Restored and regenerated forest in the Reserva Ecológica de Guapiaçu, Brazil illustrates how forests can regain maximum potential canopy cover.

ECOLOGY

Restoring forests as a means to many ends

An urgent need to replenish tree canopy cover calls for holistic approaches

By **Robin Chazdon** *and* **Pedro Brancalion**

Earth is approaching environmental thresholds that, if crossed, will create serious disruptions to ecosystems, economies, and society (*1*). To avoid the devastating effects of climate change and biodiversity loss, humanity must protect and restore native ecosystems (*2*). International conventions and organizations support forest restoration as a method for mitigating hazardous environmental shifts, but questions remain as to where and how to focus such restoration efforts. On page 76 of this issue, Bastin *et al.* (*3*) describe a new approach that advances our understanding of global tree distribution.

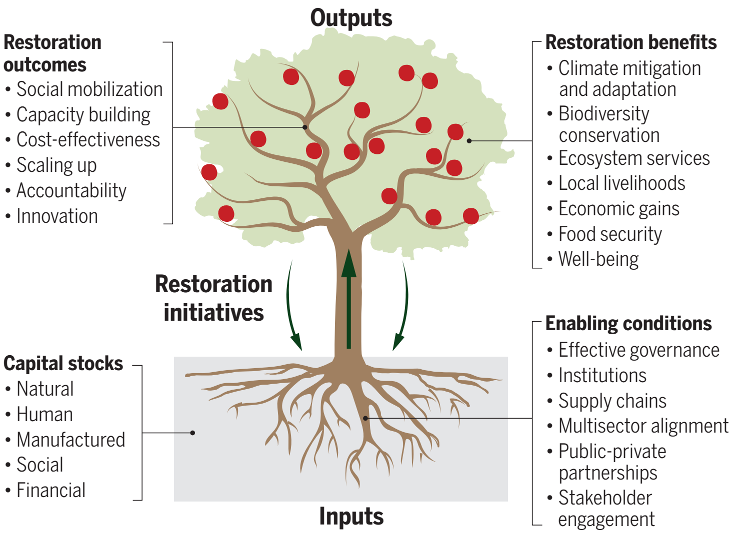
Prevailing views on how to assess forest cover and restoration opportunities might actually hinder rather than promote the large-scale action needed. Traditional methods for evaluating changes in forest cover are based on a dualistic (forest/nonforest) classification of land use that applies a subjective threshold to the 0 to 100% canopy-cover gradient (*4*). Bastin *et al.* move beyond this strict dichotomy with the use of direct photo-interpretation measurements that account for wooded lands that lie outside forest areas. The authors identify the global potential for enhanced tree canopy cover on the basis of variations in natural levels of tree cover, which has immediate implications for restoring forest cover. Test scenarios that assess how future climate change will affect global canopy cover emphasize the urgency of taking action now, as shifting responses of forests to climate change are already under way and will become far more pronounced.

This urgency of restoring tree cover does not justify short-term fixes that fail to produce environmentally and socially beneficial long-term outcomes. An assessment of the biophysical capacity for restoring global tree cover provides a necessary but insufficient foundation for evaluating where tree cover can be feasibly increased. The kinds of trees as well as how and where they are grown determine how and which people benefit. In some contexts, increasing tree cover can elevate fire risk, decrease water supplies, and cause crop damage by wildlife. Reforestation programs often favor single-species tree plantations over restoring native forest ecosystems. This approach can generate negative consequences for biodiversity and carbon storage (*5*), threaten food and land security, and exacerbate social inequities. How restored lands are governed determines how reforestation costs and benefits are distributed.

Without attention to these social and environmental issues, efforts to rapidly increase tree cover are likely to prove ineffective in the long term. Initiated by Germany and the International Union for Conservation of Nature in 2011, the Bonn Challenge has garnered 58 commitments to restore 170 million hectares of forest land by 2030. The Global Deal for Nature (*6*) proposes that 30% of Earth's surface be formally protected and an additional

Restoration systems deliver multiple benefits

A forest restoration system integrates structures (trunk and roots) that assimilate and distribute resources (soil) to initiate and sustain restoration outcomes (branches). The system ultimately delivers myriad benefits for nature and humanity (fruits). Restoration outcomes and benefits sustain the system, stabilize enabling conditions, and increase capital stocks.



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20% designated as climate stabilization areas by 2030, to stay within 1.5°C of global temperature rise.

Reaching ambitious restoration targets appears unlikely, however, without practical guidelines and tools, institutional alignment, accountability mechanisms, and a robust evidence base. Enormous gaps remain between high-level focus on restoration and implementation on the ground (*7*). Forest restoration programs are largely conducted on project scales by using approaches that are neither cost effective nor designed to achieve multiple benefits and long-term sustainable outcomes (*8*). In contrast to such site-based practices, forest and landscape restoration is a holistic approach; it aims to balance diverse types of tree cover to achieve multiple benefits, based on the local socio-ecological context and stakeholder engagement (*9*). This landscape approach is vital to reaching the global scales needed to reverse the effects of deforestation and land degradation.

Forest restoration is a mechanism to achieve multiple goals, including climate mitigation, biodiversity conservation, socioeconomic benefits, food security, and ecosystem services. A living tree helps to illustrate the components of a holistic forest restoration system (see the figure). The above-ground portions of the tree are the trunk (restoration initiatives), branches (restoration outcomes), and fruits (restoration benefits). Below the ground are soil (capital stocks) and root structures that assimilate and distribute resources and create enabling conditions.

Viewing restoration as a system also guides appropriate mechanisms to mobilize resources in cost-effective ways. One such mechanism is to use the best available spatial information to identify areas where the most beneficial and feasible restoration outcomes intersect (*10*). Bastin *et al.* clearly show that we have a narrow window of time in which to restore global tree cover. We need to act quickly, intelligently, holistically, and globally. Doing so will require planting and sustaining of restoration systems wherever new types of canopy cover are needed. ■

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GENOME EDITING

Inserting DNA with CRISPR

Inserting large DNA segments with CRISPR holds great promise for genetic engineering

By **Zhonggang Hou** *and* **Yan Zhang**

Most prokaryotes rely on the CRISPR-Cas system for adaptive immunity against viruses and mobile elements (*1*, *2*). Small RNAs produced from CRISPR direct Cas effector proteins to seek and destroy nucleic acids from invaders that have complementary target sites (*3*). There are multiple types of CRISPR, which are defined on the basis of their protein composition. Recently, RNA-guided nucleases from types II and V CRISPR systems, Cas9 and Cas12, have revolutionized genome editing by allowing programmed DNA sequence alterations (*4*). However, robust and targeted insertion of a large DNA segment into eukaryotic genomes has remained challenging. On page 48 of this issue, Strecker *et al.* (*5*) show that a CRISPR-associated transposase (CAST) mediates highly efficient, RNA-guided insertion of cargo DNA into the bacterial *Escherichia coli* genome. Moreover, Klompe *et al.* (*6*) report another CRISPR-guided DNA transposase system that operates similarly. These studies offer new tools that could transform genetic engineering and gene therapy research.

In a typical CRISPR application, Cas9 or Cas12 is directed by its guide RNA to the intended complementary genomic site flanked by a protospacer-adjacent motif (PAM), and creates a DNA double-strand break (DSB) (*4*). The outcome of gene editing largely depends on the repair of this DSB by the endogenous pathways in the host cell. Repair by end-joining DNA repair pathways often predominates and tends to introduce heterogeneous small DNA insertions or deletions (indels) that disrupt a gene's function. However, many applications require site-specific knock-in of large DNA segments, such as a therapeutic gene or reporter genes. This can be achieved through accurate DSB repair by the homology-directed repair (HDR) pathway, which requires long sequence homology between a supplied DNA template and the genomic regions flanking the insertion site. HDR is inefficient and often restricted to certain stages of the cell cycle or by cell type (*7*, *8*). Therefore, there is an urgent need for tools that enable robust and targeted DNA integration.

ILLUSTRATION: STEPHAN SCHMITZ

DNA transposons, also known as "jumping genes," can move from one genomic location to another, and their relocations and insertions are catalyzed by an enzyme complex called transposase. Since 2017, the CAST systems have been discovered through bioinformatics mining of new CRISPR variants (*9*, *10*). CAST comprises a miniature type I or V CRISPR-Cas encoded within a Tn7-like transposon. The mini-CRISPR systems encode protein machineries that either lack the Cas3 nuclease-helicase of the type I CRISPR system or carry inactivating mutations in the catalytic residues of Cas12k of the type V system. Accordingly, they could be competent for target sequence binding but not for DNA cleavage. It is speculated that these defective CRISPRs have been hijacked by Tn7-like transposons to serve a role other than

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