

Use of Radiolabelled Thymidine and Leucine To Estimate Bacterial Production in Soils from Continental Antarctica

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Tritiated thymidine incorporation (TTI) into DNA was used to examine bacterial production in two soil types from the Roberts-kollen group of nunataks in northwestern Dronning Maud Land, providing the first estimates of bacterial production in soil habitats on the Antarctic continent. Although estimates of bacterial productivity in soils near to bird nests (344 ± 422 ng of C g [dry weight]⁻¹ h⁻¹) were higher than those for soils from beneath mosses (175 ± 90 ng of C g [dry weight]⁻¹ h⁻¹; measured by TTI at 10°C), these differences were not significant because of patchiness of bacterial activity ($P > 0.05$). TTI- and [¹⁴C]leucine ([¹⁴C]Leu)-derived estimates of bacterial production were similar when incubations of 3 h were used, although incubations as short as 1 h were sufficient for measurable uptake of radiolabel. Dual-label incorporation of [³H]thymidine ([³H]TdR) into DNA and [¹⁴C]Leu into protein indicated that TTI did not reflect bacterial production of in situ assemblages when incubations were longer than 3 h. Isotope dilution analysis indicated that dilution of the specific activity of exogenously supplied [³H]TdR by de novo synthesis of TdR precursor could be limited by additions of [³H]TdR at a concentration of 1 nmol per ca. 115 mg of soil. TTI exhibited a psychrotrophic response to variation in temperature, with a temperature optimum of ca. 15°C and a Q₁₀ value for 0 to 10°C of 2.41.

Bacteria are important agents of decomposition and nutrient regeneration in many diverse ecosystems (2, 4, 30, 34, 40) and therefore play a significant role in controlling the availability of nutrients for autotrophic processes and food web dynamics. Although the biology of continental Antarctica is considered to be microbially dominated (15, 64, 69), ecological studies of nutrient cycling in soils on this continent have not, to date, attempted to quantify heterotrophic bacterial production. Recycling of nutrients by bacteria is likely to be of importance in the relatively simple, isolated ecosystems of continental Antarctica, where nutrient inputs are largely limited to primary production by microalgae and sparsely distributed plants such as mosses and lichens (27, 55, 67). In some areas, where guano imported by breeding birds represents a major nutrient source (52, 61), bacterial mediation may regulate the availability of these nutrients for autotrophic production. In addition, as temperature and moisture are major factors controlling bacterial activity (10), it is of particular interest to investigate bacterial production and nutrient cycling in continental Antarctic habitats which are defined by extremely low temperatures and the frequent and prolonged lack of water.

Quantitative studies of carbon cycling through bacteria are now possible because of the development of methods for quantifying bacterial productivity. Rate measurements of [*methy*-³H]thymidine ([³H]TdR) incorporation (TTI) into bacterial DNA may be used to estimate the productivity of bacteria in natural systems (16). Although TTI has gained widespread acceptance in microbial ecology (for reviews see references 35 and 46) and has been adopted as a standard technique for international, collaborative scientific programs, such as the Joint Global Ocean Flux Study (1990; JGOFS protocols, Kiel,

Germany), the validity of assumptions underlying TTI has been the subject of some debate (6, 20, 25, 37, 57). Essentially, this debate has emphasized the need to verify the assumptions of the TdR technique in relation to different habitats, if confidence in the accuracy of TTI-derived estimates of bacterial production is to be gained.

Calculation of bacterial productivity from TTI requires knowledge of the specific activity of the dTTP pool prior to incorporation into DNA. Direct measurement of the bacterial dTTP pool of natural assemblages is difficult, and isotopic dilution of exogenously supplied [³H]TdR by intracellular and extracellular sources of unlabelled precursor may occur (35, 46). Such dilution would result in an underestimation of bacterial production if not accounted for in subsequent calculations. The final extent of dilution can be measured by isotope dilution analysis (42). Isotopic dilution can be limited by the addition of [³H]TdR at concentrations in excess of the precursor pool size measured by isotope dilution analysis, since sufficiently high concentrations of TdR inhibit the de novo synthesis of TdR nucleotides (35, 42). Thus, isotope dilution analysis need not be performed for every sample from a particular habitat.

Further assumptions of the TTI technique include the following: (i) that TTI measurements represent balanced growth and (ii) that all growing bacteria incorporate exogenously supplied [³H]TdR into DNA. Exceptions to both of these assumptions have been noted (9, 12, 17, 21, 22, 42, 53, 60). Dual-label incorporation of [³H]TdR and [¹⁴C]leucine ([¹⁴C]Leu) can be used to measure rates of DNA and protein synthesis, respectively, in the same sample (7, 60). When possible, such studies are useful, since they provide two estimates of bacterial production based on essentially independent biochemical processes in the same sample. Furthermore, a relatively constant ratio of Leu to TdR (protein synthesis to DNA synthesis) over the time of incubation reflects balanced growth of bacteria

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incorporating both substrates and can thus be used to confirm both assumptions outlined above.

Microbiological studies in Antarctica to date have focused on maritime habitats, cold deserts, and lakes and streams (14, 15, 18, 56, 64, 65, 69). Thus, there is a relative paucity of data concerning the microbiology of inland nunataks, the rocky peaks of mountains that jut out through the glacial ice sheet. These nunataks represent extremely isolated habitats which, depending on their southerly latitude and altitude, vary in the environmental harshness imposed by low temperatures, lack of free water, and resource input. The northern nunataks of the Ahlmannryggen in Dronning Maud Land experience relatively mild weather conditions, with summer temperatures frequently above 0°C. This results in the thawing of frozen water and conditions suitable for biological activity. A biological survey of Roberts skollen has revealed the presence of mites, tardigrades, nematodes, protozoa, fungi, microalgae, lichens, and mosses (50, 52). In addition, their proximity to the edge of the ice shelf (a few hundred kilometers), and therefore marine food resources, makes some of these nunataks favorable breeding sites for seabirds, particularly the snow petrel *Pagodroma nivea* (26, 49, 51). While the birds, mosses, and microalgae are expected to provide major nutrient inputs to the system, nitrogen fixation and nutrient cycling by bacteria are likely to be important in controlling the availability of nutrients for primary production and other processes (64). The present study provides the first estimates of bacterial productivity, i.e., the rate of bacterial carbon cycling, in soils of these inland nunataks.

In this study we used the TTI technique to measure bacterial productivity on isolated, inland nunataks in Dronning Maud Land, Antarctica. As soils represent the most extensive microbial habitat on these nunataks, we compared levels of production in two different soil habitats. Interesting habitats in extreme environments include those which act as "hot spots" for increased biological activity (e.g., hydrothermal vents in the deep sea [29]). Thus, we selected soil types (viz., soil from beneath moss beds and soil from the vicinity of the nests of breeding birds) known to support enhanced microbial activity and increased nutrient concentrations (3, 47). Since we expected low rates of activity in nunatak soils, this choice also facilitated the measurement of radio signals for uptake of [³H]TdR and [¹⁴C]Leu. In addition, this study provides methodological guidelines specific to the measurement of bacterial production in Antarctic soils that are exposed to favorable moisture conditions during the summer growth season. Dual-isotope labelling experiments were conducted to determine the optimum period for which incubations should be carried out if the assumptions, of balanced growth and label incorporation into DNA, of the TTI technique are to be met. Furthermore, isotope dilution was examined to determine the concentration of radiolabelled TdR required to limit this artifact. The temperature of optimum growth of Antarctic soil bacteria was compared with that of temperate soil bacteria, and this comparison, coupled with in situ temperature data, provided information as to the temperature at which incubations should be carried out if meaningful estimations of optimum in situ bacterial productivity in Antarctic habitats are to be obtained from laboratory experiments.

MATERIALS AND METHODS

Study site. Roberts skollen is a group of nunataks situated at 71°28'S, 3°15'W in the northwestern Ahlmannryggen, Dronning Maud Land, Antarctica (Fig. 1). This group includes five major nunataks: Glacier's Edge, Ice Axe Peak, Peaceful Hill, Petrel's Rest, and Tumble Ice. The Ice Axe Peak complex includes four discrete peaks, including Cairn Peak on the northern side of the complex. View Rocks, where an automatic weather station was positioned, is a smaller complex

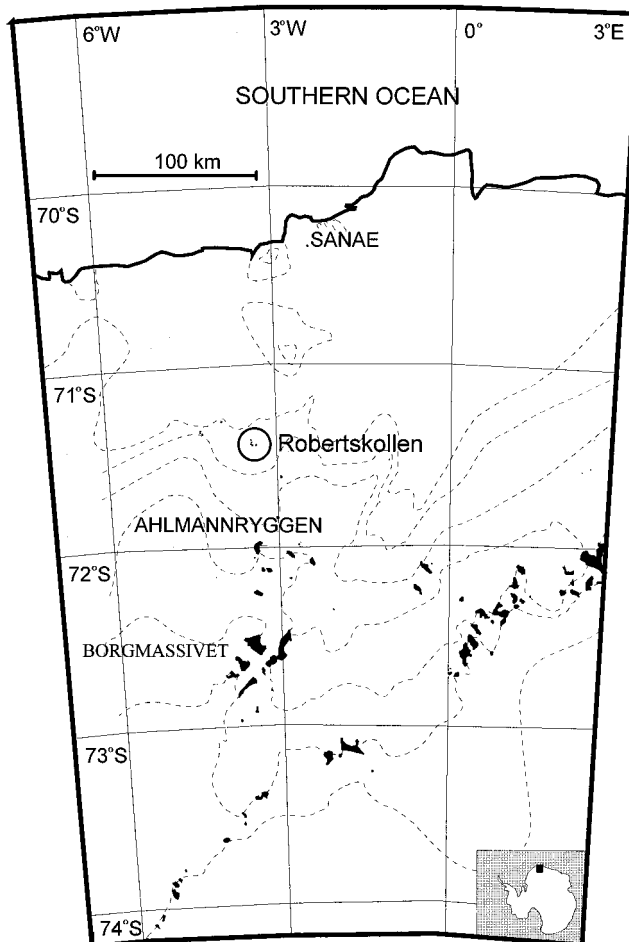


FIG. 1. Map of Dronning Maud Land, Antarctica, showing the position of the Roberts skollen group of nunataks in the northern Ahlmannryggen. SANAE (South African National Antarctic Expedition) is the coastal research station from which inland excursions were made.

that lies to the west of Cairn Peak. Preliminary biological studies of the area were reported by Ryan et al. (52). Some 500 pairs of snow petrels, *P. nivea*, breed on Ice Axe Peak, Petrel's Rest, and Tumble Ice during the summer. This species forages at sea, and thus the birds nesting at Roberts skollen may import a potentially significant quantity of nutrients to this system. Dominant plants in this system include five species of moss, which have been reported to be more common on bird-inhabited nunataks than on nunataks without breeding birds (52). Exposed patches of soil between the boulders on the scree slopes of the nunataks represent an extensive habitat (18a), harboring a community of organisms which range from tardigrades and nematodes (52) to protozoa, microalgae, fungi, and bacteria (64).

Sample collection. Replicate, surface (down to a depth of 1 cm) soil samples were aseptically collected in 25-ml glass bottles. Antarctica soil samples were stored frozen in the field by burial under ice and were stored at -20°C in a freezer during transit to Cape Town. Soil temperatures at Roberts skollen remain at ca. -20°C during the winter (ca. 6 months of the year), while soils thaw daily during the summer, when temperatures may reach 35°C. Thus, the procedure used here for collection and storage of samples is not likely to exert a greater stress on in situ assemblages than are conditions experienced in the field. Moisture contents of Roberts skollen soils were 4.29% ± 3.24% (ornithogenic) and 3.55% ± 1.49% (moss) at the time of collection. Logistical constraints precluded seasonal collection of samples for soil moisture content analysis. Antarctica soil samples for bacterial production measurements were collected from two soil habitats (i.e., they consisted of soil from within 1 m of bird nest entrances ["ornithogenic" soil] and soil from beneath moss patches) on Ice Axe Peak nunataks.

Measurement of TTI in soils. Frozen soil samples were defrosted to incubation temperature (see below), and slurries were made as follows: 4 g of soil was weighed into 25-ml glass bottles, 10 ml of sterile distilled water was added, and the mixture was homogenized with a vortex mixer. Aliquots of the slurry (250 µl

containing approximately 115 mg [dry weight] of soil) were measured into 2-ml polyethylene microcentrifuge tubes with a pipette (Gilson, France) and a wide-mouth pipette tip. A working solution of tritiated TdR was made as follows: the specific activity of [*methyl-³H*]TdR (83 Ci mmol⁻¹; Amersham Corp., United Kingdom) was diluted to 10.4 Ci mmol⁻¹ with crystalline TdR (Sigma Chemical Co.) in sterile distilled water. The final concentration of TdR in the working solution was 4.8 μM. Aliquots of the working solution (200 μl containing 10 μCi and 0.96 nmol of TdR) were added to sediment samples in the microcentrifuge tubes, and the contents of the tubes were mixed. Incubations were terminated by addition of 1.2 ml of ice-cold 80% ethanol solution containing 100 mg of TdR liter⁻¹. Samples were mixed briefly and placed on ice for 15 min. Samples were then centrifuged (8,000 × g for 2 min) before the ethanol supernatant was aspirated off. The sediment samples were washed again with 1.5 ml of ethanol as described above. After the second ethanol wash was removed, 1.5 ml of 0.3 M NaOH was added to the sediment and the sample was mixed briefly. Macromolecules were extracted, and RNA was hydrolyzed at 25°C for 18 h. Sediments were pelleted by centrifugation (8,000 × g for 1 min), and the supernatants were removed, mixed with 27 μl of glacial acetic acid, and placed into dialysis bags (12,000- to 14,000-Da cutoff; Spectrum Medical Industries) by using apparatus described by Pollard (41). Samples were washed into the bags with 1 ml of distilled water, dialyzed overnight against running tap water, and transferred to scintillation vials with 18 ml of UltimaGold XR (Packard Instruments, Inc.). Disintegrations per minute of ³H were determined in a Beckman LS 5000TD liquid scintillation counter.

Determination of isotope dilution. The extent of isotope dilution of [³H]TdR was examined according to the method described by Pollard and Moriarty (42). Aliquots of Antarctica soil slurry were added to microcentrifuge tubes (as described above) with crystalline TdR at concentrations of 0, 0.5, 1, 5, 10, and 30 nmol per tube (containing ca. 115 mg [dry weight] of sediment). Tritiated TdR was added, and the samples were mixed and incubated at 10°C for 1 h. This temperature allowed uptake of label at near-maximum rates and was close to the mean diurnal temperature during the summer. The procedure then proceeded in accordance with that described above for TTI. The reciprocal of disintegrations per minute was plotted against TdR concentration to determine the dilution of [³H]TdR in dTTP (42).

Dual-label incorporation (DLI) of [³H]TdR and [¹⁴C]leucine in soils. Rates of [³H]TdR and [¹⁴C]Leu incorporation were measured simultaneously by DLI methods as described by Tibbles et al. (60). The effect of temperature on DLI was examined by incubating samples at two temperatures, 4 and 10°C. Soil samples were defrosted to 4°C, and slurries were mixed and aliquoted at ca. 30 mg (dry weight) per tube as described above. [*methyl-³H*]TdR (28 Ci mmol⁻¹) and [¹⁴C]leucine (308 mCi mmol⁻¹; Amersham Corp.) were added at concentrations of 0.15 and 0.30 nmol, respectively, per tube. The DLI procedure then proceeded in accordance with that described for TTI, except that time series incubations were undertaken at 4 and 10°C and terminated after 0, 3, 6, 9, and 18 h. Energy spectrum spillover was corrected in dual-label determinations of disintegrations per minute according to the method described in the operation manual of the LS 5000TD.

Effect of temperature on TTI. The effect of temperature on TTI in Antarctic ornithogenic soil was compared with that in a temperate soil. Temperate "fynbos" soil was collected (in the same manner as Antarctic soils were) from Devil's Peak on Table Mountain, Cape Town, South Africa (34°00'S, 18°25'E), during May 1993. These soils support the growth of fynbos flora, which is characterized by high-level plant species diversity and low nutrient concentrations, and a moss species (*Ceratodon purpureus*) found at Roberts-kollen is also found on Table Mountain (23, 48). Slurries of temperate and Antarctic soils were aliquoted at 10°C, as described above, and then preincubated at seven different temperatures (0, 5, 10, 15, 20, 25, and 30°C) for 30 min to allow acclimation of bacterial assemblages before [³H]TdR was added. Incubations at each different temperature were terminated after 1 h, and the TTI procedure was then carried out as described above.

Determinations of field temperature, bacterial abundance, organic content, and nitrogen and phosphate content. Thermistor probes coupled to a mobile Campbell Scientific CR10 data logger were set up at Cairn Peak during January 1992 and January 1993 to measure the relative temperatures of the air, the soil surface, and moss patches (down to a depth of 1 cm). In addition, an automatic weather station at View Rocks (Fig. 1) recorded soil surface temperatures for the period of January 1993 to January 1994 to provide data covering all four seasons. All temperature data were collected every 10 min and averaged over 3-h periods.

Bacteria were enumerated by using the DNA stain 4',6-diamidino-2-phenylindole (43). Soil samples were diluted in 0.01 M tetrasodium PP_i and sonicated for 5 min at 45 to 50 Hz (13, 63), and subsamples were stained with 4',6-diamidino-2-phenylindole (5 μg/ml) for 20 min (54); filtered onto prestained (Irgalan Black) 0.2-μm-pore-size polycarbonate filters (Nuclepore); and counted at a magnification of ×1,000 with a mercury light source, a Nikon Neofluar 100/1.30 oil objective, and a Nikon DM400 filter block. Results were expressed as numbers of cells per gram (dry weight) (after 48 h at 60°C) of soil.

Organic matter content of soils was determined by weight loss of dried samples after 4 h at 450°C. The total nitrogen concentration was determined for soil samples by the Kjeldahl method (33, 58), and the total phosphorus concentration was determined by the method of Murphy and Riley (39).

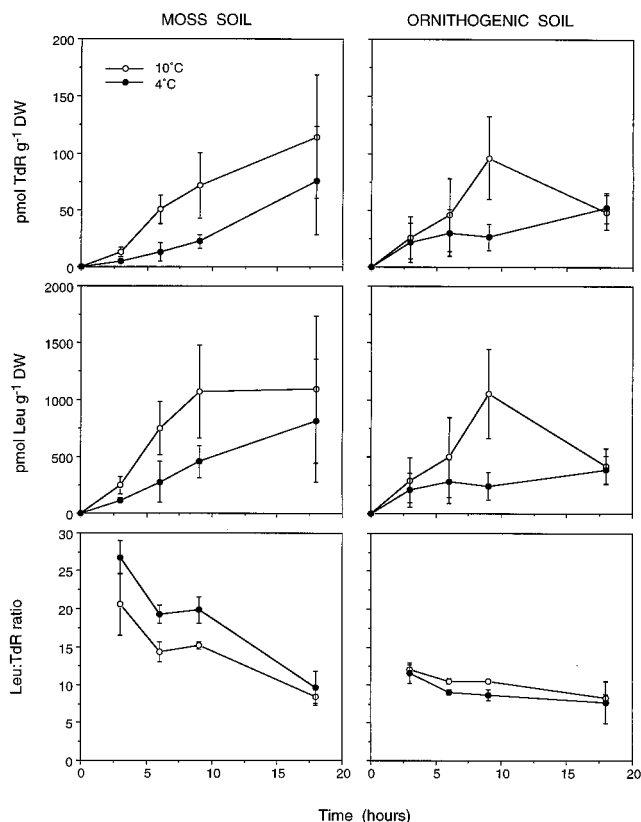


FIG. 2. Dual-label incorporation of TdR and leucine at two temperatures in two soil types (soil from under moss patches and exposed surface soil collected near bird nests) from Cairn Peak.

RESULTS

Microscopic examination of soil samples showed that in addition to heterotrophic bacteria, cyanobacteria and protozoa were also present. Cyanobacteria and protozoa do not incorporate label from [³H]TdR during short incubations, and they are thus unlikely to interfere with the measurement of heterotrophic bacterial activity (35). Time course analyses of incorporation of [³H]TdR and [¹⁴C]Leu by bacterial assemblages in ornithogenic and moss soils showed that TTI and leucine incorporation were generally linear with time over 3 to 9 h at 4 and 10°C (Fig. 2). The measured rates of incorporation of TdR and leucine in ornithogenic soil decreased over 9 to 18 h at 10°C. Rates of TTI and Leu incorporation were higher at 10°C than they were at 4°C, reflecting the results depicted in Fig. 4. Molar ratios of Leu incorporation to TdR incorporation tended to decrease with time for both soil types at both temperatures. Ratios were higher, and decreased more rapidly over time, for moss soil than for ornithogenic soil.

Bacterial productivity in moss and ornithogenic soils was estimated by TTI and [¹⁴C]Leu incorporation (Table 1). There was no significant difference ($P > 0.05$) in bacterial densities, or in production estimates, between moss and ornithogenic soils. High-level variability in densities and patchiness of distribution of microorganisms have been reported for continental Antarctica soils by other authors (5, 32). The organic and nitrogen contents of moss soils were higher (difference not significant [$P > 0.05$]) than those of ornithogenic soils (Table 2). Phosphate concentrations in ornithogenic soils were higher than those in moss soils (significant difference [$P < 0.05$]).

TABLE 1. Bacterial production in soils from two different habitats at two temperatures^a

Incubation temp	Soil habitat	Bacterial density (10 ¹⁰ cells g [DW] ⁻¹) ^b	Bacterial production (10 ⁶ cells g [DW] ⁻¹ h ⁻¹) as estimated with ^c :		Bacterial production (ng of C g [DW] ⁻¹ h ⁻¹) as estimated with ^c :		Production ratio ^d	Turnover time (days)	
			TdR	Leu	TdR	Leu		TdR	Leu
4°C	Moss	1.2 ± 0.5	3.0 ± 1.0	5.5 ± 1.8	60 ± 19	110 ± 37	0.6 ± 0.1	201 ± 145	107 ± 79
	Bird nests	1.4 ± 0.4	3.2 ± 1.0	9.7 ± 12.4	288 ± 400	195 ± 247	1.3 ± 0.3	182 ± 185	205 ± 212
10°C	Moss	1.2 ± 0.5	8.9 ± 4.7	11.7 ± 6.7	175 ± 90	230 ± 128	0.8 ± 0.4	87 ± 84	57 ± 49
	Bird nests	1.4 ± 0.4	17.4 ± 21.4	13.7 ± 15.9	344 ± 422	278 ± 323	1.2 ± 0.1	104 ± 103	116 ± 112

^a Values represent means ± standard deviations for three samples from each habitat. Each sample was assayed in duplicate.

^b DW, dry weight.

^c Values were calculated by using rates of substrate (TdR and Leu) incorporation over 3 h (Fig. 3). Factors used were as follows: 2.0 × 10¹⁸ cells mol of TdR⁻¹ (16), 1.42 × 10¹⁷ cells mol of Leu⁻¹ (7), and 20 fg of C cell⁻¹ (16).

^d Ratio of TdR-estimated production to Leu-estimated production.

Levels of bacterial production were ca. 2.5- and 1.3-fold higher at 10°C than at 4°C for moss and ornithogenic soils, respectively. TdR-derived estimates of bacterial production were similar to those derived by the leucine method (Table 1). The ratio of TdR-derived production estimates to Leu-derived production estimates was generally <1 for moss soils and >1 for ornithogenic soils, indicating that conversion factors were slightly different for the different soils. The reason for this is unclear, but possible differences in community structure may contribute to this effect. Calculated turnover times for bacterial populations in both soil types were long, ranging from 12 to 436 days at 4°C and from 11 to 236 days at 10°C; these calculations were based on total cell counts and may have underestimated specific growth rates for cases in which inactive cells formed a significant proportion of the total population.

Methodological studies also examined the extent of isotopic dilution of [³H]TdR during TTI (Fig. 3). Isotope dilution plots were biphasic for both moss and ornithogenic soils. When unlabelled TdR was added at concentrations of 1 to 2 nmol per sample, there appeared to be little or no isotopic dilution by nonexogenous sources of TdR precursor, indicating that routine isotope dilution analysis can be avoided if [³H]TdR is added at 1 nmol per sample (ca. 115 mg [dry weight]) as in the present study. Additions of TdR at higher concentrations (5 to 30 nmol per sample) apparently indicated that larger pools of precursors may be available.

The temperature response of TTI in Antarctic, ornithogenic soil was compared with that in temperate, fynbos soil (Fig. 4). TTI in Antarctic soil indicated a temperature optimum of around 15°C for bacterial production, whereas TTI in temperate soil continued to increase with increasing temperature over the range tested (0 to 30°C). Diurnal variations in temperature for air, the soil surface, and soil under moss patches were recorded during the summer of 1992 and the summer of 1993 (Fig. 5). Diurnal temperature fluctuations in exposed surface

soil were greater than those in soil from under moss patches and those in air. These data indicated that bacterial populations in exposed surface soil regularly underwent a daily freeze-thaw cycle during summer. Temperatures under moss patches were generally the most stable, seldom falling below 0°C, but they did not reach the high values (>20°C) recorded for soil. During January 1993, temperatures ranged from -9 to 5°C, from -5 to 24°C, and from -2 to 8°C for air, soil, and moss habitats, respectively.

DISCUSSION

A number of studies have investigated the density and viability of bacteria in mainland Antarctic habitats by culture (28, 45, 69) and direct counting techniques (5, 44, 45, 47). However, to our knowledge there have been no studies of heterotrophic bacterial production in continental Antarctic soils. Within these adverse environments, habitats which support enhanced biomass and rates of biological activity are interesting because they function as refugia, where environmental constraints on biological life are ameliorated to some extent. Studies in other Antarctic environments have shown that vegetated and ornithogenic soils support enhanced bacterial abundance and nutrient concentrations (3, 47). We examined the methodology of the TdR technique with respect to two soil types from nunatak

TABLE 2. Characteristics of different soils from Robertsollen^a

Soil type	Bacterial density (10 ¹⁰ cells g [DW] ⁻¹) ^b	Organic content (% of DW)	Nitrogen content (mg of N g [DW] ⁻¹)	Phosphate content (mg of P g [DW] ⁻¹)
Moss ^c	1.21 ± 0.49	6.47 ± 6.02	2.02 ± 2.93	0.008 ± 0.004
Bird ^d	1.68 ± 0.59	3.0 ± 4.6	3.8 ± 6.9	3.5 ± 3.56

^a Values are means ± standard deviations.

^b DW, dry weight.

^c Soil samples (*n* = 12) collected from beneath mosses.

^d Soil samples (*n* = 29) collected from locations 1 m away from bird nests.

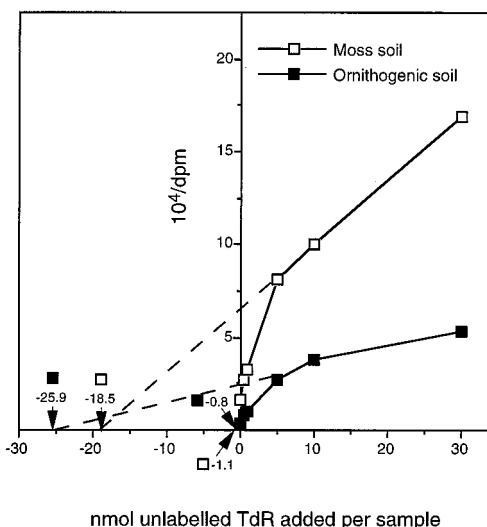


FIG. 3. Isotope dilution analysis of TTI in two Antarctic nunatak soils.

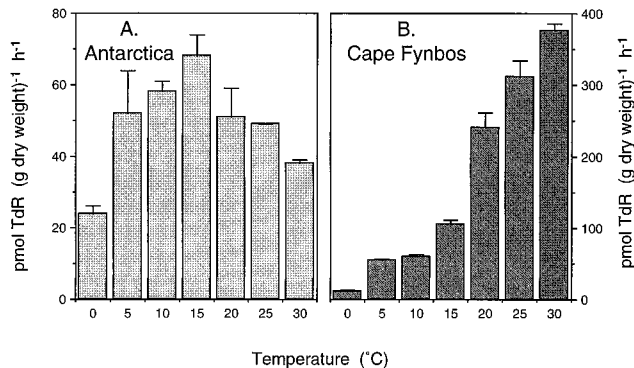


FIG. 4. Effect of temperature on TTI in soils. (A) Ornithogenic soil from Cairn Peak, Antarctica; (B) fynbos soil from Table Mountain, South Africa.

habitats, namely, soil from under moss beds and exposed surface soil from within 1 m of bird nests. These soils are influenced by different sources of nutrients; birds import nutrients from feeding grounds at sea, while mosses represent a source of nutrients from primary production. These soils also differ in terms of temperature stability, with the temperature of moss soils being far more stable than that of exposed soils during the summer (Fig. 5). However, moss and ornithogenic (i.e., exposed surface) soils were not significantly different ($P < 0.05$) in terms of nutrient concentrations (organic, phosphate, and total N contents), bacterial abundance, or production estimates. At Robertsollen, mean phosphate concentrations and levels of bacterial production were higher (by 440- and 1.4-fold, respectively) in ornithogenic soils than in moss soils. However, considerable variability and patchiness in these parameters (and other microbial fauna [18a]) were noted, and these made statistical analyses difficult for the available sample sizes. Previous studies have indicated that N, P, K, Cu, Fe, Al, and B contents of ornithogenic soils are significantly higher than those of nonornithogenic soils at Robertsollen (52). In the present study, protocol requirements for TTI assays (e.g., incubation duration and isotope dilution) were similar for ornithogenic and moss soils.

This study shows that TTI is an appropriate method for the measurement of bacterial production in Antarctica soils that are exposed to favorable moisture conditions. The TdR technique is convenient and relatively easy to perform, an important consideration for field studies in a harsh environment. It is also extremely sensitive, enabling the detection and measurement of the low rates characteristic of Antarctic habitats. This technique does have a drawback in that samples are incubated under aqueous conditions. It is generally accepted that an aqueous microenvironment facilitates bacterial activity and nutrient acquisition, and therefore this procedure would tend to overestimate rates of bacterial production for cases in which stress due to low-level water activity might otherwise constrain growth. However, in some Antarctic habitats, such as those at Robertsollen, frequent cover of soils by drift snow and subsequent melting coupled with daily freeze-thaw cycles during the summer result in regular exposure of soil microorganisms to free water. Moreover, soils on these nunataks often appear moist when thawed. Hence, we consider that the conditions of these assays do not represent extraordinary circumstances for soil assemblages at Robertsollen during the summer. Comparisons of potential maximum rates (with regard to control by water activity) between habitats is not a problem under these conditions. However, we have emphasized caution in the in-

terpretation of these data in terms of the variable condition of the environment and the need to extend the data for water profiles of these soils. We have also recognized that the volumes of water added to soils during TTI assays should be minimal to avoid excessive dilution of ambient nutrient concentrations.

The TTI technique requires that a number of assumptions be met if accurate estimates of bacterial production are to be obtained. It is also important that the method be standardized for Antarctic habitats so that valid comparisons between different studies, locations, and habitats are possible. Studies of dual-label incorporation of [^3H]TdR and [^{14}C]Leu in ornithogenic and moss soils showed that the Leu/TdR ratio decreased over time during incubations longer than 3 h. Possible explanations for the decrease of the Leu/TdR ratio over time include the following: (i) TTI does not reflect balanced growth over extended incubations (6 to 18 hours) in which unbalanced growth may be due to changing environmental conditions (8); (ii) a proportion of cells incorporating TdR were unable to incorporate leucine; and (iii) cells able to incorporate leucine but not TdR were gradually inactivated during extended incubations. It is not possible to determine the precise reasons for this effect from the present data. However, these results indicate that TTI assays of bacterial production in Antarctic soils should be restricted to incubations of less than 3 h, since TTI was apparently not linked to bacterial production during extended incubations. Similar results were obtained by Tibbles et al. (60), who also noted a decline in the ratio of molar Leu

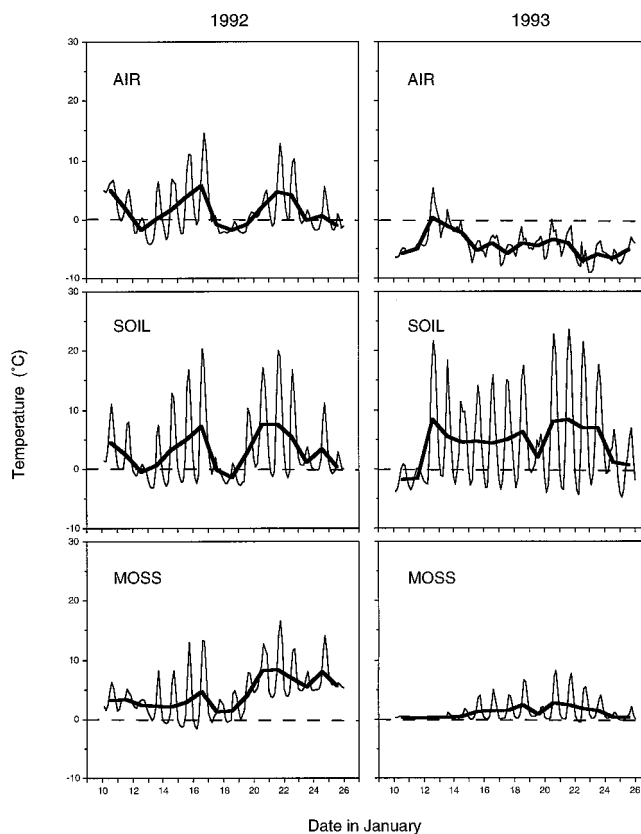


FIG. 5. Diel variations in temperature for air, exposed surface soil, and soil under moss patches during the summer at Cairn Peak, Robertsollen. Recorded temperatures, collected every 10 min and averaged over 3-h periods, are represented by the light lines, whereas the calculated mean daily temperatures are represented by the bold lines.

incorporation to molar TdR incorporation over extended incubations in temperate salt marsh sediments. In the present work, we obtained measurable uptake of radiolabel in incubations as short as 1 h, even when temperatures ranged from 0 to 15°C, and therefore we recommend that similar periods be used for TTI assays of Antarctic soil habitats.

Empirically derived conversion factors for the translation of uptake rates into bacterial productivity are not available for assemblages of Antarctica soils; the empirical determination of conversion factors for sediments or soils is extremely problematic. We therefore selected conversion factors that approximated theoretical values and were commonly used in the literature; this approach also facilitated the comparison of our data with those from studies of other systems (see below). DLI enabled verification of production estimates by measuring two essentially independent biochemical processes in the same sample. Leucine- and TdR-derived estimates differed by only 0.48- to 1.57-fold, supporting our confidence in the accuracy of the values derived by these methods and indicating that at least the ratio of the conversion factors used in these studies was correct. Further methodology indicated that isotope dilution could be limited by incubation of samples (ca. 115 mg [dry weight]) with 1 nmol of [³H]TdR. It is also recommended that the leucine method (preferably using tritiated leucine and TdR in single-label experiments [18b]) be used to validate TTI-derived estimates. We have successfully tested these methods in the field, and we obtained results which were comparable to results presented here for laboratory-based studies; TTI was not significantly different between subsamples of the same sample when subsamples were assayed first in situ and then in the laboratory after 2 months of storage at -20°C ($P < 0.05$ and $F = 1.695$ by analysis of variance [18b]). These results strengthen our confidence that data collected in the laboratory under the conditions of these methods reflect potential in situ rates.

Bacteria may be physiologically categorized according to their growth responses to temperature, and one might expect the bacterial microflora of cold, extreme environments, such as Antarctica, to be characterized by physiologies that are specifically adapted to constant low temperatures (38). Psychrotrophs are more common than psychrophiles among isolates from different habitats on and around Signy Island (19, 62) and from the coast of Terre Adélie, Antarctica (11), whereas relatively higher proportions of psychrophiles were reported in an offshore study (68). Thus, it seems that psychrotrophs dominate in Antarctic soils, freshwater, and inshore marine environments, whereas psychrophiles may dominate in offshore waters, where temperatures remain at ca. -1.8 to 2°C. However, the bias of isolation and culture techniques is now widely recognized in microbial ecology, as such techniques rarely recover more than 1% of the total bacterial population.

We examined the growth response (TTI) of in situ bacterial assemblages to temperature. The responses of TTI to temperature are reflected in the Q_{10} values for soils from Antarctic nunataks and temperate fynbos soil: an increase in temperature from 0 to 5°C had an effect on TTI in fynbos assemblages ($Q_{10} = 21.0$) greater than that which it had on TTI in Antarctic assemblages ($Q_{10} = 4.69$), whereas an increase from 5 to 10°C stimulated TTI in Antarctic ($Q_{10} = 1.24$) and fynbos ($Q_{10} = 1.15$) soils to extents similar to each other but less than those observed over the range from 0 to 5°C. Over the range from 0 to 10°C, Q_{10} values for Antarctic and fynbos soils were 2.41 and 4.91, respectively. Vincent and Howard-Williams (66) reported Q_{10} values for TTI in Antarctic freshwater streams similar to those reported here for nunatak soils. The psychrotrophic bacterial activity of Antarctic assemblages (Fig. 4) compares with

TABLE 3. Comparison of bacterial production characteristics in different sedimentary habitats

Habitat	Bacterial production (10 ⁶ cells g [DW] ⁻¹ h ⁻¹) ^a	Turnover time (days)	Temp (°C)	Reference
Nunatak soil, Antarctica	3.5–42.0	11–215	10	This study
Microbial mat, Antarctic stream	0.9–1.7 ^b	NA ^c	10	66
Subsurface sediment (lake), United States	1.1	14.7	16	54
Freshwater stream bed, United States	107.0	0.9	20	21
Seagrass bed, Australia	370.0	0.2	31	31
Mangrove sediment, Australia	5,000.0	0.2	31	1

^a Production estimates derived by TTI. DW, dry weight.

^b Units are 10⁶ cells cm⁻² h⁻¹ for mats dominated by *Phormidium*, *Nostoc*, and *Prasiola* species.

^c NA, not available.

in situ temperatures which reached relatively high levels (ca. 25°C) during summer days at Robertsollen (Fig. 5). Such temperatures are likely to be unfavorable for psychrophiles. Previous studies indicated that psychrotrophs also prevailed over psychrophiles in the Ross Desert, for apparently similar reasons (69). Moreover, acetate uptake, glucose catabolism, and TdR incorporation exhibited positive responses to temperature in freshwater stream assemblages from South Victoria Land (66).

Soil assemblages at Robertsollen experienced a regular freeze-thaw process during diurnal temperature fluctuations of the summer growth season, with an average daily fluctuation of ca. 17°C. The precise nature of the impact of freeze-thaw on in situ bacterial production is not clear. However, in this regard it was noted that assemblages exhibited TTI within 15 min after thawing (from -20°C to 4 and 10°C) and maintained linear uptake for 3 to 9 h thereafter. These findings indicate that these Antarctic soil bacteria cope well with radical temperature changes to maximize their use of optimal growth temperatures. The optimum temperature for production of soil bacteria from Robertsollen was about 15°C, indicating that psychrotolerant bacteria are likely to play the dominant microbial role in ecosystem flux of carbon and energy. Levels of bacterial productivity in nunatak soils at 10°C (near to the temperature optimum and close to the mean diurnal temperature during January) were generally lower and population turnover times were much longer relative to values reported for other sedimentary habitats in less extreme environments (Table 3). Although heterotrophic bacterial metabolism at temperatures below 0°C has been reported (31), the data of Fig. 4A suggest that the rate of production of Robertsollen assemblages at subzero temperatures is negligible relative to that at temperatures above 0°C. Therefore, the comparisons in Table 3 are appreciably reinforced by the consideration that subzero temperatures prevail for ca. 84% of the year in nunatak soils at Robertsollen. Moreover, the extremely low temperatures which characterize the Antarctic continent limit most bacterial production to a short period during the summer growth season, when relatively warmer temperatures prevail, emphasizing the slow growth and fragility of Antarctic microbial communities.

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