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In the first decade of the 20th century, two German chemists – Fritz Haber and Carl Bosch – developed a way to produce synthetic nitrogen cheaply and on a large scale. Their invention spurred the mass production of nitrogen-based fertilizers, and thus transformed farming around the globe. It also marked the beginning of our long-term interference with the Earth’s nitrogen balance. Every year, an estimated US$200 billion worth of reactive nitrogen is now lost into the environment, where it degrades our soils, pollutes our air and triggers the spread of “dead zones” and toxic algal blooms in our waterways.

It’s no wonder that many scientists are arguing that “the Anthropocene” should become the official name of the current geological era. In just a few decades, humankind has caused global temperatures to rise 170 times faster than the natural rate. We have also deliberately modified more than 75 per cent of the planet’s land surface, and permanently altered the flow of more than 93 per cent of the world’s rivers. We are not only causing drastic changes to the biosphere, we are also now capable of rewriting – and even creating from scratch – the very building blocks of life.

Every year a network of scientists, experts and institutions across the world work with UN Environment to identify and analyze emerging issues that will have profound effects on our society, economy and environment. Some of these issues are linked to new technologies that have astonishing applications and uncertain risks, while others are perennial issues, such as the fragmentation of wild landscapes and the thawing of long-frozen soil. Another issue, nitrogen pollution, represents an unintended consequence of decades of human activity in the biosphere. While the final issue analyzed here, maladaptation to climate change, highlights our failure to adequately and appropriately adjust to the shifting world around us.

There is some good news to report. As you can read in the pages that follow, a holistic approach to the global challenge of nitrogen management is beginning to emerge. In China, India and the European Union, we are seeing promising new efforts to reduce losses and improve the efficiency of nitrogen fertilizers. Ultimately, the recovery and recycling of nitrogen, as well as other valuable nutrients and materials, can help us to farm cleanly and sustainably, a hallmark of a truly circular economy.

The issues examined in Frontiers should serve as a reminder that, whenever we interfere with nature – whether at the global scale or the molecular level – we risk creating long-lasting impacts on our planetary home. But by acting with foresight and by working together, we can stay ahead of these issues and craft solutions that will serve us all, for generations to come.

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Synthetic Biology: Re-engineering the environment

Opportunities and challenges

The world is facing unprecedented challenges to a healthy and sustainable future. Habitat destruction, invasive species, and overexploitation are contributing to immense biodiversity loss. Unsustainable, extractive industry practices further burden the environment, and by extension, human welfare. Vector-borne infectious diseases pose a major threat to global health. Rapid climate change is likely to expand the geographical range of tropical diseases and further stress already taxed species and ecosystems.

A number of approaches devised to meet these challenges – some proposed and others already implemented – share a common strategy. That is, they depend upon the genetic manipulation of living organisms to acquire new functions that otherwise do not exist in nature, in order to serve human needs. Scientists can modify microorganisms like E. coli by rewriting their genetic code to turn them into tiny living factories that produce biofuel. Both baker’s yeast and E. coli can be engineered to produce adipic acid – a petroleum-derived chemical key to the fabrication of nylon – thus offering an alternative to petroleum-dependent production. Baker’s yeast can also be reprogrammed to derive an antimalarial drug called artemisinin, which is normally sourced from the sweet wormwood plant. These are all examples of products made possible by the advanced genetic-engineering technology known as synthetic biology.

The majority of commercially available synthetic biology products have been developed to provide alternatives to existing high-value commodities, especially those dependent...
Moreover, synthetic alternatives and replacements for substances conventionally derived from nature are also gaining ground in research and market spaces. Modern Meadow, a company behind the invention of a collagen-producing yeast, aims to deliver a sustainable leather alternative with properties and texture similar to animal-derived leather. Synthetic biology has also opened up a new landscape for advanced materials with novel functionalities and performance, such as materials that can self-assemble or self-repair.

The recent emergence of CRISPR (pronounced *crisper* and short for *clustered regularly interspaced short palindromic repeats*) as a gene-editing tool has enabled even more precise and inexpensive methods of engineering individual organisms, biological systems, and entire genomes. Applications of synthetic biology are advancing beyond the manipulation of microbes in the laboratory to engineering the propagation of species outside controlled settings, for specific ends. Strategies to release genetically engineered organisms into the environment to permanently alter entire populations of target species have been proposed as a means to eradicate vectors of disease, eliminate invasive species, and lend resilience to threatened plants and animals.

The intentional or accidental release of genetically engineered organisms into the environment could have significant negative impacts on both human and environmental health. Misuse of these technologies and a failure to account for unintended consequences could cause irreversible environmental damage and pose significant geopolitical threats. The potential far-reaching impacts of synthetic biology demand governance methods and research guidelines that promote its ethical and responsible use.

The filamentous fungus, *Aspergillus niger*, can naturally produce enzymes that are commercially important in the food and animal feed industries. The microorganism is genetically modified to enable the large-scale enzyme production. 180x magnification.
Rewriting the code of life

The development of recombinant DNA technology in the 1970s marked a major shift in how humans control genomes. Genetic sequencing technologies allowed for tracts of DNA to be read and understood, providing the blueprint to engineer genomes for new gene expressions. DNA sequences can be completely rewritten by deleting, adding or replacing segments. Entire portions of DNA can now be chemically synthesized and assembled, which has led to the creation of synthetic life.

The latest gene editing tool, CRISPR-Cas9, has garnered significant excitement in the scientific community and general public alike. First described in 2012, CRISPR is faster, cheaper, more accurate, and more efficient than any of its gene-editing predecessors. It has speeded up the editing process from several months to just a few days.

The CRISPR-Cas9 gene-editing technique was inspired by a naturally occurring defence system of certain bacteria against viral invasion. In nature, a bacterium can deploy the Cas9 enzyme to cut invasive genetic material inserted by a virus, effectively disabling the attack. Researchers have adapted this mechanism to cut DNA at any specific location. In CRISPR-Cas9 gene editing, scientists use a guide RNA to direct the Cas9 enzyme to a precise portion of DNA.

The Cas9 enzyme then acts as a pair of molecular scissors, cutting or deleting the targeted segment. By exploiting the natural DNA repair process, researchers can also insert a customized DNA segment into the disrupted strand.

Video: Synthetic biology explained

DNA is in every living organism’s blueprint. It guides the production of proteins needed for an organism to function. DNA, or deoxyribonucleic acid, is made up of four nucleotide bases bonding in pairs.

Just as a combination of letters form a word with a certain meaning, a string of As, Ts, Gs and Cs in a specific order form a gene that produces a specific type of protein for a specific function in the body.

When a ‘spelling mistake’, or mutation, occurs in the DNA sequence, it affects the structure and function of the synthesized proteins. A cell can become cancerous as a result of ‘mistakes’ in the DNA sequence.

Scientists can determine the precise order of the letters through DNA sequencing. The complete set of human DNA, or the human genome, has 3 billion combinations or base pairs.

Genetic engineering techniques have been used for decades to modify organisms by altering the location of genetic materials, for example in genetically modified organisms (GMOs), where a gene from one species is isolated and transferred to an unrelated species in order to achieve the desired characteristic in the target organism.

In 2010, scientists announced their success in creating the world’s first synthetic bacterial cell after a decade of learning to design, synthesize and assemble a DNA sequence from scratch.

Using the natural baker’s yeast genome as a blueprint, a consortium of scientists are now working to construct a yeast cell made out of entirely synthetic DNA.

Video link: https://www.youtube.com/watch?v=rD5uNAMbDaQ
CRISPR-Cas9 genome editing technique

In nature, CRISPR-Cas9 is the bacteria’s defense and immunity strategy against viral attacks, utilizing the system to precisely identify and cut the DNA of an invading virus, thus disabling the attack. Scientists have adapted the CRISPR-Cas9 mechanism for genome editing as it offers a more precise, relatively cheaper and faster way to modify a genome.

1. Scientists identify a section of DNA they want to modify.

2. Scientists then create a genetic sequence, called a guide RNA, that matches the targeted DNA section, and bind the guide RNA to the Cas9 enzyme, which acts as a pair of molecular scissors.

3. Guide RNA locates the targeted section and tells Cas9 where to cut.

4. A new piece of DNA can be inserted at the site to replace the cut section.

CRISPR gene editing is being used in research aiming to engineer wild organisms outside human-controlled environments. Gene drives are a synthetic biology application that depends on CRISPR gene editing to ensure the expression of desired gene edits in future generations of a wild species. The process involves an organism being engineered in a laboratory to encode a CRISPR-based gene drive and a desired gene edit. This organism is then released to mate with the normal population in the wild, forcing the inheritance of the desired gene edit along with the gene drive system in its offspring. The gene drive is a self-perpetuating process that repeats whenever the offspring mates with the wild population. And over time, the entire population of that species will all carry both the desired gene edit and the gene drive system. CRISPR-based gene drives can also ensure the inheritance of traits that disrupt reproduction, such as sterility, which could spread in a population and potentially lead to extinction. The application of CRISPR-based gene drives is most suited to sexually–reproducing species with short generation times, like most insects and some rodents.
**Synthetic Biology**

**Sustainability applications**

Many industries have made use of synthetic biology. Microorganisms, from bacteria to yeasts, are genetically engineered to become tiny factories producing more sustainable ingredients for medicines, vaccines, biofuels, green chemicals and new materials.

**Pharmaceutical products**

*E. coli* is altered to manufacture a vaccine against chlamydia, which is becoming more resistant to conventional antibiotics.

**Green and bio-based chemicals**

A variety of chemicals in everyday products are derived from petroleum. Synthetic biology enables the production of substances that can replace petroleum-based chemicals.

**Alternatives to chemicals derived from unsustainable sources**

The blood of horseshoe crabs is a major biomedical commodity used in pharmaceutical testing for bacterial contamination. A synbio substitute could reduce or replace the need for harvesting the nearly extinct species from the oceans.

**Lactic acid, succinic acid and propanediol** are among chemicals made by genetically engineered microbes that are commercially available in the global market.

**CRISPR-Cas9 genome editing technique**

The discovery of CRISPR-Cas9 has changed the entire outlook of synthetic biology research. It enables scientists to cut out a particular DNA segment of a desired sequence or replace it with a new DNA strand. Many fields of medical research require such editing precision to revolutionize treatments.

However, the technique is also subject to scrutiny for its safety as it involves a potential off-target effect, whereby it inadvertently cuts out DNA that has a similar sequence to the targeted strand, potentially triggering cancer in edited cells.

**Market and investment**

- **US$13.9 billion**
  - Projected global market value of synthetic biology applications by 2022

- **US$1.9 billion**
  - 2018 Global investment in synthetic biology startups

**Do-It-Yourself Biology or DIY Bio**

The movement of so-called 'citizen scientists' interested in performing synthetic biology experiments has gained significant traction globally. Biology enthusiasts – many without scientific background – meet in garage labs to conduct experiments using specialised DIY kits and simple protocols available online.

Some of the group have specialised equipment and hire professional staff to help citizen scientists, biohackers and biology enthusiasts in developing their projects.

**Risks and policy considerations**

There are concerns that synthetic biology could be used to re-engineer existing pathogenic viruses, making them more dangerous or produce biochemicals with only modest resources and organizational footprint.

Synthetic biology presents new challenges that need to be addressed through the consolidated action of governmental and international bodies. Development of effective methods to better manage emerging risks is essential in ensuring technological safety.
Gene drives have been made possible by the development of CRISPR-Cas9 technology.

**Applications for conservation and public health**

CRISPR-based gene drives may be key to addressing some global challenges, such as vector-borne diseases or invasive species, but they require multifaceted societal debate because of their power to modify, suppress or replace the entire population of the target species, bypassing the fundamental principles of evolution.

**Normal inheritance**

In sexual reproduction, each parent passes half its DNA to its offspring. A parent’s unique genetic trait has a 50-50 chance of being inherited by the next generation. Over many generations the unique genetic character still remains in the population but at low frequency. The normal inheritance also applies to the case of an offspring produced by a normal parent and a classic GMO parent.

**Gene drive inheritance**

A synthetic gene drive circumvents the rules of normal genetic inheritance. This self-perpetuating mechanism is designed to ensure preferential inheritance of a modified genetic trait in future generations. Over time the entire population inherits the preferred engineered trait.

During fertilization, the offspring inherits one set of DNA from the ordinary parent and one containing the CRISPR-equipped gene drive from the genetically engineered parent. CRISPR-Cas9 looks for the target site in the ordinary DNA and cuts it. When the cut DNA attempts to repair the damage, it copies the engineered strand containing the gene drive. The offspring ends up having two copies of the genetically engineered DNA with gene-drive capability to pass on to future generations.

American chestnut trees are near extinction due to chestnut blight, a fungal disease native to Asia. Pending regulatory approval, the American chestnut can be engineered to be blight-resistant and spread in the wild.

**CRISPR-based gene drives: Manipulating the wild populations of plants and animals**
Applications redefined: From laboratory to ecosystem

Synthetic biology could indirectly benefit conservation efforts by allowing the development of artificial alternatives to commercial products normally sourced from the wild. For example, the blood of the horseshoe crab is a major biomedical commodity used to test pharmaceuticals for bacterial contamination. Unsustainable harvesting is pushing the species towards global extinction. A synthetic substitute has been developed that could reduce or replace the need to harvest the endangered crabs. Likewise, engineered microbes and microalgae capable of producing alternatives to omega-3 oils could lessen pressure on declining wild fish stocks.

Conservation measures that propose a more direct application of the technology on target species have recently emerged. Releasing genetically engineered organisms into the environment could restore the health or enhance the resilience of damaged populations. For example, using an approach that predates CRISPR, scientists have synthesized the oxylate oxidase gene normally expressed by wheat, and forced its expression in the American chestnut tree. This gene can neutralize the toxin secreted by the blight that has driven the tree functionally extinct. Pending regulatory approval, blight-resistant chestnuts could be planted to re-establish this once-dominant species in eastern U.S. forests. Unlike genetically modified crops, where safety concerns largely centre around containment, the engineered American chestnut is deliberately designed to spread and flourish in the wider environment.

As climate change is predicted to increase rates of species extinction worldwide, CRISPR’s availability is likely to hasten applications for ecosystem restoration. Scientists have proposed using CRISPR for threatened species, such as corals that are under immense stress from increased ocean temperatures, acidification and pollution. Proof-of-concept CRISPR research is underway to rewrite coral genomes to express mutations that endow resilience. However, frameworks for field implementation of this research have yet to be developed.

CRISPR-based strategies could also remove invasive species from threatened ecosystems. On many Pacific islands, for example, invasive rodents are decimating native bird populations. Through international collaboration, the Genetic Biocontrol of Invasive Rodents programme is developing CRISPR-based gene drives that would spread sterility. In New Zealand, CRISPR-based gene drives are being considered to help achieve the elimination of all invasive predators by 2050. In Hawaii, gene drives have been proposed to reduce avian malaria spread by house mosquitoes that has caused serious declines in rare bird populations. However, recent research indicates that gene drives may face resistance and limited efficacy in wild mosquito populations.

It has even been suggested that extinct species could be resurrected for their ecological benefits, such as reviving a woolly-mammoth-like animal by gene editing the DNA of its closest living relative, the Asian elephant. Proposals for de-extinction of species are not only highly debatable, but also re-emphasize the importance of addressing the root cause of extinctions. Such possible genetic interventions, even if unrealized, encourage a valid debate on how biotechnology can support, coexist with, or undermine the goals of conservation.
To reduce the global disease burden, various synthetic biology strategies aim directly at suppressing populations of disease vectors. A company called Oxitec has genetically engineered mosquitoes to express a synthetic lethal gene and has released them in South America, South-East Asia, and several Caribbean nations to suppress the vector for Dengue fever, Zika virus, yellow fever, and chinkengunya. These so-called ‘self-limiting’ mosquitoes pass a lethal gene to their offspring, preventing them from surviving to adulthood. This method of suppression is, however, reversible without continual releases to sustain the engineered mosquito population in the wild. To circumvent this issue, Target Malaria, an international consortium funded by the Bill and Melinda Gates Foundation, is developing CRISPR-based gene drives to permanently control the malaria vector in sub-Saharan Africa. CRISPR-based gene drives are highly invasive as, in theory, a one-time release of a few gene-drive-bearing organisms could completely suppress an entire wild population. Another strategy is to use gene drives that do not suppress the population, but instead limit the ability of mosquitoes to transmit pathogens. CRISPR-based gene drives have also been devised to permanently immunize white-footed mice against Lyme disease on islands in Massachusetts, USA.
Innovating with wisdom

The release of genetically engineered organisms accidentally or intentionally into the environment has raised valid concerns about biosafety and unpredictable consequences. For organisms engineered in closed research or industrial facilities, containment procedures and enforced regulations on waste disposal help to avoid an escape, although this is never fail-proof. In the case of intentional release, concerns over potential genetic cross-contamination between species, ecological interactions and impacts on ecosystems and their services remain largely unresolved. Altering a disease carrier genetically could potentially cause a pathogen to evolve and become more virulent, or to be carried by a new vector.

To date, CRISPR-based gene drives have been tested only on small populations in controlled settings, with one recent experiment successfully collapsing the entire malaria-carrying mosquito population in the laboratory. As a first step towards wider trials, Target Malaria has recently gained permission to release 10,000 modified mosquitoes in Burkina Faso. These specimens will be genetically engineered to be sterile, but with no gene drives, to test how well they compete with wild males. However, such field trials to evaluate the efficacy of the gene-drive system could pose inherent risks.

Under the precautionary principle, stringent risk assessment and the inclusion of diverse stakeholder perspectives should be applied in the development and handling of innovative synthetic biology applications and products. The precautionary principle states that when human activities may lead to unacceptable harm that is scientifically plausible but uncertain, action should be taken to avoid or diminish that harm. A concept of substantial equivalence – that a genetically modified organism is as safe as its traditional counterpart – is often mentioned in conjunction with the precautionary principle. Some countries have extensive policy and regulations in place concerning genetic engineering and research, while for others, non-functional regulatory systems, policy gaps and risk-assessment capacity are major challenges.

Attempts have been made to identify, evaluate and address the ethical and biosafety concerns of synthetic biology. The U.S. National Academies of Science, Engineering, and Medicine published a report on gene drives in 2016 highlighting the need for stringent environmental risk assessments and deliberation that charters human values and necessitates rigorous public engagement.

In December 2017, the ad-hoc technical expert group on synthetic biology, established by the Parties to the Convention on Biological Diversity, concluded that organisms – developed or being developed through current methods of synthetic biology, including those containing gene drives – fall under the description of living modified organisms (LMOs), which are regulated under the legally-binding Cartagena Protocol. With 171 Party nations, the Protocol applies the precautionary approach and requires that each Party take all necessary measures to ensure the safe handling, transport and use of the resulting LMOs.

SYNBIOSAFE, an EU-funded research project, was established to identify key issues in safety, security, risk management ethics and, importantly, the science-society interface, which emphasizes public education and dialogue among scientists, businesses, government, and ethicists. Some gene-drive developers have also proposed ethical research guidelines that emphasize the need for meaningful public engagement.

Video: Why is this African village letting mosquitoes in?
Synthetic biology and genome editing have attracted interest not only from companies, but also regular citizens. Do-It-Yourself Biology, also known as “DIY Bio”, the movement of “citizen scientists” interested in synthetic biology experiments has become an international phenomenon over the last decade. Often with little prior knowledge of the field, enthusiasts meet in makeshift labs to take crash courses in biotechnology and conduct hands-on experiments. Simple protocols found online and specialized kits costing US$150–1,600 have driven the movement’s rapid expansion.

DIY Bio labs can be found in most major cities and by 2017 there were about 168 groups worldwide. Regulating the use of easily accessible and low-cost technologies like CRISPR and gene editing kits will likely be a challenge for authorities. There is also growing concern that the technology could be misused by terrorists to destroy agricultural crops or turn harmless microbes into biological weapons.

Nevertheless, the intentional release of modified organisms and their potential to permanently transform wild species and cross international borders will likely test the limits of current policy, leading some environmental groups to call for a moratorium on all gene-drive research. Other regulatory concerns focus on the potential use of synthetic biology for military offensive purposes.

Current ethical frameworks may not be able to keep pace with the rapid progress of synthetic biology and its inherent complexity, especially concerning wild species. Decisions to release engineered organisms into the wild will be shaped by the pervading environmental ethic, or how a majority of citizens relate to non-human nature. Altering the genetic code of wildlife is seen by some as a gross overstep by humans, echoing concerns about genetically modified crops. Others may feel that there is a moral responsibility to use a technology that could save lives or restore damaged ecosystems. These contrasting value systems require responsible decision-making for resolution.

Synthetic biology applications also raise questions of who has ownership of an LMO and its genome, what protection is available for vulnerable communities, and how to ensure those most impacted have a voice. It is crucial that balanced and inclusive deliberative forums steer the field of synthetic biology and ensure that its environmental applications are used to the benefit of all on our shared planet.
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Graphic references


