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Figure 2 | A long-term fire experiment in Kruger National Park, South Africa. Zhou *et al.*[4] analysed data from a large experiment that has been running since 1954. The experiment tests 12 distinct fire treatments that have been implemented in plots of around 7 hectares, which are separated by fire breaks. The treatments include annual, biennial and triennial fires; the control plots have been protected from fire for almost 70 years. This photograph provides an aerial view of a set of fire treatments near Pretoriuskop in the southwestern corner of the Park (25° 12′ S, 31° 17′ E).

those that burn annually simulate a very high fire frequency and those left unburnt, fire suppression.

Zhou *et al.* estimated the amount of carbon stored above and below ground in each of these plots. To infer the above-ground biomass of all the trees in each plot, the team measured tree sizes with a lidar (light detection and ranging) sensor mounted on a drone. They then used ground-penetrating radar and diverse sampling approaches to estimate below-ground root biomass and organic matter in the soil.

The authors found that annual burning substantially reduced the biomass and amount of carbon in vegetation above ground, but a similar decrease was not found in root and soil carbon pools. In particular, the concentration of carbon in the soil was almost as high in burnt plots as it was in unburnt ones. Zhou *et al.* estimated that fire suppression in the savannah ecosystem they studied would result in a yearly increase in sequestration of only around 0.35 tonnes of carbon per hectare from the associated increase in woody biomass. This is substantially less than the 9.4 tonnes per hectare proposed as an annual estimate by those advocating fire suppression[3] — which calls into question the idea that fire suppression would contribute meaningfully to climate-change mitigation.

Zhou and colleagues' results enhance our understanding of carbon dynamics in savannahs. However, their conclusions are limited to a few plots in the wettest corner of Kruger National Park. Many savannahs are drier than the authors' study site, particularly those in Africa. These drier areas burn less frequently and are unlikely to be greatly affected by fire suppression. Other savannahs, including many in South America and Australia, have higher rainfall than Kruger National Park, but different soils, or are dominated by plants with different evolutionary lineages. Similar studies in other regions are needed to further explore the role of fire suppression and tree planting in fire-prone savannahs.

Programmes designed for carbon sequestration and climate-change mitigation also need to consider the social and economic implications for communities that rely on savannah landscapes for grazing livestock and harvesting wood, fruits and other resources. More is not always better when it comes to trees, particularly in savannahs. Indeed, many savannah ecologists have criticized proposals for tree planting and fire suppression, arguing that increasing the cover of woody vegetation through either means reduces biodiversity[8]. Tree-planting programmes in drylands can also fail, because seed germination and seedling survival rates are low[9], particularly in larger projects in which individual plants cannot be tended individually. Similarly, fire suppression can lead to fuel accumulation and higher-intensity fires later on[10]. Ultimately, it is important to balance the costs and feasibility of fire suppression or tree planting with the potentially small carbon-storage benefits estimated by Zhou and co-workers.

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The authors declare no competing interests.

Biochemistry

Methane might be made by all living organisms

Chang Liu & Jingyao Zhang

It is textbook knowledge that some bacteria can generate methane enzymatically. A study now provides evidence that an alternative, non-enzymatic mode of methane production could occur in all metabolically active cells. **See p.482**

Methane is mainly produced enzymatically by microorganisms called methanogenic archaea, in strictly oxygen-free (anoxic) conditions. In the past decade, it has become apparent that some oxygen-dependent species, including certain plants and fungi, can also produce methane[1]. However, the mechanisms that underlie this alternative mode of methane generation have been unclear. On page 482, Ernst *et al.*[2] reveal a process of methane production, driven by reactive oxygen species (ROS), that could occur in all living organisms, regardless of whether or not they exist in an anoxic environment. This process requires no specific enzymes — just ROS, free iron and suitable donors of methyl groups.

In 2014, the group that performed the current study described[3] a three-step pathway through which methane (CH_4) can be generated in a non-biological system in the laboratory without enzymes. The process requires three main factors: a ROS called hydrogen peroxide (H_2O_2); free iron; and appropriate methyl donor compounds. First, Fenton chemistry — a reaction between H_2O_2 and reduced ferrous iron (Fe^{2+}), a component of free iron — produces a highly reactive type of ROS called a hydroxyl radical ($^{\bullet}OH$), along with other by-products[4]. Second, interactions between $^{\bullet}OH$ and methyl donors produce methyl radicals ($^{\bullet}CH_3$; ref. 5). Finally, reaction of $^{\bullet}CH_3$ with hydrogen radicals produces methane.

Ernst *et al.* reasoned that non-enzymatic methane production might also occur in living cells (Fig. 1). In support of this hypothesis, the laboratory reactions reported previously occurred under ambient conditions, like those found in cells. In addition, cells continuously generate and release ROS (including H_2O_2)[6], multiple methyl compounds[7] and free iron ions[8] during normal metabolic processes.

The authors tested their theory in the bacterium *Bacillus subtilis*, which cycles through stages of being dormant (and so metabolically inactive) and vegetative (growing and metabolically active). The group grew *B. subtilis* on a medium containing the methyl donor dimethylsulfoxide (DMSO). They found that metabolically active *B. subtilis* cells spontaneously and continuously produced methane. By contrast, methane production did not occur in dormant cells.

Ernst and colleagues showed that varying the levels of DMSO or free iron available to cells modulated methane production in metabolically active *B. subtilis*. Increased methane generation could also be brought about by the induction of oxidative stress — a dangerous excess of ROS that arises under many physiological and pathological conditions, when cells are unable to maintain a normal redox balance. Together, the group's data show that the key prerequisite for non-enzymatic methane formation is active metabolism — a process that occurs in all living organisms. The authors went on to provide further evidence for the universality of ROS-driven methane production by demonstrating that the process also occurs in fungal, plant and human cells.

The formation of methane by means of ROS can be regarded as an automated manufacturing process, in which ROS, free iron and methyl compounds are the dominant materials and methane is the product. ROS are produced by many central metabolic processes, and are a major signalling molecule in normal cell function. Perhaps ROS-driven methane formation

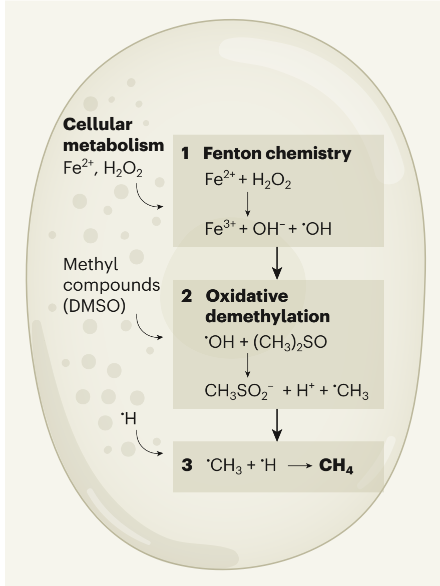

Figure 1 | Non-enzymatic methane production in cells. Ernst *et al.*[2] have demonstrated that a three-step process driven by reactive oxygen species can produce methane (CH_4) in living cells. The cells must be undergoing active metabolism, which leads to the production of hydrogen peroxide (H_2O_2), reduced ferrous iron (Fe^{2+}), methyl compounds such as dimethylsulfoxide (DMSO; $(CH_3)_2SO$) and hydrogen radicals ($^{\bullet}H$). In step one, Fe^{2+} and H_2O_2 undergo a reaction called Fenton chemistry, producing ferric iron (Fe^{3+}), hydroxide ions (OH^-) and hydroxyl radicals ($^{\bullet}OH$). In step two, oxidative demethylation of DMSO by $^{\bullet}OH$ produces methyl radicals ($^{\bullet}CH_3$). In step three, reaction of $^{\bullet}CH_3$ with $^{\bullet}H$ produces methane.

acts as a way for cells to decompose excess ROS under highly metabolically active conditions, in which prolonged accumulation of potentially toxic ROS might otherwise damage crucial molecules such as DNA, RNA, proteins and lipids[9].

This framework for biogenic, non-enzymatic methane production opens up avenues for further research in a broad range of fields, from medicine to Earth science, and even astro-

"Researchers now have the recipe for generating methane *in vitro* and *in vivo*, across all domains of life."

biology. For instance, Ernst and colleagues' work could have major implications for our understanding of cell function. Excess methane in cells or diffused throughout tissues could be indicative of cellular stress, redox imbalance or excess cellular iron. Perhaps methane is even a central signalling molecule in redox adaptation and regulation in cells. A key next step will be to try to unpack how and when methane levels fluctuate, and how the molecule is distributed throughout cells and tissues in health and disease. Another tantalizing question is whether there are undiscovered molecular sensors and transducers that respond to fluctuating methane levels to restore cellular ROS or iron levels. What pathways, known or unknown, might such molecules feed into?

Researchers now have the recipe for generating methane *in vitro* and *in vivo*, across all domains of life. A further series of procedures for methane research should now be established, including those for detecting ROS-driven methane and those for using this product in cells and tissues. This, in turn, could play into another exciting angle for future work: medical research. Perhaps ROS-driven methane formation could act as a readout for ROS levels. The detection of methane in the breath, blood or tissues could be used as a diagnostic test. A previous study in dogs[10] found that methane delivered through gas inhalation could decrease ischaemia-reperfusion injury (damage caused when oxygenated blood re-enters tissue after oxygen deprivation) by reducing oxidative stress and inflammation. This is an encouraging sign of the potential value of understanding ROS-driven methane biology.

Beyond biological research, the idea that all living organisms continuously produce methane will be of interest to Earth and climate scientists, given that it is a greenhouse gas — although whether ROS-driven methane production makes any substantial contribution to total global methane emissions will be challenging to determine. Much work is still to be done before we can understand the full implications of Ernst and colleagues' fundamental discovery.

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The authors declare no competing interests.
This article was published online on 9 March 2022.