treating them with liquid growth medium that contained chemical factors to control the signalling activities needed for blastocyst development (Fig. 1). Yu and colleagues treated the cells with two different types of culture medium in sequence, to promote differentiation of the cells into lineages representative of the trophectoderm and hypoblast.

Both groups found that human blastoids emerged after 6–8 days of culture, with a formation efficiency of up to almost 20%, comparable to the efficiencies of the mouse blastoid protocols[4–6]. The human blastoids were of a similar size and shape to natural blastocysts, with a similar total number of cells. They contained a cavity and an ICM-like cluster.

Detailed characterization of the blastoids (including genome-wide expression analysis and comparisons with human embryo data) showed that their cell lineages share molecular similarities with those of the pre-implantation human blastocyst. The spatial organization of the epiblast-, trophectoderm- and hypoblast-related lineages is consistent with that found in pre-implantation human embryos. The groups also demonstrated that the blastoid cells have key properties of blastocyst lineages — cells isolated from the blastoids could be used to generate various stem-cell types. Yu *et al*. showed that, if these stem cells were transplanted into mouse blastocysts, they gave rise to cells that could integrate with the corresponding mouse lineages in the mouse embryo.

Next, the researchers analysed further development of the blastoids using an established assay that mimics implantation into the uterus in culture dishes. Like blastocysts, when blastoids were grown in this assay for four to five days, some attached to the culture dish and continued to develop. In a portion of these attached blastoids, the cell lineage representative of the epiblast became reorganized into a structure enclosing a central cavity — reminiscent of the pro-amniotic cavity, which forms in the epiblast of post-implantation blastocysts. And in some blastoids, the trophectoderm-related cell lineage spread out and showed signs of differentiation into specialized placental cell types. Yu *et al*. also observed a second cavity in the hypoblast-related cell lineage in some blastoids, akin to the yolk-sac cavity.

Together, the groups' data demonstrate that human blastoids are promising *in vitro* models of pre-implantation and early post-implantation blastocyst development. However, there are notable limitations to overcome. For example, development of the blastoids is inefficient, and varies between cell lines produced from different donors, and between experimental batches. In addition, the three lineages seem to develop at slightly different rates in single blastoids, and development of blastoids in the same dish seems unsynchronized. Spatial organization of the hypoblast-related lineage in blastoids remains to be improved. Furthermore, the blastoids contain unidentified cell populations that do not have counterparts in natural human blastocysts.

Another challenge is that development of the blastoids is limited in post-implantation stages, unlike in mouse blastoids[4–6]. Further optimization of culture and experimental conditions will be needed to improve post-implantation-stage culturing of human blastoids *in vitro*, up to the equivalent of 14 days *in vivo*. Strict ethical rules prevent the culturing of human embryos past this stage, when structures associated with gastrulation begin to appear. Three-dimensional systems for culturing human blastocysts[10], which effectively promote post-implantation development, might help to improve our ability to culture blastoids up to this limit, by maintaining the normal 3D tissue architecture and spatial relationships between the different cell lineages in the blastoids.

Human blastoids are the first human embryo models that are derived from cells cultured *in vitro* and that have all the founding cell lineages of the fetus and its supporting tissues. As protocols are optimized, these blastoids will more-closely mimic human blastocysts. This will inevitably lead to bioethical questions. What should the ethical status of the human blastoids be, and how should they be regulated? Should the 14-day rule be applicable? These questions will need to be answered before research on human blastoids can proceed with due caution. To many people, the study of human blastoids will be less ethically challenging than the study of natural human blastocysts. However, others might view human blastoid research as a path towards engineering human embryos. Thus, the continuous development of human embryo models, including human blastoids, calls for public conversations on the scientific significance of such research, as well as on the societal and ethical issues it raises.

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This article was published online on 17 March 2021.

Biogeochemistry

Fungi are key to CO_2 response of soil

Ana Bastos & Katrin Fleischer

An analysis of experiments in which the air around terrestrial plants or plant communities was enriched with carbon dioxide reveals a coordination between the resulting changes in soil carbon stocks and above-ground plant biomass. **See p.599**

On page 599, Terrer *et al*.[1] reveal an unexpected trade-off between the effects of rising atmospheric carbon dioxide levels on plant biomass and on stocks of soil carbon. Contrary to the assumptions encoded in most computational models of terrestrial ecosystems, the accrual of soil carbon is not positively related to the amount of carbon taken up by plants for biomass growth when CO_2 concentrations increase. Instead, the authors show that carbon accumulates in soils when there is a small boost in plant biomass growth in response to CO_2, and declines when the growth of biomass is high. Terrer *et al*. propose that associations of plants with mycorrhizal soil fungi are a key factor in this relationship between the above- and below-ground responses to elevated CO_2 levels.

Rising levels of atmospheric CO_2 are thought to have driven an increase in the amount of carbon absorbed globally by land ecosystems over the past few decades, a phenomenon known as the CO_2 fertilization effect[2]. This occurs because, at the scale of leaves, higher CO_2 levels enhance photosynthesis and the efficiency with which resources (water, light

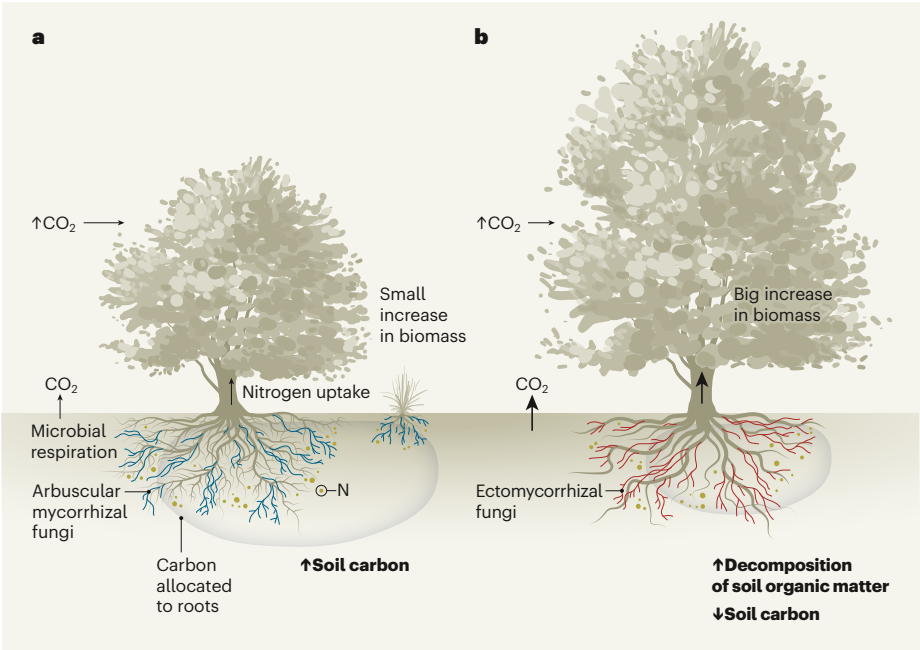

Figure 1 | Proposed effects of elevation of atmospheric carbon dioxide levels. Terrer *et al.*[1] suggest that associations of plants with different types of mycorrhizal soil fungi affect plant and soil responses to increases in atmospheric carbon dioxide levels. **a**, Plants that associate with arbuscular mycorrhizal fungi (grasses and some trees, in this study) do not 'mine' nitrogen (N, a nutrient) from the soil, and therefore do not produce much extra above-ground biomass when CO_2 levels rise. Instead, they allocate carbon to fine roots and to root-exuded substances, resulting in soil-carbon accrual. Carbon dioxide produced from the respiration of soil microorganisms returns carbon to the atmosphere. **b**, Plants that associate with ectomycorrhizal fungi (only trees in this study) mine the soil for nitrogen, the uptake of which supports a bigger increase in biomass growth than in **a**. However, nutrient mining increases the rate of decomposition of organic matter in soil. The amount of carbon in the soil therefore decreases in response to elevated CO_2 levels; microbial soil respiration is greater than in **a**.

and nutrients such as nitrogen) are used to assimilate CO_2 and support biomass growth[3]. Evidence supporting the existence of the CO_2 fertilization effect has been observed in experiments in which the atmosphere around plants or plant communities is enriched with CO_2. But at the level of whole ecosystems, responses to CO_2 enrichment are more difficult to track, because the effects are diluted throughout a chain of connected processes. Constraining estimates of the response of the global land carbon sink to rising CO_2 levels therefore remains a major challenge (see go.nature.com/3vgvhj).

Changes in soil carbon are inherently difficult to detect, and studies that assess the effects of elevated CO_2 levels on soil-carbon stocks have been equivocal[4]. Terrer and colleagues set out to investigate these effects by carrying out a meta-analysis of 108 CO_2-enrichment experiments. The authors estimate that, in these studies, soil-carbon stocks increased in non-forest sites but remained almost unchanged in forests. By evaluating the effects of multiple environmental variables, the authors found that, surprisingly, the best explanation for the observed patterns is that the changes in soil carbon stocks are inversely related to the changes in above-ground plant biomass: high accumulation of carbon in biomass was associated with soil-carbon loss, whereas low biomass accumulation was associated with soil-carbon gain. This relationship was evident only in experiments in which no nutrients had been added to the studied systems, leading the authors to propose that plant nutrient-acquisition strategies are responsible – which, in turn, depend on the mycorrhizal soil fungi associated with the plants.

A previous study reported[5] that only a small increase in above-ground biomass occurs in CO_2-enriched plants that associate with a particular family of mycorrhizae (arbuscular mycorrhizae; AM). AM-associated plants benefit from the fungi's extensive network of hyphae (branching filaments that aid vegetative growth), which support the plants' uptake of nitrogen from the soil solution. However, AM have only a limited ability to 'mine' nitrogen from organic matter in the soil. The availability of soil nitrogen therefore limits the increase of biomass growth of AM-associated plants in response to elevated CO_2 levels. By contrast, plant species that associate with a different group of soil fungi (the ectomycorrhizae; ECM) exhibit a greater increase in above-ground biomass in CO_2-enrichment studies, because some of their carbon is allocated to ECM that can mine for nitrogen[5]. Mining for nutrients by ECM is, however, thought to accelerate the decomposition of organic matter in soil.

Terrer *et al.* now find that AM-associated plants produce a bigger increase in soil-carbon stocks in CO_2-enrichment experiments than do ECM-associated plants. The authors suggest that this is because AM-associated plants allocate more carbon to fine roots and to compounds exuded by the roots, resulting in soil-carbon accrual (Fig. 1a). By contrast, nutrient acquisition by ECM-associated plants results in increased turnover – and therefore loss – of soil organic matter (Fig. 1b). Overall, this would lead to an ecosystem-scale trade-off between the amount of carbon sequestered in plants and that sequestered in soil, in a CO_2-enriched atmosphere.

Most Earth-system models that account for land carbon-cycling processes assume that rising levels of atmospheric CO_2 will increase plant growth, thus producing more plant litter and thereby increasing stocks of soil carbon[6]. The authors compared the changes in soil carbon and above-ground plant biomass predicted by various models, both in simulations of six open-air CO_2-enrichment experiments, and in global simulations of historical and future increases in atmospheric CO_2. None of the models reproduced the negative relationship between carbon sequestration by soil and growth in plant biomass that was observed in the current study.

Terrer and co-workers' findings thus provide another urgent warning that current climate models overestimate the amount of carbon that will be sequestered by land ecosystems as atmospheric CO_2 levels increase – not only because the models largely ignore the effects of nutrient limitations, but also because they overestimate the amount of carbon that could be sequestered in soil, particularly in forest ecosystems[7]. But the new study also reveals that grasslands, shrublands and other ecosystems that already have high soil-carbon stocks have great potential to accumulate more soil carbon as CO_2 levels increase. These results thus add weight to previous calls to protect existing soil-carbon stocks to mitigate the effects of climate change[8].

There are some limitations to the set of CO_2-enrichment experiments included in Terrer and colleagues' meta-analysis. The experiments are biased towards temperate systems, and most of the forests studied are associated with ECM, whereas the grasslands are all AM-associated. The authors did not find that the type of ecosystem had a substantial effect on the observed responses to CO_2, but it remains to be seen whether the reported trade-off between above- and below-ground carbon sequestration for AM- compared with ECM-associated plants applies to forests alone[9]. Further experiments, especially in tropical ecosystems, are now needed to address these issues.

Tropical ecosystems are large contributors to the global terrestrial carbon sink[10], but they are notoriously under-studied. Field observations are scarce and few manipulation experiments — such as CO_2 enrichment or nutrient additions — have been carried out in these ecosystems[11,12]. Below-ground processes are particularly challenging to assess in the tropics, where the effects of multiple nutrient scarcities often come into play[12]. Terrer and colleagues' study provides a promising framework that can be elaborated to describe diverse plant–soil interactions in various terrestrial ecosystems in the future.

CO_2-enrichment experiments generally last for just a few years, or just over a decade at most[13]. Such timescales are unlikely to capture the effects of elevated CO_2 levels on plant mortality, plant-species composition and soil-carbon turnover time, all of which can affect the sequestration of carbon by ecosystems in different ways in the longer term. Mechanistic understanding gained from experiments about the coupling between carbon and nutrient cycling can, however, be integrated into computational models. And this will allow us to constrain estimates of the size of the terrestrial carbon sink in the coming decades. The interactions between plants and their associated soil fungi, as well as other crucial below-ground agents and processes such as microbial communities, are already stirring up modelling efforts[14,15]. Terrer and colleagues' study now invites researchers to test hypotheses about the processes that drive coordinated above- and below-ground responses to rising CO_2 levels. Such studies could be a real step forwards in our understanding of the fate of the terrestrial carbon sink.

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Metrology

Atomic clocks compared with astounding accuracy

Rachel M. Godun

Three atomic clocks based on different atoms have been compared with record accuracy. The findings bring a redefinition of the second a step closer and aid the search for dark matter — an elusive component of the Universe. **See p.564**

The remarkable accuracy of atomic clocks makes them excellent instruments for timekeeping and other precision measurements. On page 564, the Boulder Atomic Clock Optical Network (BACON) Collaboration[1] reports extremely accurate comparisons of three world-leading clocks in Boulder, Colorado, housed at the National Institute of Standards and Technology (NIST) and the JILA research institute. The authors show how their clock comparisons provide insights into fundamental physics and represent substantial progress towards redefining the second in the International System of Units (SI).

Atomic clocks 'tick' at a rate determined by the frequency of light that is emitted or absorbed when an atom changes from one energy state to another. Clocks based on different atoms run at different rates, and the term 'optical clock' refers to one that runs at an optical frequency. Three of the world's best optical clocks are the aluminium-ion and ytterbium clocks at NIST and the strontium clock at JILA. The measured frequencies of all three clocks are estimated to be correct to within a fractional uncertainty of 2 parts in 10^{18} or better[2–4]. This level of uncertainty could, in principle, allow the clocks to keep time so accurately that they would gain or lose no more than one second over the age of the Universe. Such optical clocks would be 100 times more accurate than caesium clocks[5].

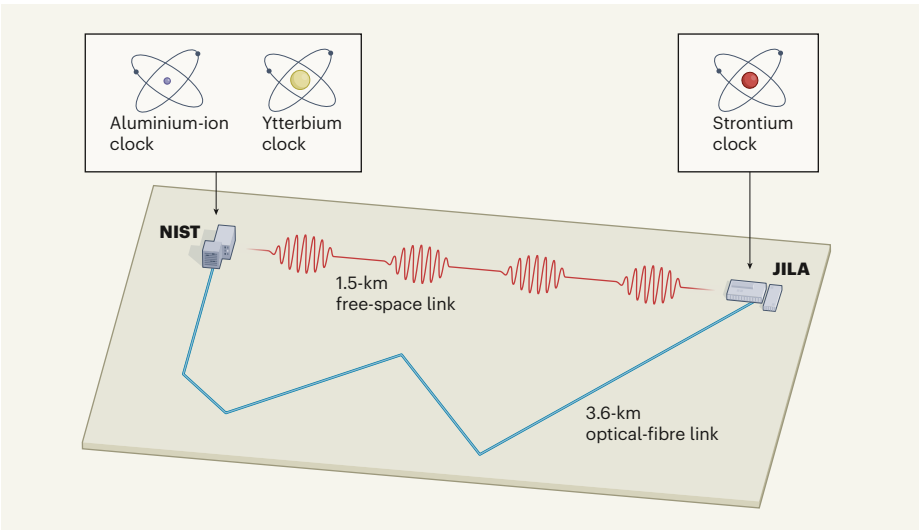

Figure 1 | Comparing a network of optical clocks. The BACON Collaboration[1] operated a network of three atomic clocks in Boulder, Colorado. The network consisted of an aluminium-ion clock and an ytterbium clock, housed at the National Institute of Standards and Technology (NIST), and a strontium clock, located at the JILA research institute. Data were transmitted between the two facilities through a 3.6-kilometre optical-fibre link and in the form of laser pulses through the air along a 1.5-km 'free-space' link. The authors used this set-up to compare the three atomic clocks with unprecedented accuracy — an achievement that has implications for fundamental physics and the future of international timekeeping. (Adapted from Fig. 1 of ref. 1.)