Prokaryotes: The unseen majority

William B. Whitman, David C. Coleman, and William J. Wiebe

PNAS 1998;95;6578-6583
doi:10.1073/pnas.95.12.6578

Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 6578–6583, June 1998

Perspective

Prokaryotes: The unseen majority

*William B. Whitman**†, *David C. Coleman*‡, *and William J. Wiebe*§

*Departments of *Microbiology, ‡Ecology, and §Marine Sciences, University of Georgia, Athens GA 30602*

ABSTRACT **The number of prokaryotes and the total amount of their cellular carbon on earth are estimated to be 4–6 × 10^{30} cells and 350–550 Pg of C (1 Pg = 10^{15} g), respectively. Thus, the total amount of prokaryotic carbon is 60–100% of the estimated total carbon in plants, and inclusion of prokaryotic carbon in global models will almost double estimates of the amount of carbon stored in living organisms. In addition, the earth's prokaryotes contain 85–130 Pg of N and 9–14 Pg of P, or about 10-fold more of these nutrients than do plants, and represent the largest pool of these nutrients in living organisms. Most of the earth's prokaryotes occur in the open ocean, in soil, and in oceanic and terrestrial subsurfaces, where the numbers of cells are 1.2 × 10^{29}, 2.6 × 10^{29}, 3.5 × 10^{30}, and 0.25–2.5 × 10^{30}, respectively. The numbers of heterotrophic prokaryotes in the upper 200 m of the open ocean, the ocean below 200 m, and soil are consistent with average turnover times of 6–25 days, 0.8 yr, and 2.5 yr, respectively. Although subject to a great deal of uncertainty, the estimate for the average turnover time of prokaryotes in the subsurface is on the order of 1–2 × 10^3 yr. The cellular production rate for all prokaryotes on earth is estimated at 1.7 × 10^{30} cells/yr and is highest in the open ocean. The large population size and rapid growth of prokaryotes provides an enormous capacity for genetic diversity.**

Although invisible to the naked eye, prokaryotes are an essential component of the earth's biota. They catalyze unique and indispensable transformations in the biogeochemical cycles of the biosphere, produce important components of the earth's atmosphere, and represent a large portion of life's genetic diversity. Although the abundance of prokaryotes has been estimated indirectly (1, 2), the actual number of prokaryotes and the total amount of their cellular carbon on earth have never been directly assessed. Presumably, prokaryotes' very ubiquity has discouraged investigators, because an estimation of the number of prokaryotes would seem to require endless cataloging of numerous habitats.

To estimate the number and total carbon of prokaryotes on earth, several representative habitats were first examined. This analysis indicated that most of the prokaryotes reside in three large habitats: seawater, soil, and the sediment/soil subsurface. Although many other habitats contain dense populations, their numerical contribution to the total number of prokaryotes is small. Thus, evaluating the total number and total carbon of prokaryotes on earth becomes a solvable problem.

Aquatic Environments. Numerous estimates of cell density, volume, and carbon indicate that prokaryotes are ubiquitous in marine and fresh water (e.g., 3–5). Although a large range of cellular densities has been reported (10^4–10^7 cells/ml), the mean values for different aquatic habitats are surprisingly similar. For the continental shelf and the upper 200 m of the open ocean, the cellular density is about 5 × 10^5 cells/ml. A portion of these cells are the autotrophic marine cyanobacteria and *Prochlorococcus* spp., which have an average cellular density of 4 × 10^4 cells/ml (6). The deep (>200 m) oceanic water contains 5 × 10^4 cells/ml on average. From global estimates of volume, the upper 200 m of the ocean contains a total of 3.6 × 10^{28} cells, of which 2.9 × 10^{27} cells are autotrophs, whereas ocean water below 200 m contains 6.5 × 10^{28} cells (Table 1).

The upper 10 cm of sediment in the open ocean is included in the oceanic habitat because, as a result of animal mixing and precipitation, it is essentially contiguous with the overlying water column. Most of the marine sediment is found in the continental rise and abyssal plain, so the numbers of prokaryotes were calculated from an arithmetic average of the cellular densities in the studies cited by Deming and Baross (ref. 9; Table 1). The Nova Scotian continental rise was excluded from this calculation because of its unusual hydrology (10).

There are fewer estimates of the number of prokaryotes in freshwaters and saline lakes (5). Given an average density of 10^6 cells/ml, the total number of cells in freshwaters and saline lakes is 2.3 × 10^{26}. This value is three orders of magnitude below the numbers of prokaryotes in seawater.

In the polar regions, a relatively dense community of algae and prokaryotes forms at the water–ice interface in annual sea ice (11). In Antarctic sea ice, the estimated number of prokaryotes (2.2 × 10^{24} cells) was based on the mean cell numbers of Delille and Rosiers (12) and the mean areal extent of seasonal ice (13). If the population size in the Arctic is similar (14), the global estimate for both polar regions is 4 × 10^{24} cells, only a fraction of the total number of prokaryotes.

Soil. Soil is a major reservoir of organic carbon on earth and an important habitat for prokaryotes. Prokaryotes are an essential component of the soil decomposition subsystem, in which plant and animal residues are degraded into organic matter and nutrients are released into food webs (15). Many studies indicate that the number of prokaryotes in forest soils is much less than the number in other soils. The total number of prokaryotes in forest soil was estimated from detailed direct counts from a coniferous forest ultisol (16), which were considered representative of forest soils in general (Table 2). For other soils, including grasslands and cultivated soils, the numbers of prokaryotes appear about the same, e.g., the number of prokaryotes in Negev desert soil is comparable to the number in cultivated soil (19). Therefore, the numbers of prokaryotes in all other soils were estimated from the unpublished field studies of E. A. Paul for cultivated soils (cited in ref. 18).

Subsurface. The subsurface is defined here as terrestrial habitats below 8 m and marine sediments below 10 cm. Few direct enumerations of subsurface prokaryotes have been made, largely because of the difficulty in obtaining uncontaminated samples. Nevertheless, circumstantial evidence suggests that the subsurface biomass of prokaryotes is enormous (20). For instance, groundwater from deep aquifers and formation

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

†To whom reprint requests should be addressed: e-mail: whitman@uga.cc.uga.edu.

Table 1. Number of prokaryotes in aquatic habitats

Habitat	Volume,* cm^3	Cells/ml, $\times 10^5$	Total no. of cells, $\times 10^{26}$
Marine			
Continental shelf	2.03×10^{20}	5	1.0
Open ocean			
Water, upper 200 m	7.2×10^{22}	5	360
Water, below 200 m	1.3×10^{24}	0.5	650
Sediment, 0–10 cm	3.6×10^{19}	4600	170
Fresh			
Lakes	1.25×10^{20}	10	1.3
Rivers	1.2×10^{18}	10	0.012
Saline lakes	1.04×10^{20}	10	1.0
Total			1180

*Marine, freshwater, and saline lake volumes were calculated from refs. 7 and 8.

water from petroleum deposits contain 10^3–10^6 prokaryotic cells/ml (21, 22).

Unconsolidated sediments represent most of the marine subsurface and about 20% of the terrestrial subsurface (23). The number and sizes of subsurface prokaryotes in unconsolidated sediments of the deep ocean and the continental shelf and slope (24–30) and the terrestrial coastal plain (31, 32) have been determined. Because the terrestrial values fall within the range of the marine values, arithmetic averages were calculated to create a depth profile to 600 m (Table 3). For deeper sediments to 4 km, the number of prokaryotes was extrapolated from the formula of Parkes *et al.* (33). At 4 km, the average temperature reaches 125°C (34), which is close to the upper temperature limit for prokaryotic life.

Of the 3.8×10^{30} prokaryotes calculated to be in the unconsolidated subsurface sediments, 97% or 3.7×10^{30} occur at depths shallower than 600 m (Table 3). The estimated number of prokaryotes for deeper sediments is only 0.13×10^{30} cells. This value is uncertain because it is based on extrapolation. In addition, the accuracy also depends on whether or not the data used to calculate the depth profile are representative of the entire subsurface. Because most of these data were obtained from regions of the Pacific Ocean, the depth profile is likely to be most accurate for those sediments.

The estimated number of terrestrial subsurface prokaryotes (Table 3, 2.5×10^{29}) is a minimum value because it is limited to unconsolidated sediments, which represent only 20% of the terrestrial subsurface. Two other approaches can be used to estimate the total number of terrestrial subsurface prokaryotes. The first approach, originally used by Gold (20), is based on the assumption that the average porosity of the terrestrial subsurface is 3%. Assuming that the percentage of the total pore space occupied by prokaryotes is 0.016% (35), the average volume of a subsurface prokaryotic cell is 1.07×10^{-12} cm^3 (36), and the volume of the upper 4 km of the terrestrial subsurface is 4.9×10^{23} cm^3, the total number of terrestrial subsurface prokaryotes is 2.2×10^{30} cells. Considering the general nature of these assumptions, the agreement within an order-of-magnitude of the estimate in Table 1 provides some confidence in the latter estimate.

Alternatively, the number of terrestrial subsurface prokaryotes can be estimated from groundwater data. Based on values from seven sites and four studies (31, 37–39), the average number of unattached cells in groundwater is 1.54×10^5 cells/ml. The total volume of groundwater in the upper 4 km of the earth's surface is 9.5×10^{21} cm^3 (40), and thus the number of unattached prokaryotes in groundwater is 1.46×10^{27} cells. However, the number of prokaryotes in aquifer sediments is probably many orders of magnitude greater than the number unattached in the groundwater per se. For an aquifer 30–200 m deep, only 0.058% of the prokaryotes are unattached (calculated from the data of refs. 31, 41, and 42). This value appears to be representative of groundwater from other deep aquifers (22, 37), which implies that the terrestrial subsurface contains about 2.5×10^{30} prokaryotic cells. This estimate contains two major uncertainties. First, about 55% of the earth's groundwater is found below 750 m (40), and the extrapolation of values from the groundwater and aquifers above 750 m may not be applicable. Second, the ratio of unattached prokaryotes in aquifers was calculated from unconsolidated sediments, and the ratio may vary in other types of aquifers where the physical properties of the rocks and sediments are very different.

In summary, the subsurface is a major habitat for prokaryotes, and the number of subsurface prokaryotes probably exceeds the numbers found in other components of the biosphere. The greatest uncertainty is in the estimate for the terrestrial subsurface because this estimate is based on only a few measurements. However, even for the terrestrial subsurface, two independent methods suggest that the number of

Table 2. Number of prokaryotes in soil

Ecosystem type*	Area, $\times 10^{12}$ m^2	No. of cells,† $\times 10^{27}$
Tropical rain forest	17.0	1.0
Tropical seasonal forest	7.5	0.5
Temperate evergreen forest	5.0	0.3
Temperate deciduous forest	7.0	0.4
Boreal forest	12.0	0.6
Woodland and shrubland	8.0	28.1
Savanna	15.0	52.7
Temperate grassland	9.0	31.6
Desert scrub	18.0	63.2
Cultivated land	14.0	49.1
Tundra and alpine	8.0	20.8
Swamps and marsh	2.0	7.3
Total	123.0	255.6

*From ref. 73.

†For forest soils, the number of prokaryotes in the top 1 m was 4×10^7 cells per gram of soil, and in 1–8 m, it was 10^6 cells per gram of soil (16). For other soils, the number of prokaryotes in the top 1 m was 2×10^9 cells per gram of soil, and in 1–8 m, it was 10^8 cells per gram of soil (18). The boreal forest and tundra and alpine soils were only 1 m deep. A cubic meter of soil was taken as 1.3×10^6 g.

Table 3. Total number of prokaryotes in unconsolidated subsurface sediments

Depth interval,* m	Cells/cm^3, $\times 10^6$	No. of cells, $\times 10^{28}$: Deep oceans†	Continental shelf and slope‡	Coastal plains§
0.1	220.0¶	66.0	14.5	4.4
10	45.0¶	121.5	26.6	8.1
100	6.2¶	18.6	4.1	1.2
200	19.0¶	57.0	12.5	3.8
300	4.0¶	12.0	2.6	0.8
400	7.8¶		10.1	3.2
600	0.95‖		3.7	1.2
1,200	0.61‖		3.2	1.0
2,000	0.44‖		2.6	0.9
3,000	0.34‖			0.7
Total		275.1	79.9	25.3

Grand Total: $380 \times 10^{28} = 3.8 \times 10^{30}$

*Depth intervals are designated by the upper boundary. Thus, "0.1" represents 0.1–10 m and "3,000" represents 3,000–4,000.
†Corresponds to seismic layer I (23).
‡Corresponds to subcontinental sediments (23).
§Corresponds to geosyncline sediments of Mesocenozoic origin (23).
¶Calculated from the arithmetic averages.
‖Calculated by extrapolation of the formula of Parkes *et al.* (33).

prokaryotes is very large, about 2.5–25 $\times$ 10^{29} cells. Thus, the total number of subsurface prokaryotes is probably 3.8–6.0 $\times$ 10^{30} cells.

Other Habitats. Although they were found not to constitute a large fraction of the total number of prokaryotes, other habitats are of interest in their own right.

Animals. Many vertebrate and invertebrate animals contain dense populations of prokaryotes that play important roles in nutrition and disease. To estimate the total number of prokaryotes on and within animals, the numbers of prokaryotes in each individual animal and the population size of the animal must be known. Unfortunately, these values are only known for a small number of mostly domestic animals.

In mammals and birds, prokaryotes are abundant on the skin and within the gastrointestinal tract. Within the gastrointestinal tract, most of the prokaryotes are anaerobes in the colon, cecum, or rumen (43, 44), and the total number found within animals whose population sizes are known can be readily calculated (Table 4). For comparison, the numbers of prokaryotes on the skin of humans can be calculated. The density of prokaryotes is about 10^3–10^4 cells/cm^2, except in the groin and axilla, where it is 10^6 cells/cm^2 (57). Based on the surface area of an adult (58), the total number of prokaryotes on the skin of an individual is about 3 $\times$ 10^8 cells, a value far below the number of prokaryotes in the colon (Table 4).

Insects, such as termites, cockroaches, and craneflies, harbor dense prokaryotic populations in their hindguts (53, 59, 60). Because the number of termites in the world has been estimated and the number of prokaryotes for at least one type of termite has been measured (53, 55), it is possible to estimate the total number of prokaryotes in termites (Table 4). Although huge, this value is much smaller than the total number of prokaryotes found in many other habitats.

Although the number of prokaryotes in the gastrointestinal tracts of animals is enormous, it is unlikely to represent a large fraction of the total prokaryotes on earth. For example, the number of prokaryotes in the bovine rumen is 4–6 orders of magnitude less than the numbers found in soil, the subsurface, and sea water. Therefore, although the numbers of prokaryotes are known for only a few groups of animals, it is unlikely that animals contain a major fraction of the total number of prokaryotes.

Leaves. Although prokaryotes associated with plant roots are measured with other soil prokaryotes for methodological reasons, leaves and other plant tissues also harbor large populations of prokaryotes. Leaf area can be estimated from the leaf area index. The numbers of prokaryotes on leaves are highly variable, but the viable count (cfu or colony-forming units) rarely exceeds 10^4–10^6 cfu/cm^2 (61–64). An upper limit for the number of prokaryotes on leaves can be estimated by assuming a dense population and a high leaf area index. Assuming a leaf area index of 10, which is typical of many forests, the maximum number of prokaryotes would be about 10^{11} cfu/m^2. A forest soil contains about 6 $\times$ 10^{13} cells/m^2 (see Table 2). Even if the viable counts are 1–10% of the direct counts, the maximum number of prokaryotes on leaves is unlikely to exceed the number in soil. In fact, in a temperate forest, the number of prokaryotes on leaves is a small fraction of the number in the underlying soil (65).

Air. By volume, the atmosphere represents the largest compartment of the biosphere, and prokaryotes have been detected at altitudes as high as 57–77 km (66). Nevertheless, the total number of airborne prokaryotes appears to be quite low. For the bottom 3 km of the atmosphere, the total number of prokaryotes over land is about 5 $\times$ 10^{19} cfu (calculated from refs. 67–69), a value so low that it is unlikely that airborne prokaryotes represent a large fraction of the total number of prokaryotes.

Carbon Content. The amount of carbon in prokaryotes can be estimated from the cell numbers in soil, aquatic systems, and the subsurface. In the soil and subsurface, the cellular carbon is assumed to be one-half of the dry weight. In soil, the average dry weight of a prokaryotic cell is 2 $\times$ 10^{-13} g or 200 fg (18). Thus, the total prokaryotic cellular carbon in soil is 26 $\times$ 10^{15} g of C or 26 Pg of C (Table 5). In the subsurface, there is only one measurement of the average dry weight of cells, that of 172 fg for cells from a terrestrial aquifer (36). This value yields an estimate of the terrestrial prokaryotic cellular carbon of 22–215 Pg of C (Table 5). The estimate for the marine subsurface, 303 Pg of C (Table 5), may be compared with 56 Pg of C, the value obtained by Parkes *et al.* (33). The difference, 5.4-fold, is due in part to how the depth integrations were calculated. Parkes *et al.* (33) used logarithmic extrapolations rather than arithmetic averages, which decreased their estimated number of cells by 3-fold. They also estimated the amount of carbon per cell at 65 fg of C rather than the 86 fg of C used here. The remaining difference occurs because the current estimate is based in part on additional marine and terrestrial data.

For aquatic systems, the average cellular carbon and volume has been a matter of considerable discussion, and the range in average cellular carbon reported is 5–20 fg of C/cell (5, 17, 70–72). To obtain the estimate of 2.2 Pg of C (Table 5), the average cellular carbon for sedimentary (9) and planktonic prokaryotes (17, 70–72) was assumed to be 10 and 20 fg of C/cell, respectively. If the average cellular carbon is assumed to be 5 fg of C/cell, the total amount of prokaryotic cellular carbon would be 0.6 Pg of C.

Discussion. The total carbon of prokaryotes on earth is enormous, approximately 60–100% of the total carbon found in plants (Tables 5 and 6). Inclusion of this carbon in global models will greatly increase estimates of the amount of carbon stored in living organisms. In addition, prokaryotes contain large amounts of N, P, and other essential nutrients. For instance, assuming a C/N/P ratio in prokaryotes of

Table 4. Total number of prokaryotes in some representative animals

Animal	Organ	Cells/ml or cells/g	Organ contents*	No. of animals†	No. of cells, $\times$ 10^{23}	Refs.
Human	Colon	3.2 $\times$ 10^{11}	220 g	5.6 $\times$ 10^9	3.9	45, 46
Cattle	Rumen	2.1 $\times$ 10^{10}	106 liter	1.3 $\times$ 10^9	29.0	47, 48
Sheep and goats	Rumen	4.4 $\times$ 10^{10}	12 liter	1.7 $\times$ 10^9	9.0	47, 48
Pigs	Colon	5.4 $\times$ 10^{10}‡	9 liter	8.8 $\times$ 10^8	4.3	49, 50
	Cecum	2.8 $\times$ 10^{10}‡	1 liter	8.8 $\times$ 10^8	0.3	49, 50
Domestic birds§	Cecum	9.5 $\times$ 10^{10}	2 g	1.3 $\times$ 10^{10}	0.024	51, 52
Termites	Hindgut	2.7 $\times$ 10^6¶		2.4 $\times$ 10^{17}	6.5	53

*Organ contents in volume or grams of wet weight. For comparison, the volume of the human colon is 0.5 liter. For domestic birds, weight wet was calculated from a volume of 2 ml assuming that 1 ml - 1 g wet weight.
†Values from the *FAO Production Yearbook* (54), except for the termites value which was from ref. 55.
‡The direct count was assumed to be 2.7 $\times$ viable count (56).
§Includes chickens, ducks, and turkeys.
¶Per termite.

Table 5. Number and biomass of prokaryotes in the world

Environment	No. of prokaryotic cells, $\times 10^{28}$	Pg of C in prokaryotes*
Aquatic habitats	12	2.2
Oceanic subsurface	355	303
Soil	26	26
Terrestrial subsurface	25–250	22–215
Total	415–640	353–546

*Calculated as described in the text.

1:0.24:0.025 (74), the entire prokaryotic pool for N and P is 85–130 Pg of N and 9–14 Pg of P. In all plants, assuming C/N and C/P ratios for the 471 Pg of plant C in forests and woodlands of 156 and 1340, respectively, and C/N and C/P ratios for the 88 Pg of plant C in other ecosystems of 12.5 and 125, respectively (73), the amounts of N and P are 10 Pg and 1.05 Pg, respectively. Thus, the plant pool for these nutrients is an order of magnitude smaller than the total prokaryotic pool. In fact, the amount of N and P in soil prokaryotes, 6.2 Pg and 0.65 Pg, respectively, is nearly equal to the amount in terrestrial plants even though terrestrial plants contain much more carbon. Other essential nutrients are probably distributed similarly, and prokaryotes may represent the largest living reservoir for these elements on earth.

The abundance of prokaryotic carbon and other elements may be compared with the statement of Kluyver that about one-half of the "living protoplasm" on earth is microbial (2). Because most of the plant biomass is made up of extracellular material such as cell walls and structural polymers, the protoplastic biomass of prokaryotes probably far exceeds that of plants, and Kluyver's well-accepted estimate is probably much too conservative.

From the estimate of prokaryotic carbon in soil and aquatic habitats, it is possible to set some limits for the average growth or turnover rates for these populations. Assuming an efficiency of carbon assimilation of 0.2 (75, 76), the amount of "net productivity" necessary to support the turnover of prokaryotes in the upper 200 m of the ocean is four times their carbon content or 0.7–2.9 Pg of C (depending on the amount of carbon per cell). Given that about 85% of the net productivity is consumed in the upper 200 m (73) and assuming that all of this carbon is used by prokaryotes, the average turnover rate cannot exceed 15–60 yr^{-1}, and the average generation time cannot be less than 6–25 days. For the upper 200 m of the open ocean, the reported average generation time is 2.5–27 days (3). Similar calculations for the deep ocean (below 200 m) and soil suggest that the average turnover rate for prokaryotes cannot exceed approximately 1.2 and 0.4 yr^{-1}, respectively. The value for soil is not greatly different from current estimates for the upper portion of the soil of 0.4–2 yr^{-1} (77–79). Thus, our estimates of the prokaryotic cellular carbon in the upper ocean and soil are consistent with published productivity estimates.

Results from a similar analysis for the subsurface prokaryotes are problematic. Assuming that 1 Pg of C/yr, or about 1% of the total net productivity, reaches the subsurface and that the net burial rate is 0.06 Pg of C/yr (73), only 0.94 Pg of C/yr is available to support the subsurface community of prokaryotes. If the efficiency of carbon assimilation is 0.20, then the calculated average turnover time is 1–2 $\times 10^3$ yr, far longer than found in other ecosystems. At present, a number of plausible explanations for this apparent anomaly exist. (*i*) The average turnover time could be on the order of 1,000 yr. If this were the case, most of the subsurface prokaryotes must be metabolically inactive and probably nonviable. Circumstantial evidence suggests that this is not the case, and viability of subsurface prokaryotes is within the range observed for prokaryotes from surface sediments and soils (cf. 24, 31). Sulfate reduction, methanogenesis, and other activities have also been detected in cores from the subsurface (24). Thus, although it is likely that the relative metabolic activity and rate of carbon consumption of subsurface bacteria are lower than that found on the surface, activity must still be sufficient to maintain culture viability. (*ii*) Lithoautotrophic processes may provide an additional source of energy for growth of subsurface prokaryotes. Although lithoautotrophy has been demonstrated in some geological formations, current evidence suggests that most of the subsurface biomass is supported by organic matter deposited from the surface (80–82). Because the data are so limited, future studies could revise this view. (*iii*) The subsurface biomass may be overestimated. The estimate of subsurface carbon is based on a conversion factor derived from data at one site, which may not be representative. However, given that some of the smallest cells so far described in nature contain 5 fg of C, the magnitude of this error is unlikely to be more than 10- to 20-fold. (*iv*) The efficiency of carbon assimilation may be underestimated. Pure culture studies with rich media suggest that the efficiency of carbon assimilation can be as high as 0.85 (83). However, the error associated with this factor cannot be more than 4-fold. These points, when considered together, emphasize that our current understanding of subsurface prokaryotes is incomplete. Because of their numerical importance, more extensive examination of this habitat is imperative.

The large population size of prokaryotes implies that events that are extremely rare in the laboratory could occur frequently in nature. For instance, prokaryotes have an enormous potential to accumulate mutations and, thus, to acquire genetic diversity. However, the population size itself is not altogether an accurate measure of the potential for mutational change, which must also include the growth rates of the populations. Large, slowly growing populations may produce fewer cells and fewer mutational events than smaller, rapidly growing populations do. Even with the uncertainties for the average growth rates for many natural populations discussed above, it is still possible to estimate the cellular production rates and hence the frequency of these rare events (Table 7). Although subsurface prokaryotes predominate numerically, their cellular productivity is comparable to that of the much smaller but more rapidly growing population associated with domestic animals

Table 6. Relationship of plant and prokaryotic biomass to primary productivity

Ecosystem	Net primary productivity,* Pg of C/yr	Total carbon content, Pg of C		
		Plant*	Soil and aquatic prokaryotes	Subsurface prokaryotes
Terrestrial	48	560	26	22–215
Marine	51	1.8	2.2	303

*From ref. 73.

Table 7. Annual cellular production of prokaryotes in various habitats

Habitat	Population size	Turnover time, days	Cells/yr, $\times 10^{29}$
Marine heterotrophs			
Above 200 m	3.6×10^{28}	16*	8.2
Below 200 m	8.2×10^{28}	300*	1.1
Marine autotrophs	2.9×10^{27}	1.5†	7.1
Soil	2.6×10^{29}	900*	1.0
Subsurface	4.9×10^{30}*	5.5×10^5*	0.03
Domestic mammals	4.3×10^{24}‡	1§	0.02

*The value or mean of the range discussed in the text.
†Based on the median generation time of *Prochlorococcus* (84).
‡Sum of the number of prokaryotes in cattle, sheep, goats, and pigs from Table 4.
§From ref. 85.

(Table 7). The highest cellular productivity is found in the open ocean (Table 7). Thus, mutations and other rare genetic events are more likely to occur in the population of marine prokaryotes than in populations in other habitats.

Genes that are widely distributed in prokaryotes have a tremendous opportunity for mutational change, and the evolution of conserved genes must be otherwise greatly constrained. Assuming a prokaryotic mutation rate of 4×10^{-7} mutations per gene per DNA replication (86, 87), four simultaneous mutations in every gene shared by the populations of marine heterotrophs (in the upper 200 m), marine autotrophs, soil prokaryotes, or prokaryotes in domestic animals would be expected to occur once every 0.4, 0.5, 3.4, or 170 hr, respectively. Similarly, five simultaneous mutations in every gene shared by all four populations would be expected to occur every 60 yr. The capacity for a large number of simultaneous mutations distinguishes prokaryotic from eukaryotic evolution and should be explicitly considered in methods of phylogenetic analyses.

For essentially asexual, haploid organisms such as prokaryotes, mutations are a major source of genetic diversity and one of the essential factors in the formation of novel species. Given prokaryotes' enormous potential to acquire genetic diversity, the number of prokaryotic species may be very large. Recent estimates for the number of prokaryotic species range from 10^5 to 10^7 (88). However, the current definition of a prokaryotic species, which includes strains whose genomic DNAs form hybrids with a change in the melting temperature (ΔT_m) of less than 5°C (89), may be misleading. Application of the same definition to eukaryotes would lead to the inclusion of members of many taxonomic tribes into the same species (90). Similarly, phylogenetic groups such as humans, orangutans and gibbons would also belong to the same species (91). Thus, a simple comparison of the number of eukaryotic and prokaryotic species greatly underestimates prokaryotic diversity. Given prokaryotes' numerical abundance and importance in biogeochemical transformations, the absence of detailed knowledge of prokaryotic diversity is a major omission in our knowledge of life on earth.

We are grateful to our colleagues, whose understanding, generosity, and sense of humor made this project possible. They include, but are not limited to, M. Azain, B. Binder, J. F. Dowd, R. P. Freeman-Lynde, T. C. Hazen, T. Hollibaugh, S. Kayar, M. Lee, S. Martin, M. Moran, W. J. Payne, L. Pomeroy, J. B. Risatti, and J. Russell. We acknowledge support from National Science Foundation Grants BIR-94-13235 (W.B.W. and D.C.C.), DEB 96-32854 (D.C.C.), and DEB 94-12089 (W.J.W.) and Department of Energy Grant DE-FG02-97ER20269 (W.B.W.).

1. Romankevich, E. A. (1988) *Geokhimiya* **(2),** 292–306.
2. Kluyver, A. J. & van Niel, C. B. (1956) *The Microbe's Contribution to Biology* (Harvard Univ. Press, Cambridge, MA), p. 3.
3. Ducklow, H. W. & Carlson, C. A. (1992) *Adv. Microb. Ecol.* **12,** 113–181.
4. Simon, M. (1994) *Arch. Hydrobiol.* **130,** 283–302.
5. Fry, J. C. (1988) in *Methods in Aquatic Bacteriology*, ed. Austin, B. (Wiley, London), pp. 27–72.
6. Campbell, L. & Vaulot, D. (1993) *Deep-Sea Res. I* **40,** 2043–2060.
7. Garrison, T. (1994) *Essentials of Oceanography* (Wadsworth, New York), p. 353.
8. Mitsch, W. J., Mitsch, R. H. & Turner, R. E. (1994) in *Global Wetlands: Old World and New*, ed. Mitsch, W. J. (Elsevier, New York), pp. 3–56.
9. Deming, J. W. & Baross, J. A. (1993) in *Organic Geochemistry: Principles and Applications*, eds. Engel, M. H. & Macko, S. A. (Plenum, New York), pp. 119–144.
10. Aller, J. Y. & Aller, R. C. (1986) *Deep-Sea Res.* **33,** 755–790.
11. Vincent, W. F. (1988) *Microbial Ecosystems of Antarctica* (Cambridge Univ. Press, New York).
12. Delille, D. & Rosiers, C. (1995) *Polar Biol.* **16,** 27–34.
13. Gordon, A. L. (1981) *J. Geophys. Res.* **86,** 4193–4197.
14. Smith, R. E. H., Clement, P. & Cota, G. F. (1989) *Microb. Ecol.* **17,** 63–76.
15. Coleman, D. C. & Crossley, D. A., Jr. (1996) *Fundamentals of Soil Ecology* (Academic, San Diego).
16. Richter, D. D. & Markewitz, D. (1995) *Bioscience* **45,** 600–609.
17. Bratbak, G. & Dundas, I. (1984) *Appl. Environ. Microbiol.* **48,** 755–757.
18. Paul, E. A. & Clark, F. E. (1989) *Soil Microbiology and Biochemistry* (Academic, New York).
19. Fliessbach, A., Sarig, S. & Steinberger, Y. (1994) *Arid Soil Res. Rehabil.* **8,** 353–362.
20. Gold, T. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6045–6049.
21. Nilsen, R. K., Beeder, J., Thorstenson, T. & Torsvik, T. (1996) *Appl. Environ. Microbiol.* **62,** 1793–1798.
22. Pedersen, K. (1993) *Earth-Sci. Rev.* **34,** 243–260.
23. Ronov, A. B. & Yaroshevsky, A. A. (1969) in *Geophysical Monograph 13: The Earth's Crust and Upper Mantle*, ed. Hart, P. J. (Am. Geophys. Union, Washington, DC), pp. 37–57.
24. Cragg, B. A., Harvey, S. M., Fry, J. C., Hebert, R. A. & Parkes, R. J. (1992) *Proc. Ocean Drill. Program: Sci. Results* **127/128 (Pt. 1),** 761–776.
25. Cragg, B. A. (1994) *Proc. Ocean Drill. Program: Sci. Results* **135,** 147–150.
26. Cragg, B. A. & Kemp, A. E. S. (1995) *Proc. Ocean Drill. Program: Sci. Results* **138,** 599–604.
27. Cragg, B. A. & Parkes, R. J. (1994) *Proc. Ocean Drill. Program: Sci. Results* **139,** 509–516.
28. Cragg, B. A., Parkes, R. J., Fry, J. C., Hebert, R. A., Wimpenny, J. W. T. & Getliff, J. M. (1990) *Proc. Ocean Drill. Program: Sci. Results* **112,** 607–619.
29. Cragg, B. A., Parkes, R. J., Fry, J. C., Weightman, A. J., Rochelle, P. A., Maxwell, J. R., Kastner, M., Hovland, M., Whiticar, M. J. & Sample, J. C. (1995) *Proc. Ocean Drill. Program: Sci. Results* **146 (Pt. 1),** 399–411.
30. Cragg, B. A., Parkes, R. J., Fry, J. C., Weightman, A. J., Maxwell, J. R., Kastner, M., Hovland, M., Whiticar, M. J., Sample, J. C. & Stein, R. (1995) *Proc. Ocean Drill. Program: Sci. Results* **146 (Pt. 2),** 139–144.
31. Hazen, T. C., Jimenez, L., Lopez de Victoria, G. & Fliermans, C. B. (1991) *Microb. Ecol.* **22,** 293–304.
32. Chapelle, F. H., Zelibor, J. L., Jr., Grimes, D. J. & Knobel, L. L. (1987) *Water Resources Res.* **23,** 1625–1632.
33. Parkes, R. J., Cragg, B. A., Bale, S. J., Getliff, J. M., Goodman, K., Rochelle, P. A., Fry, J. C., Weightman, A. J. & Harvey, S. M. (1994) *Nature (London)* **371,** 410–413.
34. Garland, G. D. (1971) *Introduction to Geophysics: Mantle, Core, and Crust* (Saunders, Philadelphia).
35. Harvey, R. W., Smith, R. L. & George, L. (1984) *Appl. Environ. Microbiol.* **48,** 1197–1202.
36. Balkwill, D. L., Leach, F. R., Wilson, J. T., McNabb, J. F. & White, D. C. (1988) *Microb. Ecol.* **16,** 73–84.
37. Pedersen, K. & Ekendahl, S. (1990) *Microb. Ecol.* **20,** 37–52.
38. Stevens, T. O., McKinley, J. P. & Fredrickson, J. K. (1993) *Microb. Ecol.* **25,** 35–50.
39. Pedersen, K., Arlinger, J., Hallbeck, L. & Pettersson, C. (1996) *Mol. Ecol.* **5,** 427–436.
40. Berner, E. K. & Berner, R. A. (1987) *The Global Water Cycle* (Prentice–Hall, Englewood Cliffs, NJ).
41. Balkwill, D. L. (1989) *Geomicrobiol. J.* **7,** 33–52.
42. Sinclair, J. L. & Ghiorse, W. C. (1989) *Geomicrobiol. J.* **7,** 15–31.
43. Drasar, B. S. & Barrow, P. A. (1985) *Intestinal Microbiology* (Am. Soc. Microbiol., Washington, DC).
44. Smith, H. W. (1965) *J. Pathol. Bacteriol.* **89,** 95–122.
45. van Houte, J. & Gibbons, R. J. (1966) *Antonie van Leewenhoek* **32,** 212–222.
46. Cummings, J. H., Banwell, J. G., Segal, I., Coleman, N., Englyst, H. N. & Macfarlane, G. T. (1990) *Gastroenterology* **98,** A408.
47. Hungate, R. E. (1966) *The Rumen and Its Microbes* (Academic, New York), p. 34.
48. Habel, R. E. (1975) in *The Anatomy of the Domestic Animals*, ed. Getty, R. (Saunders, Philadelphia), 5th Ed., p. 861.
49. Butine, T. J. & Leedle, J. A. Z. (1989) *Appl. Environ. Microbiol.* **55,** 1112–1116.
50. Dukes, H. H. (1955) *Physiology of Domestic Animals* (Comstock Pub. Assoc., Ithaca, NY), 7th Ed.
51. Barnes, E. M., Mead, G. C., Barnum, D. A. & Harry, E. G. (1972) *Br. Poult. Sci.* **13,** 311–326.

52. Salanitro, J. P., Fairchilds, I. G. & Zgornicki, Y. D. (1974) *Appl. Microbiol.* **27,** 678–687.
53. Schultz, J. E. & Breznak, J. A. (1978) *Appl. Environ. Microbiol.* **35,** 930–936.
54. Food and Agricultural Organization (1994) *FAO Production Yearbook* (Food and Agricultural Organization of the United Nations, Rome), Vol. 48.
55. Zimmerman, P. R., Greenberg, J. P., Wandiga, S. O. & Crutzen, P. J. (1982) *Science* **218,** 563–565.
56. Eller, C., Crabill, M. R. & Bryant, M. P. (1971) *Appl. Microbiol.* **22,** 522–529.
57. Willett, H. P. (1992) in *Zinsser Microbiology*, eds. Joklik, W. K., Willett, H. P., Amos, D. B. & Wilfert, C. M. (Appleton & Lange, Norwalk, CT), 20th Ed., pp. 393–400.
58. Marples, M. J. (1965) *The Ecology of the Human Skin* (Charles C. Thomas, Springfield, IL), p. 6.
59. Cruden, D. L. & Markovetz, A. J. (1984) *Arch. Microbiol.* **138,** 131–139.
60. Klug, M. J. & Kotarski, S. (1980) *Appl. Environ. Microbiol.* **40,** 408–416.
61. Corpe, W. A. & Rheem, S. (1989) *FEMS Microbiol. Lett.* **62,** 243–250.
62. Dickinson, C. H., Austin, B. & Goodfellow, M. (1975) *J. Gen. Microbiol.* **91,** 157–166.
63. Ruinen, J. (1961) *Plant Soil* **15,** 81–109.
64. Van Outryve, M. F., Gossele, F. & Swings, J. (1989) *Microb. Ecol.* **18,** 175–186.
65. Holm, E. & Jensen, V. (1972) *Oikos* **23,** 248–260.
66. Imshenetsky, A. A., Lysenko, S. V. & Kazakov, G. A. (1978) *Appl. Environ. Microbiol.* **35,** 1–5.
67. Fulton, J. D. (1966) *Appl. Microbiol.* **14,** 241–244.
68. Fulton, J. D. (1966) *Appl. Microbiol.* **14,** 245–250.
69. Lighthart, B. & Stetzenbach, L. D. (1994) in *Atmospheric Microbial Aerosols*, eds. Lighthart, B. & Mohr, A. J. (Chapman & Hall, New York), pp. 68–98.
70. Lee, S. H. & Fuhrman, J. A. (1987) *Appl. Environ. Microbiol.* **53,** 1298–1303.
71. Cho, B. C. & Azam, F. (1990) *Mar. Ecol. Prog. Ser.* **63,** 253–259.
72. Carlson, C. A., Bales, N. R., Hansell, D. A. & Ducklow, H. W. (1998) *EOS* **79,** OS171 (abstr.).
73. Schlesinger, W. H. (1997) *Biogeochemistry* (Academic, New York), 2nd Ed.
74. Duboc, P., Schill, N., Menoud, L., van Gulik, W. & von Stockar, U. (1995) *J. Biotechnol.* **43,** 145–158.
75. Cole, J. J., Findlay, S. & Pace, M. L. (1988) *Mar. Ecol. Prog. Ser.* **43,** 1–10.
76. Pomeroy, L. R. & Wiebe, W. J. (1993) *Marine Microb. Food Webs* **7,** 101–118.
77. Coleman, D. C. (1995) in *Food Webs: Integration of Patterns and Dynamics*, eds. Polis, G. A. & Winemiller, K. O. (Chapman & Hall, New York), pp. 39–50.
78. Jenkinson, D. S., Ladd, J. N. & Rayner, J. H. (1980) in *Soil Biochemistry*, eds. Paul, E. A. & Ladd, J. N. (Dekker, New York), Vol. 5, pp. 415–471.
79. Schnuerer, J., Clarholm, M. & Rosswall, T. (1985) *Soil Biol. Biochem.* **17,** 611–618.
80. Ekendahl, S. & Pedersen, K. (1994) *Microbiology* **140,** 1565–1573.
81. Stevens, T. (1997) *FEMS Microbiol. Rev.* **20,** 327–337.
82. Wellsbury, P., Goodman, K., Barth, T., Cragg, B. A., Barnes, S. P. & Parkes, R. J. (1997) *Nature (London)* **388,** 573–576.
83. Payne, W. J. & Williams, M. L. (1976) *Biotechnol. Bioengineer.* **18,** 1653–1655.
84. Binder, B. J., Chisholm, S. W., Olsen, R. J., Frankel, S. L. & Worden, A. Z. (1996) *Deep-Sea Res. II* **43,** 907–931.
85. Russell, J. B. & Bruckner, G. G. (1991) in *Microbiology of Animals and Animal Products*, ed. Woolcock, J. B. (Elsevier, New York), pp. 1–17.
86. Hutchinson, F. (1996) in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington, DC), 2nd Ed., pp. 2218–2235.
87. Jacobs, K. L. & Grogan, D. W. (1997) *J. Bacteriol.* **179,** 3298–3303.
88. Hammond, P. M. (1995) in *Microbial Diversity and Ecosystem Function*, eds. Allsopp, D., Colwell, R. R. & Hawksworth, D. L. (CAB International, Wallingford, Oxon, U.K.), pp. 29–71.
89. Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E., Stackebrandt, E., *et al.* (1987) *Int. J. System. Bacteriol.* **37,** 463–464.
90. Sibley, C. G., Ahlquist, J. E. & Monroe, B. L., Jr. (1988) *Auk* **105,** 409–423.
91. Sibley, C. G. & Ahlquist, J. E. (1987) *J. Mol. Evol.* **26,** 99–121.