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Correspondence and requests for materials should be addressed to W.H.S. (e-mail: schlesin@duke.edu).

Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere

Ram Oren*, David S. Ellsworth†‡, Kurt H. Johnsen§, Nathan Phillips||, Brent E. Ewers*, Chris Maier§, Karina V.R. Schäfer*, Heather McCarthy*, George Hendrey‡, Steven G. McNulty¶ & Gabriel G. Katul*

* Nicholas School of the Environment and Earth Sciences, Duke University, Durham, North Carolina 27708, USA

† School of Natural Resources and Environment, University of Michigan, Ann Arbor, Michigan 48109, USA

‡ Department of Environmental Sciences, Brookhaven National Laboratory, Upton, New York 11973-5000, USA

§ Southern Research Station, US Forest Service, Research Triangle Park, North Carolina 27709, USA

|| Department of Geography, Boston University, Boston, Massachusetts 02215, USA

¶ Southern Global Climate Change Program, US Forest Service, Raleigh, North Carolina 27606, USA

Northern mid-latitude forests are a large terrestrial carbon sink^{1–4}. Ignoring nutrient limitations, large increases in carbon sequestration from carbon dioxide (CO₂) fertilization are expected in these forests⁵. Yet, forests are usually relegated to sites of moderate to poor fertility, where tree growth is often limited by nutrient supply, in particular nitrogen^{6,7}. Here we present evidence that estimates of increases in carbon sequestration of forests, which is expected to partially compensate for increasing CO₂ in the atmosphere, are unduly optimistic⁸. In two forest experiments on maturing pines exposed to elevated atmospheric CO₂, the CO₂-induced biomass carbon increment without added nutrients was undetectable at a nutritionally poor site, and the stimulation at a nutritionally moderate site was transient, stabilizing at a marginal gain after three years. However, a large synergistic gain from higher CO₂ and nutrients was detected with nutrients added. This gain was even larger at the poor site (threefold higher than the expected additive effect) than at the moderate site (twofold higher). Thus, fertility can restrain the response of wood carbon sequestration to increased atmospheric CO₂. Assessment of future carbon sequestration should consider the limitations imposed by soil fertility, as well as interactions with nitrogen deposition.

By burning fossil fuel and forests, and converting land to intensive agriculture use, humans have elevated the atmospheric concentration of CO₂ (ref. 3) and the deposition of atmospheric nitrogen (N)^{9,10}. Growth of many tree species is enhanced with provisions of both CO₂ and N in the suboptimal range¹¹, making it difficult to assess the effect of increased availability of either one when the supply of both increases concurrently¹⁰. Experiments indicate decreased nutrient availability¹², owing to increased carbon (C)/N ratios in elevated CO₂-grown foliage and litter¹³, but increased nutrient uptake with elevated CO₂-induced growth enhancement of fine roots¹⁴. Because most forests occur on low-nutrient soils, the uncertain effects of elevated CO₂ on nutrient supply hinders our ability to estimate forest C sequestration for future global C budgets.

If tree nutrient uptake does not increase in proportion to the growth response to elevated CO₂, then maturing pine trees should show less elevated CO₂-induced growth enhancements on low-fertility sites than on moderate sites, and thus should respond more to elevated CO₂ under improved nutrition. To evaluate these predictions, we used two field experiments with related loblolly pine (*Pinus taeda* L.) genotypes: the longest running forest-based free-air CO₂ enrichment (FACE) experiment conducted on a moderately fertile site; and a whole-tree chamber CO₂ enrichment experiment on an infertile site. The large difference in fertility of the two sites is indicated by a much greater increase in growth at the infertile site (130%) than at the moderate site (15%) when added nutrients augmented the sites to the same optimal fertility⁷. Additional site and soil characteristics that reflect fertility are provided in Methods.

The FACE prototype (FACE_p) was built in 1993 in a 10-year-old, 8.5-m tall loblolly pine plantation in the lower Piedmont plateau, growing on moderately low fertility, acidic clay-loam, at the Duke Forest of Duke University, North Carolina (35° 58' N, 79° 08' W; elevation 130 m). Currently the pines are about 15-m tall, and comprise 98% of the basal area¹⁵. FACE_p has enriched (550 p.p.m.v. CO₂) a 30-m diameter circular patch in the forest since 1994, during daylight hours of the growing season¹⁶. Before CO₂ enrichment in FACE_p commenced, growth (that is, the amount of C sequestered in woody biomass increment) was similar in FACE_p and an adjacent,

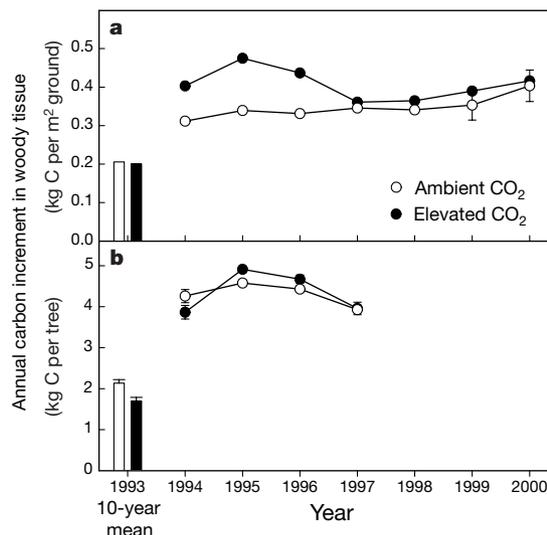


Figure 1 A comparison of annual carbon increment under elevated atmospheric CO₂ concentration (initiated in 1994) and ambient concentration without nutrient addition. **a**, Plot-level comparison between the free-air CO₂ enrichment prototype (FACE_p) and a nearby untreated, ambient CO₂ plot (in the past 2 yr, the number of untreated plots was increased to five). **b**, Individual tree comparison between trees in FACE_p and trees selected at random from the entire stand. Data for 1993 are shown as means for the first 10 yr of the stand's life.

untreated plot (Fig. 1a), as were foliar N concentration and physiological capacity¹⁶.

Averaged over the first 3 yr, the elevated CO₂ plot had a 34% increase in growth relative to the ambient CO₂ plot, reaching a maximum of 40%, but only a 6% increase over the next 4 yr. After four growing seasons of enrichment, we cored all pine trees in FACE_p (*n* = 78) and trees selected at random from the entire stand (*n* = 56; none in the untreated plot), and calculated annual individual tree growth for every year since 1993. The temporal pattern in individual tree growth was fundamentally the same as that of the stand with lower growth of FACE_p trees before the commencement of CO₂ enrichment, a large initial relative response to elevated CO₂, and a subsequent decrease in the response (Fig. 1b).

The CO₂-induced growth enhancement over the first 2 yr in the adjacent replicated FACE study (*n* = 3)¹⁵, which began operation in 1996, averaged about 24%, and ranged from 7 to 57% (1 s.d. = 15%). The initial growth enhancement in the FACE_p plot was not different from that at replicated FACE plots, after matching annual growth for the same year since enrichment commenced in both studies (2 yr, *t*-test, *P* > 0.1; see Methods for statistical approach). Thus, the early growth responses to elevated CO₂ were consistent in both experiments, but these responses are transient (Fig. 1).

The decrease in response to elevated CO₂ (Fig. 1) might be caused by nutrient limitation that can develop fairly rapidly in this moderate-fertility site with a limited rooting depth (< 0.3 m)¹⁷. The January foliar N concentration was similarly low in FACE_p and in the surrounding stand growing under ambient CO₂ (0.97 ± 0.04% by weight; mean ± s.e., *n* = 16). Plant-soil models predicted responses to an experimental stepwise increase in elevated CO₂ in nutrient-limited forests¹⁸, similar to that shown in Fig. 1. To test whether nutrient limitations reduced the tree response to elevated CO₂, we partitioned FACE_p and its unenriched counterpart in 1998, and added a balanced fertilizer to half the area (> 265 m²)

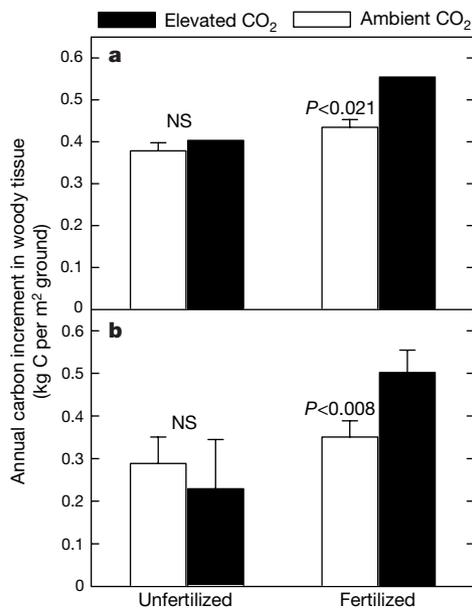


Figure 2 Effect of atmospheric CO₂ concentration and nutrient supply on annual carbon increment in woody tissue of genotypically similar loblolly pine. **a**, A moderate-fertility Duke Forest site (averaged for 1999 and 2000); **b**, an infertile SETRES site (1997 and 1998). *P* values represent test results between ambient and elevated CO₂ in each fertility level. In both sites, elevating only CO₂ had no significant effect (in **a**, data without nutrient addition are the mean of the past 2 yr shown in Fig. 1a); fertilizing in ambient CO₂ had a significant effect; and fertilizing under elevated CO₂ had significantly higher effects than the sum of the single CO₂ and N effects.

at the end of the growing season, aiming to attain optimal nutrition (1.4% N)^{7,19}. We also established four plot pairs, randomly positioned in the stand, with one member of each fertilized similarly, thus producing five plot pairs under ambient CO₂. After 1 yr, fertilization increased foliar N concentration under ambient CO₂ to 1.18 ± 0.08% (mean ± s.e.), but foliar N remained low under elevated CO₂ (1.02%), similar to unfertilized plots under both ambient and elevated CO₂ (1.05 ± 0.07%).

Averaged over 1999 and 2000, about 378 ± 16.0 g C m⁻² yr⁻¹ (mean ± s.e.) was incorporated into woody tissue under ambient CO₂ and without fertilization (Fig. 2a). Annual woody growth under elevated CO₂ without nutrient addition sequestered only 7% more C (*P* > 0.05), and improved nutrient supply in ambient CO₂ atmosphere increased growth by 15% (*P* = 0.024). However, the combination of improved nutrition and elevated CO₂ increased growth by 47% (~175 g C m⁻² yr⁻¹; *P* = 0.007)—more than twice the sum of the separate responses to each factor (total ~80 g C m⁻² yr⁻¹). This clearly indicates a synergistic effect of CO₂ and nutrient supply that raised the relative CO₂-induced growth response to the maximum previously seen in FACE_p (~40%).

We assessed whether this synergism increases with decreasing site fertility by evaluating the response of trees (*n* = 3, whole-tree open-top chambers) in another North Carolina loblolly pine plantation of a similar age and genetic stock growing on an infertile sandy soil (USDA Forest Service Southeast Tree Research and Education Site, SETRES; 34° 48' N, 79° 12' W). At this infertile site, foliar N was 1.02 ± 0.04% (mean ± s.e.) in both CO₂ environments, increased with fertilization to 1.29 ± 0.05%, and decreased to 1.15 ± 0.04% when fertilized trees were subjected to elevated CO₂ for two growing seasons (550 p.p.m.v.). Without added nutrients, annual growth did not respond to elevated CO₂ in the 2-yr period (*P* > 0.05; Fig. 2b). Under optimal nutrition¹⁹ and ambient CO₂, the growth of these trees increased by 21% (*P* < 0.05). However, the synergistic enhancement from improved nutrition and higher atmospheric CO₂ was 74% (*P* = 0.008)—more than three times the sum of the separate responses. This suggests that CO₂ responses of growth in pine forests will be highly variable and depend greatly on site fertility, perhaps to the point of not responding at all on the nutritionally poorest sites.

In addition to nutrient limitations, water deficits could also limit forest response to elevated CO₂. The early part of the 1999 growing season was unusually dry along the eastern seaboard of the USA,

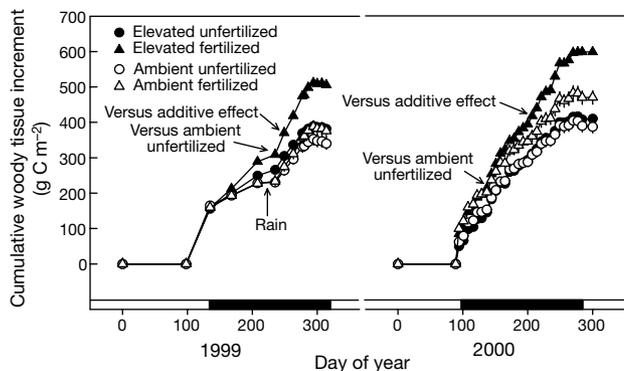


Figure 3 Cumulative carbon increment in woody tissue under ambient and elevated atmospheric CO₂ concentration with and without the addition of nutrients. The carbon increment under elevated CO₂ without nutrient addition was never significantly greater than under ambient CO₂ (*P* > 0.05). Arrows depict the earliest time in each growing season in which the carbon increment under elevated CO₂ with nutrient addition was significantly higher than the indicated treatment (*P* < 0.05). The period of CO₂ enrichment in each year is indicated as a horizontal bar at the bottom. In 1999, drought-breaking rains (indicated) raised soil moisture in the root zone from 0.14 to 0.32 m³ m⁻³. Error bars represent s.e.

with the May–July rainfall 84% below the 30-yr site average. In contrast, rain was ample and evenly distributed during the 2000 growing season. Weekly measurements of stem diameter at the Duke Forest site permitted us to quantify drought-induced interaction effects with the CO₂ and nutrition treatments on the seasonal dynamics of C investment in woody tissue. A fraction of the stem increment that occurred after each rainfall event (such as that in 1999; Fig. 3) was due to actual wood growth and a fraction was due to stem swelling with hydration; we estimated the latter, reversible ‘increment’ as $\leq 17.4 \text{ g C m}^{-2}$ after a given event. Without drought in 2000, growth enhancement in fertilized elevated CO₂ conditions became significant earlier (Fig. 3), and sequestered $45 \text{ g C m}^{-2} \text{ yr}^{-1}$ (27%) more than with early season drought in 1999. Hence, the synergistic effect of CO₂ and nutrients is especially strong when seasonal water limitations are alleviated.

Treatments imposed in a stepwise manner may invoke different responses than gradual changes⁸. Where site fertility is very low, responses to stepwise CO₂ enrichment experiments may be muted (Fig. 2b). Where fertility is moderate, responses may be transient and decrease when soil nutrients are depleted by the initial increase in growth rate¹⁹ (Fig. 1). Thus, the short-term dynamics of the elevated CO₂ response¹⁵ may not portray the longer-term, multi-year responses shown in this study, particularly where dynamic changes in nutrient cycles are involved¹². There are many reasons to expect the long- and short-term responses to be different^{18,20}, and this study highlights the need for caution in broadly extrapolating from short-term results.

Along a gradient of decreasing native-site fertility, nutrient amendments will become increasingly necessary if forest ecosystems (planted or natural) are intended to both take advantage of elevated atmospheric CO₂ and aid in reducing the rate at which atmospheric CO₂ concentrations increase. Under current atmospheric CO₂, nitrogen deposition has been estimated to contribute little to enhanced C sequestration in temperate forests^{10,21,22}, and N deposition may ultimately cause a growth reduction by causing nutritional disharmony²³. Nevertheless, a model comparison has concluded that interaction between CO₂ and N deposition may be involved in the response of terrestrial ecosystems to future levels of atmospheric CO₂ (ref. 24).

Our amendment of $\sim 11 \text{ g N m}^{-2} \text{ yr}^{-1}$ is more than 10 times the current deposition rates of $\sim 0.65 \text{ g N m}^{-2} \text{ yr}^{-1}$ at both sites. At the moderate-fertility site, C sequestration was enhanced by ~ 14.1 and $\sim 17.3 \text{ g C g}^{-1} \text{ N}$ addition under elevated atmospheric CO₂ in the dry and wet year, respectively (that is, an average of $15.7 \text{ g C g}^{-1} \text{ N}$). At the nutritionally poor site, the enhancement under elevated CO₂ was $19.1 \text{ g C g}^{-1} \text{ N}$ addition. This provides a first estimate of the effect of N supply on CO₂-induced C sequestration in woody biomass increment at the scale of forest stands. We note, however, that industrial N fertilizer production and use already has an impact on the global N cycle²⁵, and any plan to enhance C sequestration that relies on a significant increase in N fertilization must be carefully evaluated for both local and global effects. □

Methods

Setting

The FACE and SETRES sites are mid-rotation plantations without density-related mortality. The summers are warm and humid, and winters are moderate. Annual precipitation (1,100–1,200 mm) is evenly distributed throughout the year, but occasional deficits in growing-season water occur. The sites are considered moderate (FACE) and low (SETRES) fertility, as reflected in the heights of dominant *P. taeda* of 15.7 and 10.4 m at age 25 yr, respectively. Soil incubations for net N mineralization at these sites yielded roughly 2.7 (upper 7.5 cm) and 1.3 (15 cm) $\text{g N m}^{-2} \text{ yr}^{-1}$, respectively, and cation exchange capacity was about 12 (upper 15 cm) and 2 (20 cm) $\text{cmol}(+) \text{ g}^{-1}$. Seasonal CO₂ enrichment in FACE_p, typically from late April to late October, was achieved in 1994–2000 by the free-air CO₂ enrichment technique²⁶.

Annual growth of trees at the FACE_p and its paired untreated plot was measured from 1993 onwards, at the end of each growing season. In 1998, an impermeable barrier to 1-m depth, about three times the depth of fine roots in this forest¹⁷, partitioned both plots into two sections containing roughly the same mass of above-ground biomass per unit ground. Concurrently, four pairs of 10 m × 10 m plots were established nearby with a minimum

distance of 20 m between plots. One randomly selected section (FACE_p and its counterpart) and one randomly chosen plot of each newly established plot pair were fertilized to meet optimal values^{7,19}. An application rate of $11.2 \text{ g N m}^{-2} \text{ yr}^{-1}$ in the form of urea began in July 1998.

The SETRES stand was planted in 1985 in the Sandhills of North Carolina¹⁹, a site with sandy, siliceous, well-drained soil. Annual nutrient additions to meet optimal values commenced in March 1992, with annual applications averaging $11.2 \text{ g N m}^{-2} \text{ yr}^{-1}$ over the 7 yr (ref. 18). Six fertilized trees and six unfertilized trees of a similar diameter range and mean height of $8.6 \pm 1 \text{ m}$ were enclosed in open-top chambers²⁷ in 1996. CO₂ enrichment to $\sim 550 \text{ p.p.m.v.}$ in three chambers per treatment commenced in August 1996, and lasted for two complete growing seasons.

Carbon sequestration

Woody biomass (that is, in branches, bole and roots down to 5 mm in diameter) was estimated at both sites from diameter and allometric equations for ambient CO₂ conditions^{19,28}. At Duke Forest, estimates included trees with diameters > 50 mm at 1.4 m above ground, representing 99% of the biomass, less than 5% of which is in hardwood species. At SETRES, equations were available for trees with and without fertilization¹⁹. From SETRES, the density of wood decreased from 0.51 to 0.47 g cm^{-3} with fertilization. From cores taken at FACE_p, the density of wood decreased from 0.52 to 0.48 g cm^{-3} with elevated CO₂. Thus, when growth estimated from diameter was increased by either treatment, the values were adjusted to reflect these reductions. Woody biomass above ground was converted to C content by multiplying by 0.48, and below ground by 0.44, as determined from combustion (CHN Analyzer; Perkin Elmer). In SETRES, biomass was converted to a ground-area value on the basis of 1,260 trees ha^{-1} . At Duke Forest, diameter was measured weekly during the growing season (Fig. 3) with stainless steel dendrometer bands¹⁵ installed 1.4 m above ground on all trees. Edge conditions created when a 3-m wide strip was cleared around FACE_p did not affect growth: there was no pattern in relative growth rate with distance from the edge, nor was there a difference between the population of trees positioned less than one-third of the radius from the periphery and that composed of the rest of the trees ($P > 0.5$).

Statistical considerations

We analysed the SETRES data by paired *t*-test after pairing trees on the basis of initial biomass. Data from FACE_p and its counterpart were not compared statistically (Fig. 1a); however, data from FACE_p and its unenriched counterpart were compared with data from the nearby replicated FACE ($n = 3$), and the 1999 and 2000 data from FACE_p were compared with data from all nearby unenriched plots ($n = 5$) by a *t*-test between a single observation and a population mean²⁹. This approach permits testing whether the response of FACE_p is different from that of the replicated unenriched plots, but without replications it is not possible to estimate the mean response to elevated supplies of both CO₂ and N. Furthermore, we note that the statistical scope of inference of most controlled experiments, including the replicated FACE and SETRES, is limited³⁰.

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Correspondence and requests for materials should be addressed to R.O. (e-mail: ramoren@duke.edu).

An Early Cambrian tunicate from China

D.-G. Shu, L. Chen, J. Han & X.-L. Zhang

Early Life Institute & Department of Geology, Northwest University, Xi'an, 710069, China

Like the Burgess Shales of Canada, the Chengjiang Lagerstätte from the Lower Cambrian of China is renowned for the detailed preservation as fossils of delicate, soft-bodied creatures^{1–9}, providing an insight into the Cambrian explosion. The fossils of possible hemichordate chordates^{5–7} and vertebrates⁹ have attracted particular attention. Tunicates, or urochordates, comprise the most basal chordate clade¹⁰, and details of their evolution could be important in understanding the sequence of character acquisition that led to the emergence of chordates and vertebrates^{11–18}. However, definitive fossils of tunicates from the Cambrian are scarce or debatable^{4,9,19–24}. Here we report a probable tunicate *Cheungkongella ancestralis* from the Chengjiang fauna. It resembles the extant ascidian tunicate genus *Styela* whose morphology could be useful in understanding the origin of the vertebrates.

Phylum Chordata
 Subphylum Urochordata
 Class Ascidiacea
Cheungkongella ancestralis gen. et sp. nov.

Type species. *Cheungkongella ancestralis*.

Etymology. Genetic name is a metaphor of China and is also in honour of the Cheungkong Scholars Programme that supports this work; the specific name is a reference to its possible primitive position.

Holotype. Early Life Institute (ELI), Northwest University, Xi'an: ELI-0000195.

Stratigraphy and locality. Qiongzhusi Formation, Yu'an-shan Member (*Eoredlichia* Zone); Lower Cambrian. The specimen was collected by L.C. and J.H. from the same locality and horizon as the

animal *Xidazoon*⁸ and agnathan vertebrate *Myllokunmingia*⁹.

Diagnosis. The body is club-shaped, reminiscent of extant ascidian *Styela*, with two-fold division: an upper main body and a lower thick supporting stem attached to hard substratum (Fig. 1). The body is wholly enclosed within a structure interpreted as a secreted tunic. The stem tapers downward, and the main body is bucket-shaped in outline, bearing a large oral siphon with short tentacles on its top and a small cloacal one on the lower dorsal side. A pharynx occupies over two-thirds of the body volume.

Description. *Cheungkongella ancestralis*, new genus and species, is known from a single specimen, with a total length of about 25 mm. The whole body consists of two regions: a stout stem, which in life supported a sub-spherical main body. The stem (about 15 mm long) tapers distally, and is attached to the exterior surface of the left, free cheek of a trilobite *Eoredlichia intermedia*, an index fossil for the Lower Cambrian. The stem bears some transverse creases, consistent with an enclosing tunic, and prominent longitudinal 'ribs'. The distal section has a conspicuously coarse texture, and has several patches of agglutinated sediment including quartz grains.

The main body (roughly 10 mm long) was probably sub-spherical in life. Wrinkling of the compressed body on the ventral side (opposite to cloacal siphon) is consistent with folding of a tough

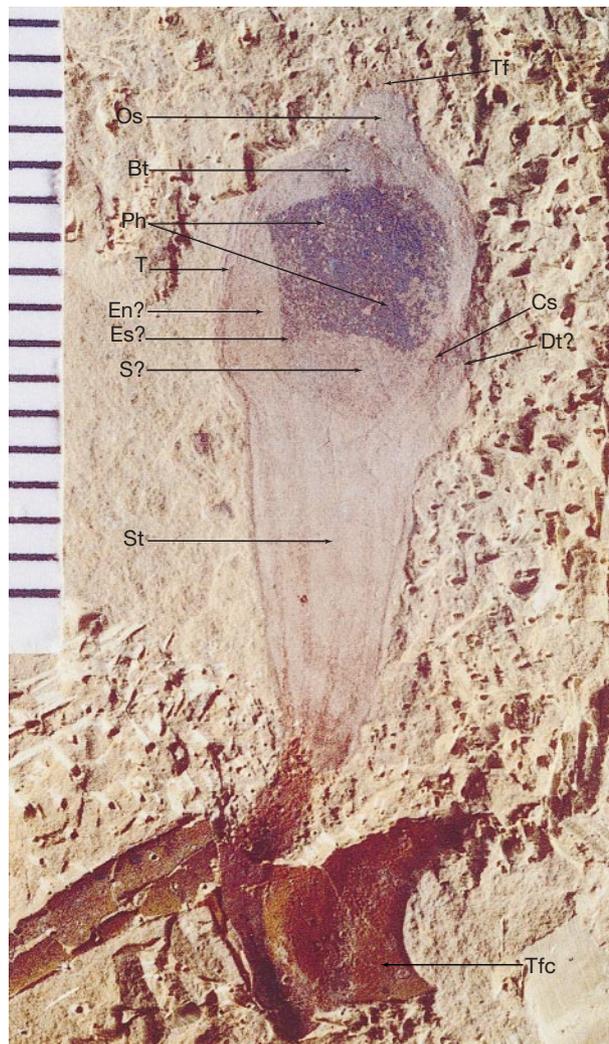


Figure 1 The Lower Cambrian urochordate *Cheungkongella ancestralis* gen. et sp. nov. from Haikou, Kunming, Yunnan. Specimen ELI-0000195, viewed from the left. Scale bar, 1 mm. Bt, buccal tentacles; Cs, cloacal siphon; Dt?, degenerating tail; Os, oral siphon; En?, presumed endostyle; Es?, possible esophagus; Ph, pharynx; S?, presumed stomach; St, stem; T, tunic; Tf, tentacle-like fringe; Tfc, trilobite free cheek.