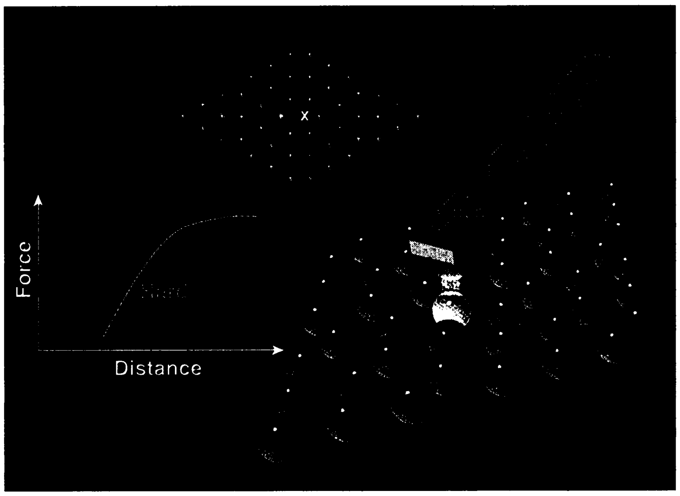

Gently does it. In their AFM experiments, Lantz *et al.* can distinguish between silicon atoms at different surface locations on the basis of their force response curves (**left**). Shown here are four unit cells of the silicon 7 × 7 surface, with a corner hole at the center (marked with an x). This site serves as a reference to measure background forces. The corner adatom (green) and the center adatom (red) have different stiffnesses, as indicated by the curves of the same color in the graph. This difference is exaggerated for clarity. The tip is shown at the point of closest approach, where the chemical bond (yellow cloud) starts to form.

which is likely if the tip picks up some of the material from the surface, a perfect image showing the periodicity of the sample is obtained, but local defects do not show up. This can be easily understood by taking two washboards and sliding one on top of the other. The resulting up/down motion will be the same even if you remove one of the ridges from one washboard.

"True" atomic resolution was not achieved until 1995, when the 7 × 7 reconstruction of silicon (a beautiful rearrangement of the surface atoms) was imaged with an AFM (*3*, *4*). It was the atomic-scale imaging of this surface that gave the scanning tunneling microscope (STM), the older sibling of the AFM, instant fame in 1983 (*5*). Lantz *et al.* chose this classic surface for their experiment. It is ideal for this first demonstration because the atoms are widely spaced and each has a "lone" atomic orbital sticking out of the surface. All the atoms are silicon, but their stiffness differs nevertheless depending on their position, which determines the way they bond to their neighbors underneath. This type of force spectroscopy has been done on surfaces for some time (*6*), but this is the first time that the surface is also imaged with atomic resolution in the same experiment (after all, you have to take the blindfold off to check that your identification of the fruit is correct!).

In a recent related study, Foster *et al.* identified the fluorine atoms in CaF_2 on the basis of the shape of the features in the AFM image, aided by theoretical modeling (*7*). The observed triangular shape of the surface fluorine atoms is caused by the contribution to the image from fluorine atoms just below. This result is important because this type of sample, being an insulator, cannot be imaged with STM. Giessibl *et al.* have also shown that under special conditions, it is possible to image individual orbitals such as those of an atom on the tip (*8*) This work has elicited criticism (*9*) and a technically compelling response (*10*, *11*). The idea of squeezing microscopic samples with an AFM tip is also being applied to biological specimens. Mahaffy *et al.* have recently made quantitative viscoelastic measurements on fibroblasts at the submicrometer level and concluded that the response is dominated by the cytoskeleton (*12*).

The AFM studies discussed here were performed in the so-called noncontact mode, which uses the weak attractive forces between tip and sample for imaging. The area of noncontact AFM with true atomic resolution is starting to blossom (*13*, *14*). Over the short term, we can expect improvements through the use of stiffer cantilevers and smaller oscillation amplitudes (*8*). The use of carbon nanotubes as tips (*15*) promises to improve the weakest link in these experiments. Once we have identified surface atoms and their bonding strengths, we will be able to do more atomic manipulation (*16*), even at room temperature (*17*). The use of sophisticated tactile ("haptic") interfaces (*18*, *19*) will allow us to "feel" the atoms in real time and gently push them to desired locations. We may at last be able to take off the boxing glove.

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PERSPECTIVES: CARBON CYCLE

Discriminating Plants

F. I. Woodward

The increasing concentration of carbon dioxide (CO_2) in the atmosphere (*1*) is the surest evidence that humans are changing the global environment. Atmospheric CO_2 would accumulate even faster if the oceans and the terrestrial biosphere did not absorb about half the CO_2 emissions from burning fossil fuels and deforestation. The amounts of CO_2 absorbed by these sinks are difficult to quantify, however, and this is hampering a detailed understanding of the carbon cycle.

Recent studies suggest that plants discriminate in a systematic way against CO_2 containing the stable isotope ^{18}O, offering an exciting opportunity to differentiate the terrestrial from the oceanic sink (*2*, *3*); the greater the terrestrial sink the lower the ^{18}O content of atmospheric CO_2 (*4*). However, Gillon and Yakir (*5*) suggest on page 2584 of this issue that current understanding of this discrimination may be incomplete, resulting in incorrect sink estimates.

Both transpiration and photosynthesis are involved in the discrimination of plants against CO_2 containing ^{18}O ($C^{18}O^{16}O$) (see the figure). $H_2{}^{16}O$ transpires more rapidly from leaves than the heavier $H_2{}^{18}O$. The site of evaporation therefore becomes enriched in the heavier isotope (*3*). This

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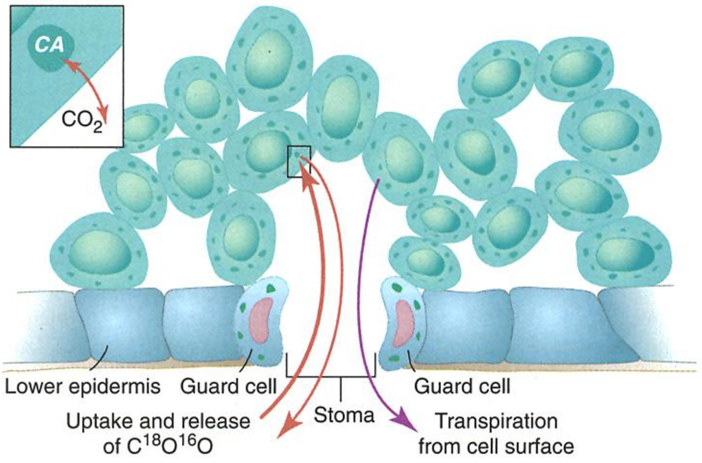
The author is in the Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK. E-mail: F.I.Woodward@Sheffield.ac.uk

evaporative ^{18}O enrichment in water is transferred first to the water in the chloroplast (where photosynthesis takes place) and then, by the enzyme carbonic anhydrase, to dissolved CO_2 in the chloroplast (*2*). CO_2 entering the leaf thus equilibrates isotopically with chloroplast water.

Plants fall into two groups determined by their photosynthetic pathway. About 85% of plants use the C_3 pathway, with the remainder using the more recently evolved C_4 pathway. A fraction *F* of the CO_2 diffuses out of the leaf without being fixed photosynthetically but after isotope exchange with chloroplast water. In C_3 plants, *F* is about 50% of the inward flux of CO_2. In contrast, *F* is only about 30% in C_4 plants, where the photosynthetic rate is higher and the CO_2 concentration in the chloroplast lower. This outward flux discriminates against $C^{18}O^{16}O$; that is, $C^{18}O^{16}O$ is preferentially retained in the plant. Previous studies have assumed complete isotopic equilibrium between water and CO_2 when calculating this discrimination.



Discriminating leafs. The fluxes of $H_2{}^{18}O$ (purple) and $C^{18}O^{16}O$ (red) into and out of a leaf through a stoma to the sites of evaporation (cell surfaces). Expanded inset: CO_2 exchange in the chloroplast. *CA*, carbonic anhydrase.

On the basis of measurements of over 50 species, Gillon and Yakir propose that the assumption of complete isotopic equilibrium, due to sufficient carbonic anhydrase activity, requires reconsideration. Their in situ measurements of carbonic anhydrase indicate that isotopic exchange between water and CO_2 is virtually complete for C_3 and C_4 dicotyledonous species (a large class of flowering plants comprising many forest trees, potatoes, beans, and cabbages, as well as roses and clematis). These data are in keeping with previous research (*3, 6*), including studies that considered the possibility that isotopic exchange between water and CO_2 was incomplete (nonequilibrium) in C_3 species (*2, 7*). For grasses, however, Gillon and Yakir find that isotopic exchange is incomplete, at about 75% for C_3 grasses and just 38% for C_4 grasses, where carbonic anhydrase activity, *CA*, is both low and variable between species.

For C_4 species with low chloroplast CO_2, low *CA*, and therefore a lack of isotopic equilibrium, these new results indicate a smaller impact on atmospheric $C^{18}O^{16}O$ than indicated by the original equilibrium theory. Gillon and Yakir have expanded these estimates using data on the distribution of vegetation types, including C_4 species, to estimate the global fluxes of ^{18}O-enriched CO_2. The ^{18}O-enriched CO_2 flux from global vegetation was only 78% of that expected for equilibrium with leaf water. This indicates the substantial impact of the incomplete ^{18}O equilibrium in vegetation dominated by C_4 species. Back calculations from these fluxes assuming isotopic equilibrium would underestimate gross primary production (GPP) by about 22%, with particularly marked underestimates of C_4 activity.

The authors' comparisons between nonequilibrium and equilibrium estimates of ^{18}O-enriched CO_2 fluxes assume that the calculated discrimination against $C^{18}O^{16}O$ (Δ_A) is unchanging for the two cases. This can only be achieved by changing the global estimates of the ^{18}O composition at the site of evaporation in leaves. This is contentious but could explain discrepancies between different methods of estimation. Even when Δ_A is the same for nonequilibrium and equilibrium conditions, there is still the need to account for the forcing of atmospheric $C^{18}O^{16}O$ concentration by the outward flux of CO_2 from the leaf. This requires that the gradient for $C^{18}O^{16}O$ exchange is considered from the chloroplast surface rather than the chloroplast itself, as suggested previously by Gillon and Yakir (*6*). This would lead to an 18% underestimate of GPP calculated from changes in atmospheric $C^{18}O^{16}O$ and, assuming equilibrium, slightly less than the 22% calculated by Gillon and Yakir but still a substantial error.

One of the key points raised by Gillon and Yakir is that grasses, and C_4 grasses in particular, show the greatest disequilibrium between ^{18}O in water and CO_2. C_4 dicotyledons do not show this effect, indicating that nonequilibrium is not simply associated with the C_4 pathway of photosynthesis. Water supply to grass leaves occurs linearly and sequentially from base to tip; the ^{18}O composition of source water should become enriched by evaporation along this pathway. It has been shown for C_3 and C_4 grasses (*8*) that the longer the leaf, the greater the enrichment of the source water and the greater the deviation of the ^{18}O concentration of the enriched water calculated from a constant source water composition. Longer leaves (typifying C_4 species) therefore have a greater enrichment. Perhaps the large scatter and high disequilibrium values for C_4 grasses estimated by Gillon and Yakir result, at least in part, from variations in leaf length and the necessity to account for the linear evaporative enrichment of ^{18}O. Helliker and Ehleringer (*9*) have also found low and very variable *CA* in C_4 grasses, in keeping with Gillon and Yakir's results.

Precise quantification of ^{18}O discrimination by plants and ecosystems (*10*) is still not possible, but the work of Gillon and Yakir has brought us one step closer to this important goal. Ultimately, this approach will provide information about the carbon cycle unmatched by any other current method.

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