Randy J. Seeley *and* **Darleen A. Sandoval** *are at the Metabolic Diseases Institute, University of Cincinnati, Cincinnati, Ohio 45237, USA.*
e-mail: randy.seeley@uc.edu

1. Chiolero, A., Faeh, D., Paccaud, F. & Cornuz, J. *Am. J. Clin. Nutr.* **87,** 801–809 (2008).
2. www.cdc.gov/mmwr/preview/mmwrhtml/mm5935a3.htm
3. Williamson, D. F. *et al. N. Engl. J. Med.* **324,** 739–745 (1991).
4. Mineur, Y. S. *et al. Science* **332,** 1330–1332 (2011).
5. Schwartz, M. W. *et al. Nature* **404,** 661–671 (2000).
6. Huang, H., Xu, Y. & van den Pol, A. *J. Neurophysiol.* doi:10.1152/jn.00740.2010 (2011).
7. Greenfield, J. R. *et al. N. Engl. J. Med.* **360,** 44–52 (2009).

GLOBAL CHANGE

Indirect feedbacks to rising CO_2

There have been many studies on the effects of enriched levels of atmospheric carbon dioxide on soils. A meta-analysis shows that emissions of other greenhouse gases increase under high-CO_2 conditions. SEE LETTER P. 214

ALEXANDER KNOHL & EDZO VELDKAMP

Human activities have caused atmospheric concentrations of carbon dioxide, a major greenhouse gas, to increase at an accelerating pace. Starting at around 280 parts per million (p.p.m.) in pre-industrial times, they have now exceeded 390 p.p.m., and are expected to reach 600–800 p.p.m. by the end of the century[1]. On page 214 of this issue[2], van Groenigen and colleagues add to our awareness of the complex consequences of this trend, in terms of the effect that it will have on emissions of other greenhouse gases from various ecosystems.

In producing global warming, CO_2 is responsible for the largest part of the anthropogenic impact on Earth's energy balance. It is, of course, also an essential nutrient for plant metabolism. Numerous CO_2-enrichment experiments over the past two decades have demonstrated the positive effect of elevated CO_2 on plant growth — increased biomass and increased carbon storage in soils[3]. The vegetation response to elevated CO_2 might be constrained by various interactions with water and nutrients such as nitrogen[4,5]. However, experiments and model projections suggest that accelerated plant growth due to CO_2 fertilization could draw down some of this gas from the atmosphere, and hence could weaken future rates of CO_2 increase and lessen the severity of climate change[6].

Van Groenigen *et al.*[2] present evidence that rising levels of CO_2 are not only resulting in an increased carbon sink in terrestrial ecosystems, but could also cause increased emissions of other, much more potent, greenhouse gases such as methane (CH_4) and nitrous oxide (N_2O) from soils. Methane is produced by anaerobic methanogenic microorganisms that thrive in wetlands, including rice paddies, where labile (biologically accessible) carbon is available and diffusion of oxygen into the soil is severely restricted. Nitrous oxide is mainly produced in soils by aerobic nitrifying and anaerobic denitrifying bacteria. The interaction between nitrogen availability and soil water content controls the rate of N_2O production. The respective global-warming potentials of CH_4 and N_2O are 25 and 298 times greater than that of CO_2, and thus they influence Earth's energy balance even though they occur in much smaller concentrations.

Van Groenigen and colleagues collected information from 49 published studies that reported the effect of atmospheric CO_2 enrichment on CH_4 and N_2O fluxes from soils. Using a meta-analysis, they show that elevated CO_2 stimulated N_2O emissions by 18.8%, and that CH_4 emissions from wetlands increased by 13.2% and from rice paddies by as much as 43.4%. Notably, they also suggest the mechanisms that are probably responsible for these observed increases in greenhouse-gas emissions (Fig. 1).

Their suggestion goes as follows. Elevated CO_2 led to reduced plant transpiration (the evaporation of water from plant surfaces, leaves in particular), which increased soil water content and promoted the existence of anaerobic microsites in soils. This, together with increasing biological activity, probably stimulated denitrification and consequently N_2O production. Also, the CO_2-induced increase in root biomass may have contributed by increasing the availability of labile carbon, a crucial energy source for denitrification. The CO_2-induced stimulation of CH_4 emissions from wetlands and rice paddies was probably the result of higher net plant production, leading to increasing carbon availability for substrate-limited methanogenic microorganisms. Extrapolating their

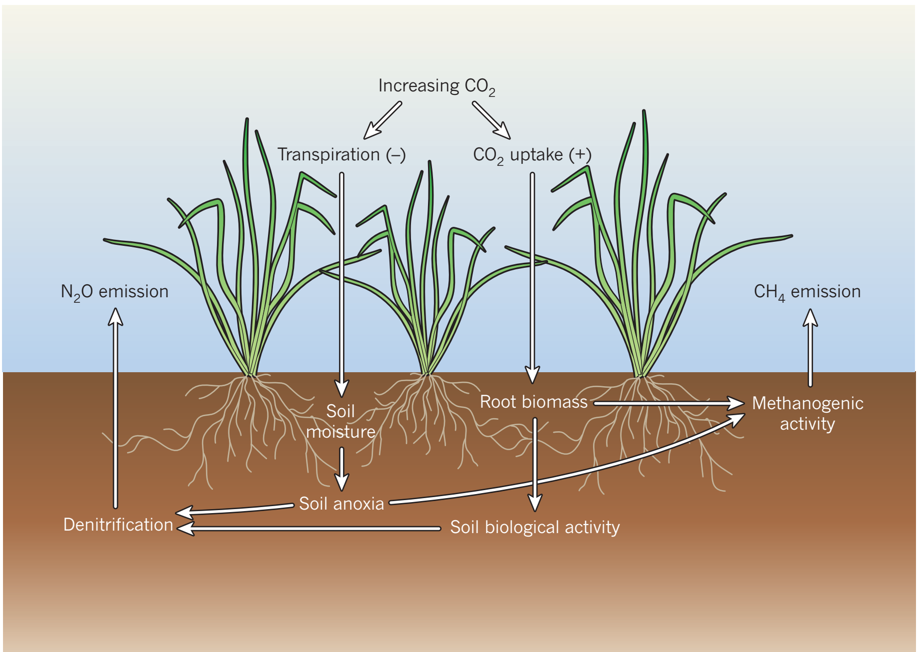

Figure 1 | Proposed mechanisms of increased N_2O and CH_4 emissions from soils. From their meta-analysis, van Groenigen *et al.*[2] estimate that rising levels of atmospheric CO_2 will result in more output of N_2O from upland soil (at a rate equivalent to 0.57 Pg CO_2 yr^{-1}) and of CH_4 from rice paddies and wetlands (equivalent to 0.56 Pg CO_2 yr^{-1}). They suggest that these increases are caused by reduced plant transpiration under conditions of elevated CO_2, resulting in increased soil moisture. Together with increased root biomass, this leads both to greater denitrification (and hence increased N_2O emission) and to more methanogenic activity (and hence increased CH_4 emission). The increase in these greenhouse gases will thus partially offset the predicted enhanced uptake of carbon by terrestrial ecosystems in a high-CO_2 world.

results to the global scale, van Groenigen *et al.*[2] estimate that the combined effect of stimulated N_2O and CH_4 emissions could be equivalent to at least 1.12 Pg CO_2 yr^{-1} (Pg = petagrams = 10^{15} grams). This is around 17% of the expected increase of the terrestrial CO_2 sink as a result of higher CO_2 concentrations.

Earlier studies have shown that long-term carbon sequestration in a CO_2-enriched atmosphere can be constrained by nitrogen availability[5,6]. Critics may wonder how these studies and van Groenigen and colleagues' analysis fit together, as it seems unlikely that denitrification would be stimulated by elevated CO_2 in nitrogen-limited ecosystems. This apparent discrepancy may be explained by the geographical bias in the present paper. The large majority of the 49 studies included in the meta-analysis were located in temperate regions, in areas — the United States, Europe, China and Japan — that are nowadays subject to considerable deposition of atmospheric nitrogen[7]. Some ecosystems included in the meta-analysis, such as agricultural areas receiving little or no fertilizer, and regions of natural vegetation, may thus have been subject to the input of considerable anthropogenic nitrogen through the atmosphere. Because nitrogen deposition is predicted to increase in the coming decades, the studies may therefore be more representative of future conditions, when nitrogen deposition will have become a global feature.

Another striking point is the almost complete lack of studies in the tropics and subtropics, where the strongest increases in nitrogen deposition are expected to occur[7]. Some tropical ecosystems may react differently from temperate ecosystems to elevated CO_2 concentrations. Many intact tropical forests tend to cycle large quantities of nitrogen[8], and an increase in soil-moisture content may have strong effects on N_2O emissions even without nitrogen deposition. Tropical grasslands are dominated by grasses using the C_4 photosynthetic pathway, which may improve their water-use efficiency to different extents from that of plants using the C_3 pathway. There is a clear need for field studies in these ecosystems, in order to improve our ability to evaluate the overall effect of elevated CO_2 on the budgets of greenhouse-gas emissions.

Obviously, the report by van Groenigen *et al.*[2] is not the end of the story, and future research may provide evidence of other feedbacks that have not yet been quantified or even hypothesized. Nevertheless, this study provides the first comprehensive analysis of available data that shows the importance of indirect feedbacks of elevated CO_2 on CH_4 and N_2O emissions on a global scale. It is now up to the scientific community to include these feedbacks in global climate models and to fill in the large gaps in information that still exist. ■

Alexander Knohl *is in the Section of Bioclimatology, and* **Edzo Veldkamp** *is in the Section of Soil Science of Tropical and Subtropical Ecosystems, University of Göttingen, 37077 Göttingen, Germany.*
e-mails: aknohl@uni-goettingen.de; eveldka@uni-goettingen.de

1. Solomon, S. D. *et al.* (eds) *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (Cambridge Univ. Press, 2007).
2. van Groenigen, K. J., Osenberg, C. W. & Hungate, B. A. *Nature* **475,** 214–216 (2011).
3. Hungate, B. A. *et al. Glob. Change Biol.* **15,** 2020–2034 (2009).
4. Reich, P. B. *et al. Nature* **440,** 922–925 (2006).
5. Oren, R. *et al. Nature* **411,** 469–472 (2001).
6. Thornton, P. E., Lamarque, J.-F., Rosenbloom, N. A. & Mahowald, N. M. *Glob. Biogeochem. Cycles* **21,** GB4018, doi:10.1029/2006GB002868 (2007).
7. Galloway, J. N. *et al. Science* **320,** 889–892 (2008).
8. Hedin, L. O., Brookshire, E. N. J., Menge, D. N. L. & Barron, A. R. *Annu. Rev. Ecol. Evol. Syst.* **40,** 613–635 (2009).

REGENERATIVE MEDICINE

Drawing breath after spinal injury

New work on a rat model suggests that, after spinal-cord injury, restoration of sustained and robust respiratory function is possible using strategies that promote both neuronal plasticity and regeneration. **SEE ARTICLE P.196**

KATHERINE ZUKOR & ZHIGANG HE

'Above C4, breathe no more.' This is the memory aid that reminds medical students that damage to the spinal cord above the fourth cervical vertebra (C4) — that is, the neck — can interrupt breathing. Injuries at the cervical level are the most common type of spinal-cord injury and account for more than half of all cases. Individuals who survive such injuries usually need ventilators to breathe, and so face a host of complications to their overall health and quality of life. A study by Alilain *et al.*[1] on page 196 of this issue offers hope that we may one day know how to treat this problem, so that patients with spinal-cord injuries above C4 can breathe on their own.

Breathing rate, rhythm and depth are controlled automatically by specialized regions of the brainstem[2] (Fig. 1a). The neurons in these regions send their axonal processes down the spinal cord to control the activity of other neurons in the phrenic motor nuclei (PMN) of the cervical spinal cord (C3–C6). The axons of the PMN neurons form the phrenic nerves, which, in turn, innervate the muscles of the diaphragm. Thus, contraction and relaxation of the diaphragm enable rhythmic breathing. When the spinal cord is injured above the C4 level, axons connecting the brainstem to the PMN are damaged, and breathing is disrupted. To make matters worse, axons in the adult spinal cord do not regenerate well, one of the main reasons being the inhibitory environment of the injured spinal cord[3].

Over the years, researchers have invoked many strategies to provide axons with a more supportive environment. These include either removing inhibitory molecules, such as chondroitin sulphate proteoglycans (CSPGs) in the extracellular matrix[4], or grafting in a piece of peripheral nerve that could serve as a bridge for axonal growth[5]. Combinations of these approaches have yielded encouraging results. For example, after a cervical spinal-cord injury (SCI) in rats, applying a peripheral nerve graft, together with injection of the enzyme chondroitinase ABC (chABC) to degrade CSPGs, allows spinal-cord axons to regenerate through the graft, re-enter the spinal cord and form synaptic connections with neurons on the opposite side of the injury[6].

Alilain *et al.*[1] applied a similar treatment strategy to recover respiratory function in rats after SCI. The authors made a partial injury at the C2 level to paralyse the diaphragm on one side of the animals' body (Fig. 1b). They then removed a piece of the rats' tibial nerve and grafted one end of it in the injury site at C2 and the other end in a small slit at the C4 level — near the PMN. Finally, they injected chABC at both ends of the graft, as well as in the PMN area, to degrade CSPGs (Fig. 1b).

Twelve weeks after injury, the group receiving this treatment had the highest percentage of recovered animals and the best quality of recovery in respiratory function compared with controls. Specifically, in many animals the paralysed half of the diaphragm muscle recovered nearly normal rhythmic electrical activity. Moreover, neurons from breathing centres of the brainstem grew axons into the graft. To demonstrate that recovery was largely due to axons that had regenerated through the graft and not just the rewiring of circuits in the portions of the spinal cord that were uninjured, Alilain *et al.* cut the graft; this treatment