O}$  emissions lies one of the greatest inventions of the twentieth century: the Haber–Bosch chemical process, which enables the large-scale production of synthetic nitrogen-containing fertilizers<sup>4</sup>. The amount of fertilizers produced by this process more than equals the amount of nitrogen that is captured naturally in agroecosystems through a process termed biological nitrogen fixation<sup>5</sup>. This implies that about half of the nitrogen atoms in a person living in a developed nation have passed through a chemical plant running the Haber–Bosch process (see go.nature.com/4bhywae).

Synthetic fertilizers are essential for providing the food needed to sustain the estimated 8 billion people on Earth. However, excessive fertilizer application and limitations in the efficiency of nitrogen use (less than half of the nitrogen in applied fertilizer is taken up by crops) has dire environmental consequences<sup>6</sup>. In addition to emission of climate-warming

$\text{N}_2\text{O}$  (which occurs when soil microorganisms break down fertilizer), fertilizer run-off leads to a rise in the concentrations of the compound nitrate in groundwater. This, in turn, promotes the process of eutrophication and harmful algal blooms, which causes oxygen depletion and the emergence of 'dead zones' that cannot support aquatic life in bodies of water.

Microorganisms that can take up  $\text{N}_2\text{O}$  are a natural 'sink' for this gas. Such microbes make an enzyme called  $\text{N}_2\text{O}$  reductase (NosZ), which can convert  $\text{N}_2\text{O}$  to environmentally benign dinitrogen ( $\text{N}_2$ ). However, until now,



**Figure 1 | Lowering agricultural greenhouse-gas emissions by applying a bacterium to soil.** Fertilizer added to soil can result in release of the potent greenhouse gas nitrous oxide ( $\text{N}_2\text{O}$ ) in waves that are influenced by daily variations in temperature. Hiis *et al.*<sup>3</sup> have built on previous work of theirs<sup>7</sup> that identified a bacterial strain – *Cloacibacterium* sp. strain CB-01 – that can consume  $\text{N}_2\text{O}$  and release nitrogen gas ( $\text{N}_2$ ), which is not a greenhouse gas. When the authors tested the effect of adding the bacterium along with fertilizer to field plots, they observed a substantial decrease in the release of  $\text{N}_2\text{O}$ . For example, they saw a 64% drop in  $\text{N}_2\text{O}$  emissions in this field plot trial using live bacteria when compared with the results for the heat-killed control. (Figure adapted from Fig. 5 of ref. 3.)

researchers have not successfully unlocked the potential of this microbial process for controlling  $\text{N}_2\text{O}$  emissions. Hiis *et al.* present an innovative approach called bioaugmentation that lowered  $\text{N}_2\text{O}$  emissions from farmland by 50–95%.

Bioaugmentation refers to the introduction of microorganisms into a natural or engineered environment with the goal of increasing the rate of the process of interest – in this case, the conversion of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (an enzymatic reaction known as  $\text{N}_2\text{O}$  reduction). A key challenge for successful bioaugmentation in this context is to find microorganisms that can both consume  $\text{N}_2\text{O}$  and survive in a real-world environment such as agricultural soil. The authors built on previous work of theirs<sup>7</sup> that used a dual-enrichment strategy to find such microbes. The strategy enriched naturally occurring microbes by alternating between two types of substrate: agricultural soil and partially treated sewage (a cheap fertilizer called digestate) obtained from a municipal wastewater treatment plant. This previous work by the authors<sup>7</sup> yielded *Cloacibacterium* sp. strain CB-01, an  $\text{N}_2\text{O}$ -consuming bacterium that can be rapidly propagated on digestate.

Strain CB-01 has the genes needed to make NosZ, but lacks other genes for nitrogen metabolism (such as those for dissimilatory nitrate/nitrite reductase), indicating that the bacterium can consume  $\text{N}_2\text{O}$  but not produce it. Although the  $\text{N}_2\text{O}$ -reducing performance of strain CB-01 is merely average among characterized  $\text{N}_2\text{O}$  reducers, it grows to high cell densities in sterilized digestate when oxygen is provided, readily switches to  $\text{N}_2\text{O}$  consumption when oxygen is depleted and has robust  $\text{N}_2\text{O}$ -reducing activity in agricultural soils.

In three independent field experiments, the authors demonstrated that soils fertilized with digestate containing live CB-01 cells lowered  $\text{N}_2\text{O}$  emissions by 50–95%, depending on soil type, compared with fertilized control soils that received heat-killed CB-01 cells (Fig. 1). A high inoculation density could explain such impressive short-term results, but the activity was sustained over the growing season – the authors show that the decline of strain CB-01 was gradual and cells survived for several months. This highlights the power of the dual-enrichment strategy for isolating microbes that combine desirable functions with tenacity and robustness for real-world bioaugmentation applications in different types of agricultural soil.

The authors estimated  $\text{N}_2\text{O}$  emissions for Europe in 2030 using a model called GAINS (greenhouse gas and air pollution interactions and synergies). This is an analytical framework for assessing emissions- and pollution-reduction strategies that simultaneously mitigate air pollution and climate change. Assuming that the bioaugmentation approach achieves a 60% reduction in  $\text{N}_2\text{O}$  emissions and that

bacteria are applied to all liquid manure systems in Europe, the total human-associated  $\text{N}_2\text{O}$  emissions from the region would decrease by 2.7%. The decrease would be 4% for the 27 member countries of the European Union, which have a higher share of liquid manure systems than do European countries outside the EU. If the bioaugmentation approach of bacterial supplementation could be extended to all types of mineral and natural fertilizer applied to agricultural fields, the authors estimate that Europe could decrease its  $\text{N}_2\text{O}$  emissions by 24%.

As a proof of concept, the authors demonstrate the utility of bioaugmentation with *Cloacibacterium* sp. strain CB-01 grown in digestate used as a nitrogen fertilizer. The effectiveness of the approach was validated using four soils that have different physical and geochemical properties. To achieve the projected 2030 emission reductions, the bioaugmentation agent must be grown in manure and other forms of mineral and natural fertilizers before application to agricultural fields. In addition, a variety of factors will affect the success of this approach – for example, the method should be suitable for a range of soil types, management practices, dynamic biogeochemical properties (such as pH)<sup>8</sup> and fluctuating climate and weather patterns. Given the factors that might influence performance of the bioaugmentation approach, a suite of  $\text{N}_2\text{O}$ -reducing microorganisms adapted to various environmental conditions and fertilizers will be needed to achieve effective and cost-efficient soil inoculation on a large scale.

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## Robotics

# Leg exoskeleton trained through simulation alone

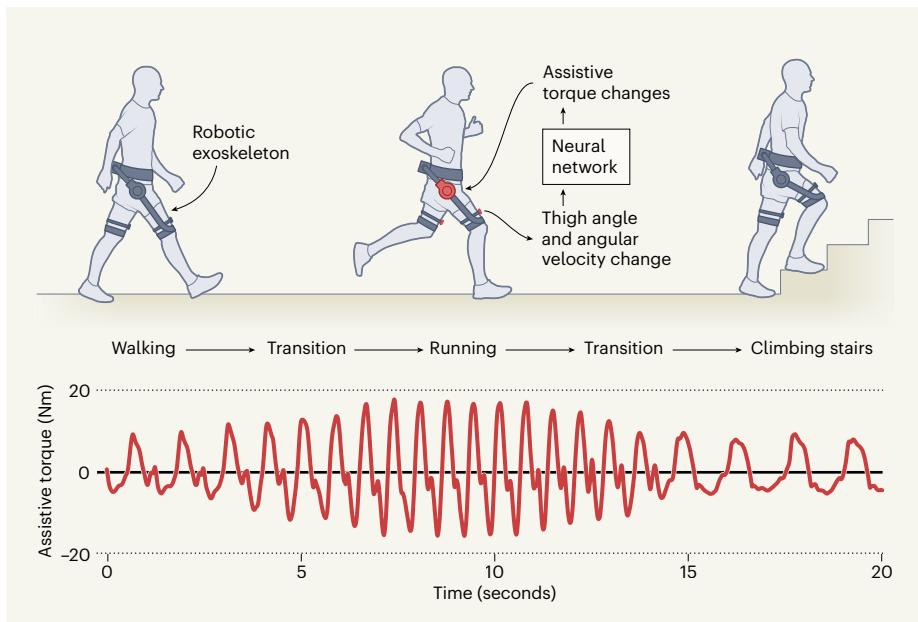
**Alexandra S. Voloshina**

A strategy for training a robotic exoskeleton through simulation takes the user out of the equation – saving users of wearable devices time and energy, and smoothing the transition between different types of movement. **See p.353**

Wearing a robotic exoskeleton can restore mobility for individuals with motor disabilities, or enhance a person's ability to perform daily activities. The ways that humans move are both complex and varied, yet these devices<sup>1</sup> are often designed to assist with only one activity, such as walking or running, and can require extensive tuning for each wearer. On page 353, Luo *et al.*<sup>2</sup> introduce a framework for developing more-versatile and adaptable devices. It relies on simulating human–device interactions without the need for copious data amassed from a human using the device. The authors show that this simulation-based approach leads to effective assistance for a range of tasks and can be adapted to

different individuals – paving the way towards the integration of robotic exoskeletons into everyday life.

Robotic devices designed to improve a person's gait can be worn on one or more joints of the leg, and usually have embedded motors that apply torques to either bend or extend the joint. This assistance – when applied properly – reduces the energy that the wearer expends when using their muscles. The device moves in sync with the joint, enabling the leg muscles to relax and allowing the device to assume their workload. However, for the device to offer optimal assistance and save maximum energy for the wearer, it must provide the right amount of assistive torque at the correct time.



**Figure 1 | A multitasking robotic exoskeleton.** Luo *et al.*<sup>2</sup> developed a strategy for programming the assistive torque (measured in Newton metres, Nm) that a robotic leg exoskeleton provides while its wearer is walking, running and climbing stairs. The method trains the device controller using simulations, in contrast to other approaches, which often rely on hand-tuned controllers. Basic kinematic measurements, such as the angle and angular velocity of the thigh, are fed into a set of interconnected neural networks that enable the trained controller to calculate the required torque for each type of movement. Luo *et al.* showed that this strategy allows the controller to transition smoothly between the three locomotive tasks and saves energy for the wearer. (Adapted from Fig. 4 of ref. 2.)