

The Role of Bacterial-based Protist Communities in Aquatic and Soil Ecosystems and the Carbon Biogeochemical Cycle, with Emphasis on Naked Amoebae

O. Roger ANDERSON

Biology Lamont-Doherty Earth Observatory of Columbia University, Palisades, New York, U.S.A.

Abstract. Current research is reviewed on aquatic and soil microbial ecology with attention to the fate of organic carbon in bacterial-based protist food webs, including some new data. Particular attention is given to the effects of pulsed sources of low-molecular weight organic sources of carbon on soil respiration, changes in bacterial, nanoflagellate, and naked amoeba C-biomass, and evidence for throughput of carbon in microbial food webs in Arctic and some low-latitude, temperate soil environments. The proportion of pulsed sources of glucose-C that is sequestered in microbial biomass relative to loss as CO₂ is examined in laboratory experimental studies, and implications of the research for microbial community dynamics and global warming due to terrestrial sources of respiratory CO₂ are discussed.

Key words: Global warming, microbial ecology, microbial respiration rates, microbial trophodynamics, respiratory CO₂, soil respiration, terrestrial carbon flow.

INTRODUCTION

This is a review of some recent findings of the role of microbial communities, including especially amoeboid protists, in nutrient-derived carbon flow and its fate in aquatic and particularly soil environments, with a focus on the functional ecology of carbon budgets and the biogeochemical carbon cycle. Naked amoebae refer to amoeboid protists without tests, walls, tecta or porous outer shells, but may have a surface coat or

glycocalyx, sometimes composed of scales. The term naked or sometimes non-testate amoebae has been used previously in prior publications (e.g. Patterson 1984, Page 1987) to distinguish them from testate amoebae enclosed in an external organic or mineralized test, and this terminology is adopted here. For simplicity, the terms “amoeba” or “amoebae” will be used in this review to refer to naked amoebae, unless the full terminology is required for clarity.

The role of amoebae in soils (especially agricultural soils) has received considerably more attention than their role in aquatic ecosystems, probably because of the importance of understanding the contributions of microbial communities to soil fertility and productivity. This paper largely focuses on the ecological role of

Address for correspondence: O. R. Anderson, Biology Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964, U.S.A.; Tel.: 845 365-8452; Fax: 845 365-8150; E-mail: ora@LDEO.columbia.edu

naked amoebae in soil ecosystems, especially natural soil sites, including some new relevant data. However, attention is given to their role in aquatic habitats first to highlight some of the unique aspects of the solid, particulate soil environment relative to the more continuous aquatic milieu. It has been known for some time that species of amoebae isolated from soils are also present in freshwater environments (e.g. Page 1988). This is attributed partially to the similarity of the properties of soil water and freshwater systems, but also due to the transportability of encysted stages of amoebae that can be carried by wind and other means between terrestrial and aquatic environments. Contiguity of soil with margins of freshwater habitats may also contribute to transfer of cysts and trophic stages of amoebae between the two. To some extent, amphibians and other animals that inhabit freshwater and terrestrial environments also may carry amoebae and other microbes between the two locales.

AQUATIC ENVIRONMENTS

Aquatic environments compared to most soils provide an ample and sustained source of water to support eukaryotic microbial growth, except during periods of drying as in temporary bodies of water. The continuity of the fluid environment also favors more rapid diffusion of dissolved gases and nutrients compared to the more compartmentalized, particulate environment of soils. However, amoebae require surface attachment to feed. Soil particles, when moisture is sufficient, are particularly suitable, providing a relatively large surface area for support of bacteria, microflagellates, and other prey where the amoebae can attach and engulf the food particles. In aquatic environments, sufficient densities of suspended appropriately size particles are essential for amoeba growth and establishment of sizable populations. For example, in a highly productive freshwater pond in northeastern U.S.A., with ample suspended mineral and organic particulates, the amoebae can account for more carbon biomass than ciliates on some occasions, especially in early spring and autumn; but ciliate biomass was overall higher in mid summer (Anderson 2007). Similarly in the Hudson estuary, throughout the spring season, amoeba carbon biomass was substantially higher than ciliates (5- to 6-fold), and overall on average was higher (c. 3-fold) than ciliates throughout the sampling interval April to October (Anderson

2007). In both locales, when the temperature was more moderate, amoebae were typically higher in abundance than the ciliates. Other studies, however, in rivers (e.g. Kiss *et al.* 2009) have shown that amoebae biovolume may be an order of magnitude lower than ciliates. The apparent discrepancies between the abundance data of Anderson (2007) and Kiss *et al.* (2009) may be attributed to differences in available sources of prey as well as the availability of suitable particulates for attachment of amoebae in these two contrasting aquatic environments. In addition to the density of suspended particulates, the particle size is directly related to the size and hence biomass (carbon content) of amoebae, at least as assessed in the Hudson Estuary (Anderson 2011). Some rivers may be less turbid with appropriate-sized particles to support amoeba communities compared to an eutrophic pond or coastal water mass. For example, in the Hudson estuary, the mean \pm s.e. C content ($\mu\text{g/l}$ of particles) for amoebae dwelling on larger suspended particles ($> 200 \mu\text{m}$) was 0.34 ± 0.07 , but for smaller particles ($< 200 \mu\text{m}$) was 0.06 ± 0.02 . The densities of amoebae (number/l of particles), however, were not substantially different on the two different sized particles; the main difference was the larger size of individuals on larger particles. In general, the preceding data indicate the importance of suspended particle densities and size on aquatic amoeba diversity and C-biomass.

Prior research on aquatic amoebae in lakes and rivers includes only occasional reference to the presence and/or abundance of suspended particulates, but sometimes indicate that suspended particles are significant sites for colonization by amoebae. For example, Arndt (1993), reviewing the literature on lake plankton, noted that although amoeboid protists (sarcodines) seem to contribute only a minor contribution (1–15%) of annual mean protozooplankton biomass, they were sporadically an important constituent of plankton communities. Naked amoebae appeared to be frequently associated to particles and aggregates, and Arndt suggested that amoebae may be important functional components of lake snow. Studies of diverse water masses at widely different geographic locales provide further evidence of particle-associated protist communities containing naked amoebae, including Antarctic lakes (e.g. Laybourn-Parry *et al.* 1991, 1992), Salton Sea, California (e.g. Rogerson and Hauer 2002), Lake Constance during peak phytoplankton blooms (Weisse and Mueller 1998), Clyde estuary, Scotland (Rogerson and Laybourn-Parry 1992); coastal marine sites (Rogerson *et al.* 2003), and

suspended detritus in small ponds (e.g. Kyle and Noblet 1986). However, no information is often provided on the size and density of suspended particles. Future research on planktonic amoeba ecology, especially related to carbon biomass, should include more attention to the kind and density of suspended particles in the water column (Anderson 2011). This is especially true if nutrient concentrations, temperature, seasonal variables, and other significant ecological variables are to be differentiated from edaphic factors (such as the suspended particle density) that affect amoeba productivity.

Few studies have examined the role of naked amoebae, among other protists, in carbon budgets or the carbon cycle in aquatic and soil environments. However, some have investigated the role of amoebae during predation pressure on, and consumption of, bacteria. Rogerson *et al.* (1996) estimated the mean bacterial ingestion rate of surface-dwelling marine amoebae based on amoeba biovolume and derived a regression equation relating the two variables. Among other trophic relationships, they reported that the smallest amoeba (estimated volume of $57.3 \mu\text{m}^3$) consumed 10 bacterial cells/h; while the largest cell examined, *Trichosphaerium sieboldi* (estimated volume of $61,021 \mu\text{m}^3$), consumed approximately 1,465 bacteria/h. Despite the wide range of morphotypes examined, amoeba consumption was well described by the relationship, $\log C = 0.124 + 0.636 \log V$; where C = number of bacteria consumed/h and V = amoeba cell volume (μm^3). Butler and Rogerson (1997) published the first estimate of grazing rates of marine amoebae on bacteria using samples from the Clyde Sea area. The number of fluorescently labeled bacteria consumed was determined at 5, 10, 15 and 20°C . Consumption rates varied markedly, ranging from 0.2 to 194.7 bacteria/h (equivalent to 0.16–155.8 μm^3 bacterial biovolume). Not all of these values were considered to be optimal; several of the uptake values were too low to account for the measured growth rates of amoebae. Based on optimal consumption rate data, the mean specific rates increased from 0.042 bacteria/h/ μm^3 at 5°C to 0.131 bacteria/h/ μm^3 at 20°C . Thus, for the smallest amoebae ($45 \mu\text{m}^3$) the consumption rate was ~ 6 bacteria/h, and for the largest amoebae ($3,258 \mu\text{m}^3$), ~ 430 /h at 20°C . Other studies have documented amoeba feeding rates and growth at temperatures below 5°C for some Antarctic species (e.g. Mayes *et al.* 1997). A review of amoeba bacterial consumption rates (bacteria/h) in biofilms including data for 17 different amoeba species or isolates, as well as other protists, has

been published by Parry (2004). More recently, Lessen *et al.* (2010) analyzed the bacterial carbon consumption rate of amoebae from the Hudson estuary using several approaches simultaneously including serial dilution culture experiments. They reported an ingestion rate between 1.2 to ~ 2.4 ng bacterial C/d/ng amoeba C.

Lack of good estimates of amoeba respiration rates, as well as those of other protists (especially under conditions more typical of the natural environment), hampers making a more comprehensive analysis of their role in carbon cycling. Although we are making progress in reliably estimating carbon uptake during predation, a complete analysis of the fate of the ingested carbon must include the amount respired. This is particularly important given the growing emphasis on understanding the various sources of atmospheric CO_2 contributed by biota under varying environmental conditions. Some estimates of amoeba respiration rates in aqueous cultures during laboratory experiments (e.g. Baldock *et al.* 1982, Crawford *et al.* 1994, Laybourn-Parry *et al.* 1980) have provided good benchmarks for likely estimates of amoeba respiration in relation to biovolume, including more comprehensive estimates based on regression equations for amoebae and other protists (Fenchel and Finlay 1983).

SOIL ENVIRONMENTS

The role of amoebae and other protists in soil environments has received substantial attention, including treatises on soil decomposition and nutrient recycling (e.g. Adl 2003, Adl and Gupta 2006), abundance, diversity and ecology in a variety of soil environments and physiological ecology roles (Darbyshire 1994), and significance in the fertility of soil. Some of these latter studies, beginning well before the twentieth century, are nicely summarized in a critical review Sherman (1916), including additional experimental evidence. More recent publications present evidence, among other topics, about the role of protists in soil fertility at varied geographic locales, including arid soils (e.g. Robinson *et al.* 2002), temperate forest soils (e.g. Clarholm 2002), tropical soils (Swift 1996), and agroecosystems (Swift and Anderson 1993). Increasing interest is developing on examining the flow of carbon and its respiratory loss in protist communities in soil ecosystems, based on experimental and field-based studies (e.g. Fitter *et al.* 2005, Griffiths 2001) and laboratory simulations

(e.g. Rutherford and Juma 1992). Fitter *et al.* (2005) summarized the key findings of the UK NERC Soil Biodiversity Programme, including significance of the carbon dynamics: 1) small organisms were extremely diversified, i.e. over 100 species of bacteria, 350 protozoa, 140 nematodes and 24 distinct types of arbuscular mycorrhizal fungi were identified, 2) stable isotope (^{13}C) analyses indicated a rapid movement of carbon through the food web, and 3) the soil system was highly resistant to perturbations, probably due to the combination of taxonomic diversity, rapid carbon flux, and the redundant roles of the diverse biota.

Abundance and diversity of soil amoebae

Prior research on the abundance and diversity of amoebae in terrestrial environments has laid a strong foundation for more recent studies of the functional ecology of amoebae and protist communities in the carbon budget and carbon biogeochemical cycle. Documentation of amoeba abundance during a four-year study at a grassy site in Northeastern U.S.A. (Anderson 2000) showed that overall abundances tended to peak reaching densities greater than 2,000/g in early spring (March and April) and again with a more modest peak in late summer (August), then generally increased sporadically toward late autumn into early December, with densities greater than 1,500 to slightly less than 2,000/g. There was a significant correlation ($r = 0.36$, $N = 43$, $p < 0.02$) between monthly precipitation and total amoeba density (number/g soil), but no significant correlation between the soil moisture content and density at the time of sampling ($r = 0.15$, $N = 43$, $p = 0.34$). This can be explained by the sporadic nature of precipitation and soil moisture at a given sampling day, each month. The monthly precipitation is more likely to represent the modal moisture content of the soil, than a particular value on a given day. However, the moisture content of the soil is a good indicator of the percent active (non-encysted) amoebae based on regression analyses: $P = 2.84 M - 5.59$ ($r^2 = 0.95$); where P = percent active amoebae and M is the weight-based percent water content of the soil (Anderson 2000).

A laboratory experimental and field-based study of the role of soil porosity (granularity) and organic content (Anderson 2002) showed that mean abundance and diversity of amoebae were consistently higher in organically enriched soil and in soil of increasing granularity, both in laboratory culture microcosms and in field samples varying in granularity. Triplicate soil samples, obtained with a 1 ml syringe that was cut open at the

end to form a micro-corer, showed substantial differences in abundances in samples taken at distances of several millimeters in the same laboratory microcosm. Field samples, collected directly from two of the sites, showed similar patterns of spatial abundance and diversity as found in the experimental studies, indicating substantial small-scale compartmentalization of soil protist communities. These data provided evidence of soil eukaryotic microbiocoenoses and indicated that soil microfauna may encounter wide variations in resources and prey communities as they migrate within small distances of several millimeters or less.

Relatively less is known about the carbon content of naked amoebae in soil of varying composition, nutrient concentrations and in relation to changing patterns of precipitation. This can be attributed partially to prior limitations in estimating the carbon content of individual amoebae based on biovolume. More recently, biovolume-based regression estimates of amoeba carbon content (e.g. Anderson 2006, Pelegri *et al.* 1999) have provided an efficient means of assessing amoeba C-biomass in experimental and field-based studies. Some current studies on the role of amoebae and microbial communities in carbon budgets and the biogeochemical carbon cycle in terrestrial habitats, including some new data from recent research, are reviewed.

Soil moisture studies and carbon dynamics

Based on prior evidence that soil moisture significantly affects the percentage of amoebae that are active (Anderson 2000), the effects of soil moisture on microbial biomass and respiratory loss of carbon as CO_2 in microbial communities, including amoebae, is a topic that has been addressed in laboratory microcosm studies when dry soil is rewetted (e.g. Anderson 2011). Bacteria, heterotrophic nanoflagellates (hereafter referred to simply as flagellates), and amoebae densities were monitored in relation to total soil respiratory CO_2 efflux using laboratory cultures of soil obtained at three temperate, Northeastern U.S.A. forest sites (Torrey Cliff, NY), i.e. a subalpine elevated berm planted with mountain laurel and red cedar, broad leaf forest, and a white pine stand. Organic content of the three soil samples expressed as percent (w/w) was as follows: subalpine elevated berm (15%), broad leaf forest (13%), and white pine stand (6%). Illustrative data for the three sites are presented. Dried soil samples were moistened to near field capacity with 0.45 pore-size filtered water and analyzed at 24 and 72 hours post wetting to monitor effects on respiration and on the abundance and car-

bon biomass of bacteria, heterotrophic flagellates and amoebae. Wetting of the soil produced a pronounced peak in respiration ($\text{nmol CO}_2/\text{min/g soil}$) during the first 24 h, followed by a decline at 72 h, as follows (24 h and 72 h): berm site (9.0 ± 0.7 and 3.8 ± 0.6), forest (10.7 ± 0.06 and 5.5 ± 0.7), and pine stand (3.0 ± 0.1 and 1.2 ± 0.05). Bacterial densities (number/g) and C-biomass decreased for all sampling sites at 72 hours compared to 24 hours; while densities of naked amoebae consistently increased (by as much as five-fold) at 72 hours for each of the three sampling sites. The heterotrophic flagellate densities and C-biomass varied, sometimes increasing marginally (e.g. berm and forest soil samples) or decreasing marginally (pine stand). Naked amoebae are known to prey on flagellates (Anderson 1994, Bovee 1985) and some of the decline in heterotrophic nanoflagellates densities may be attributed to predation by amoebae or other microbiota.

With respect to partitioning of carbon resources, the experimental results indicate that, commensurate with pulsed precipitation events, there is a shift in the carbon fractions from a large respiratory loss associated with the initial peak in the CO_2 flux, toward a more distributed component in eukaryotic microbial particulate fractions. With increasing interest in possible sources of terrestrial respiratory CO_2 in the atmosphere, and its potential for enhancing the greenhouse effect driving global warming, the importance of accounting for the partitioning of carbon in soil microbial communities (especially estimates of the balance between particle sequestration within biota versus loss as CO_2 to the atmosphere) is becoming of greater significance. The current results point toward a significant effect of protistan predation on bacteria, resulting in increased biotic particle-bound carbon resources. Simultaneously, this may account for the diminished respiratory CO_2 efflux, particularly during early phases after a pulsed rewetting of soil.

The carbon budgets of soil in high and low latitudes

High latitude studies. With increasing evidence of global warming and climate change, particular attention has been given to Arctic ecosystems, where some of the most dramatic changes are happening in recent decades, including increased melting of the permafrost each year and consequent subsidence of land, both inland and near the ocean margins. The latter often results in loss of seaside buildings that slide into the sea. Furthermore, there is increasing evidence of major changes in terrestrial habitats, including evidence that regions

previously net carbon sinks (due to primary production by moss and tundra vegetation) may become net CO_2 sources as warming favors soil microbial growth and increased respiratory release of atmospheric CO_2 (e.g. Anderson 2010a, Billings *et al.* 1982, Loya and Grogan 2004). Given that tundra and boreal ecosystems occupy millions of square kilometers globally at high latitudes, the contribution of soil microbial respiration to atmospheric CO_2 during permafrost melting and increased soil temperatures can be substantial.

In the tundra, where moss-rich surface soil is thawed and sufficiently moist to support microbial activity, CO_2 efflux is higher for mesic sites compared to wet sites where water-logging and anaerobic conditions can suppress aerobic respiration (e.g. Anderson 2010b, Illeris *et al.* 2004, Oberbauer *et al.* 1991). Illeris *et al.* (2004), studied subarctic heath soil and reported that optimum moisture content for CO_2 efflux was in the moderate range of 240% soil dry weight, consistent with a range between 200 and 500% reported by Heal *et al.* (1981). Laboratory measurements of tundra soil respiration from a mesic upslope location compared to a wetter downslope location (Anderson 2010b) also supported the conclusion that respiratory efflux (nmol/min/cm^3) was greater at the mesic site relative to the wetter site when measured at two different temperatures of 15°C (9.1 ± 0.6 vs. 4.1 ± 0.7) and 25°C (21.4 ± 0.2 vs. 7.8 ± 0.5). The Q_{10} also varied between the two sites: mesic site (2.4) and wet site (1.7).

The flow and fate of organic nutrients in microbial communities, including naked amoebae, at high latitudes may gain increasing attention if global warming continues to produce longer summer months of permafrost melting, and elevated microbial metabolism. Increasing warmth, and additional niches opened up to vascular plants as well as increased growth of moss, potentially increases likely pulsed inputs of low molecular weight, soluble organic compounds into the soil; some of it leaching from moss during precipitation (e.g. Wilson and Coxson 1999), and more substantially from root exudates of vascular plants, especially if the warming is accompanied by increased atmospheric CO_2 (e.g. Anderson and Griffin 2001, Leake *et al.* 2006). To examine the effects of pulsed release of low-molecular weight organic compounds on the carbon budgets of tundra soil microbial communities, laboratory experimental studies were done to determine the effects of a pulsed application of glucose solution in samples of tundra moss-rich soil on the fate of the added car-

bon in the microbial communities (bacteria, heterotrophic nanoflagellates and naked amoebae), including the amount lost through respiration (Anderson 2012). The basic protocol is outlined in Fig. 1.

Soil was obtained from three sampling sites in Arctic tundra, two in early spring during late May (one with sparse surface moss and one with more substantial moss) and one in summer during July. Samples of each

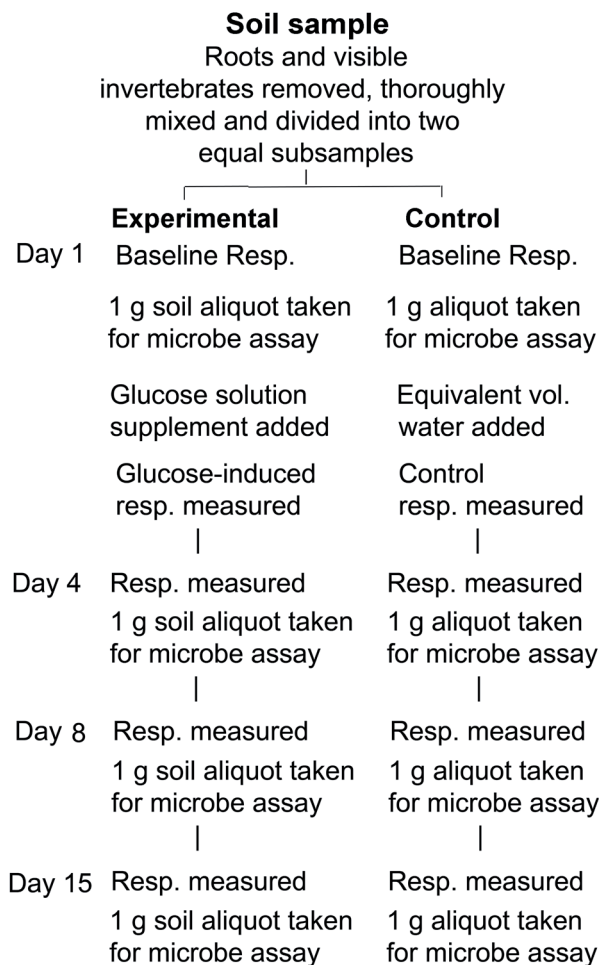


Fig. 1. Protocol for respiration and microbial biomass experiments. Respiration was measured with an infrared CO₂ gas analyzer, glucose (444 µg/g soil) in solution was added as a supplement. Microbial assays for naked amoebae used a culture observation method (COM) for living amoebae routinely employed in our laboratory (e.g. Anderson 2000), glutaraldehyde-fixed portions of the soil sample were examined microscopically for bacteria and heterotrophic nanoflagellates using a fluorescent staining technique (Anderson *et al.* 2001). For experiments of one-week duration, sampling was done on Days 1, 4, and 8. For two-week duration experiments, sampling was done on Days 1, 8, and 15.

were prepared according to the basic protocol using the seven-day plan for data collection and analysis (i.e. Days 1, 4, and 8). In addition to the respiration measurements at 20°C, similar to summer daytime temperatures in the Arctic, subsamples obtained at each sampling day were used to assess bacterial, heterotrophic nanoflagellate and naked amoeba abundances and estimates of their C-content. a summary of the results (glucose treatments and controls without glucose) is presented, combining data from all three samples. Respiration measurements are presented in Table 1, and microbial densities (number/g) and C-biomass (µg/g) are presented in Table 2. The glucose supplement on Day 1 (Day 1+) produced an immediate spike in respiration more than twice the respiration rate in the control without glucose (12.9 and 5.2 nmol/min/g, respectively). However, subsequently, the rate decreased to near baseline during the remainder of the week (Days 4 and 8, combined data). During the measurement of the respiration spike in the glucose-treated sample on Day 1, further measurements were taken at hourly intervals, post-supplementation. Respiration rate declined approximately linearly, reaching baseline levels within 4 to 5 hours. The linear regression equation relating decline in respiration rate (R_s) to time in hours (h), for a spring sample was $R_s = -2.6 h + 22.8$ ($r = -0.98$, $p < 0.001$), and for the summer sample $R_s = -4.1 h + 20.3$ ($r = -0.97$, $p < 0.005$). The larger negative slope for the summer sample may reflect a higher metabolic activity of the microbes. The total amount of CO₂ respired during 4 hours after glucose supplementation, calculated from the rate of decline over four hours, was: 5.1 nmol/g soil for a spring soil sample and 8.1 nmol/g for the summer sample. The amount of C respired expressed as equivalent weight for the two respective samples was: 1.4 ng C/g and 2.2 ng C/g. Overall, the amount of C respired

Table 1. Grand means ± s.e. respiration data (nmol/min/g) for treatment and control preparations during an eight-day experiment from three Arctic sites.

Day ^a	Treatment	Control
1	5.6 ± 0.8	5.2 ± 0.5
1(+)	12.9 ± 1.6*	5.2 ± 0.7
4 & 8	4.7 ± 0.4	4.3 ± 0.3

^a A (+) sign after Day 1 indicates 2 ml of 0.05% (w/v) glucose solution added. Controls received 2 ml of filtered distilled water. Asterisk indicates a statistically significant difference ($p < 0.05$).

Table 2. Statistical analyses of microbiota means \pm s.e. for densities (number/g) and carbon content for bacteria, flagellates, and naked amoebae, on Day 1 and Days 4 and 8, combining data from three Arctic tundra site experiments.

	Treatment		Control	
	Densities	Carbon	Densities	Carbon
<u>Bacteria</u>	($\times 10^8$)	($\mu\text{g/g}$)	($\times 10^8$)	($\mu\text{g/g}$)
Day 1	2.1 \pm 0.8	27.0 \pm 10.6	2.0 \pm 0.8	26.5 \pm 10.2
Days 4 & 8 ^a	5.2 \pm 1.2	67.3 \pm 15.2	3.3 \pm 0.6	42.8 \pm 7.8
<u>Flagellates</u>	($\times 10^5$)	($\mu\text{g/g}$)	($\times 10^5$)	($\mu\text{g/g}$)
Day 1	4.3 \pm 1.5	4.1 \pm 1.4	4.7 \pm 1.7	4.4 \pm 1.6
Days 4 & 8 ^a	7.4 \pm 1.7	6.9 \pm 1.6	4.3 \pm 0.9	4.0 \pm 0.8
<u>Naked Amoebae</u>	($\times 10^3$)	(ng/g)	($\times 10^3$)	(ng/g)
Day 1	4.2 \pm 2.6	127.4 \pm 60.2	3.5 \pm 1.5	123.5 \pm 49.1
Days 4 & 8 ^a	10.0 \pm 2.5	446.6 \pm 110.2	7.3 \pm 2.1	214.5 \pm 43.5

^a All mean data for the treatment group compared to the control group at Days 4 & 8 are significant at $p < 0.05$. Adapted from Anderson (2012).

during the 4-hour spike represents less than 1% (w/w) of the amount of glucose-C added in the supplement.

This raises the important question of how much of the remaining added glucose-C was sequestered in the microbial fractions. The data in Table 2 (Days 4 and 8 combined) indicate that a substantial amount of the glucose carbon is sequestered in the microbial fractions (comparing treatment mean data to control data). The difference in C gain (treatment – control) was 24.5 $\mu\text{g/g}$ for bacteria; 2.9 $\mu\text{g/g}$ for flagellates, and 232 ng/g for amoebae. It is important to note, however, that the increase in densities for amoebae subsequent to Day 1 glucose supplementation was substantially greater ($\sim 2.4 \times$ the initial amount) compared to flagellates ($1.7 \times$). This also is reflected in the relative increases in C-biomass. Control sample changes were less marked. Overall, the amount of glucose-C sequestered in the total microbial fraction for the three samples compared to the amount added in the supplement ranged from only 5% in the spring sample to 39% in the summer sample, indicating that a significant amount of the added carbon can become sequestered in the microbial biomass compared to the amount lost by respiration, and more likely increases as seasonal warming progresses. The remainder of the added C, not accounted for in the microbial fractions, may be sequestered in higher trophic levels; and some undoubtedly is bound to soil particles, and not immediately available for assimilation (e.g. Park *et*

al. 2007). More detailed data, for each sampling site, are presented in Anderson (2012).

Low latitude studies. To gain additional evidence of the generality of the experimental results, beyond the high latitude sampling sites, two additional studies were done at a northeastern U.S.A. site using the same procedures as Anderson (2012) based on the protocol in Fig. 1. Two sampling sites on Torrey Cliff, Palisades, NY were used: a raised berm planted with subalpine mountain laurel and red cedar trees, and a deciduous forest grove (red maple, oak, and tulip trees) in a hollow at approximately 3 m depth relative to the surrounding terrain. These will be referred to as the “Berm site” and the “Forest site,” respectively. However, in contrast to the tundra soil studies, the two Torrey Cliff studies used a two-week design with analyses done on Days 1, 8, and 15 (Fig. 1). This provided additional evidence of effects beyond one week. Glucose solution supplementation in the treatment group was done on Day 1 as in the tundra experiments.

Results mirrored those in the tundra experiments. Respiration spiked after addition of the glucose supplement (Day 1+), increasing nearly two-fold for the Berm site sample and over two-fold for the Forest site sample (Table 3), both substantially higher than the control respiration rate. However, as with the tundra data, the respiration rate declined to near baseline values on Day 8 (but higher than the control values), and

Table 3. Means \pm s.e. respiration data (nmol/min/g) for Treatment and Control preparations in the Berm and Forest experiments during two weeks.

Day ^a	Berm experiment		Forest experiment	
	Treatment	Control	Treatment	Control
1	3.4 \pm 0.3	2.9 \pm 0.2	4.9 \pm 0.7	4.8 \pm 0.6
1(+)	6.2 \pm 0.7	3.4 \pm 0.03	12.1 \pm 0.9	4.5 \pm 0.6
8	2.3 \pm 0.1	1.8 \pm 0.2	5.3 \pm 0.6	5.1 \pm 1.0
15	1.7 \pm 0.1	1.9 \pm 0.2	3.7 \pm 0.2	4.4 \pm 0.6

^a A (+) sign after Day 1 indicates supplementation with 2 ml of 0.05% (w/v) glucose solution. Controls received 2 ml of micropore-filtered deionized water.

subsequently decreased further on Day 15, within the range of the control sample respiration rate. As with the tundra experiments, measurements of respiration rate during four to five hours post glucose supplementation showed a nearly linear decrease with time. The regression equation for the rate of decline for the Berm experiment was $R_s = -0.61 h + 8.14$ ($r = -0.99$, $p < 0.001$), and for the Forest experiment $R_s = -1.16 h + 13.47$ ($r = 0.99$, $p < 0.001$); where R_s is respiration rate in nmol/min/g, and h is hours. The larger negative slope for the Forest site equation compared to the Berm may reflect the greater metabolic activity of the microbes in the Forest soil. It was enriched with substantial amounts of dark organic matter (52% w/w) derived from decaying leaves and plant detritus compared to 17% (w/w) for the Berm site. The markedly larger respiration rate of the Forest sample when supplemented with glucose (12.1 nmol/min/g) compared to the Berm (6.2 nmol/min/g) is further evidence of enhanced metabolic activity as well as greater densities of bacteria and protists in the Forest sample. The amount of CO₂ respired during the decline of the respiration rate after supplementation with glucose was 42.1 μ mol/g for the Berm site, and 71.9 μ mol/g for the Forest site. In sum, the total loss of C by respiration during the about four-hour decline in the activity of the spike (Day 1+) was 11.4 μ mol/g for the Berm experiment, and 19.4 μ mol/g for the Forest experiment.

Detailed data for the microbial densities and C-biomass are presented in Table 4 for the Berm site and Table 5 for the Forest site. Bacterial densities and carbon content increased in both the Treatment and Control preparations, but the total amount in the Treatment preparations was less than in the Controls, probably

reflecting top-down predation effects by the increasing abundances of flagellates and amoebae, both increasing substantially in the first week after addition of the glucose solution. The mean flagellate densities tended to plateau within the limits of the error of measurement, and may have been top-down controlled by predatory amoebae that peaked in abundance by the eighth day. Amoebae are known predators of flagellates (Anderson 1994, Bovee 1985) as well as bacteria. Overall, there was a substantial increase in the C-biomass of flagellates and amoebae in the Treatments compared to the Controls on Days 8 and 15 for both the Berm and Forest soil experiments. In general, the additional data collected at the end of the second week in these two experiments, showing a decline in abundance and C-biomass of flagellates and amoebae on Day 15 compared to Day 8, suggest that the carbon gains from the glucose supplement flow upward through the trophic levels, peaking first in the flagellates and amoebae, and then presumably moving further upward through the food web by the end of the second week. However, further research is needed to document changes in the abundance and C-biomass of higher-level predators before a definite conclusion can be made. There is limited evidence of how higher-level predators may change with glucose supplementation in soil. However, Kaneda and Kaneko (2004) showed that glucose supplementation in laboratory studies of soil from a broad-leafed forest, significantly enhanced the growth of collembolans (spring-tails) within a period of 7–8 days, directly proportional to the amount of glucose added. It is also possible that there is some mortality of the protists as the available organic supplement declines and competition pressures increase for available prey.

Table 4. Statistical analyses of microbiota means \pm s.e. for densities (number/g) and carbon content for bacteria, flagellates, and naked amoebae, on Days 1, 8 and 15 for the subalpine Berm site experiment at Palisades, NY.

	Treatment		Control	
	Densities	Carbon	Densities	Carbon
<u>Bacteria</u>	($\times 10^8$)	($\mu\text{g/g}$)	($\times 10^8$)	($\mu\text{g/g}$)
Day 1	3.1 \pm 0.2	40.3 \pm 1.9	3.1 \pm 0.2	40.3 \pm 1.9
Day 8	5.7 \pm 0.2	75.2 \pm 2.2	7.4 \pm 0.3	98.1 \pm 4.3
Day 15	7.5 \pm 0.3	98.0 \pm 4.9	8.2 \pm 0.9	97.1 \pm 13.5
<u>Flagellates</u>	($\times 10^6$)	($\mu\text{g/g}$)	($\times 10^6$)	($\mu\text{g/g}$)
Day 1	1.0 \pm 0.2	14.9 \pm 1.9	1.0 \pm 0.2	14.9 \pm 1.9
Day 8	1.3 \pm 0.3	20.2 \pm 4.0	0.6 \pm 0.1	7.8 \pm 1.0
Day 15	1.2 \pm 0.1	16.7 \pm 0.9	0.7 \pm 0.1	8.1 \pm 1.7
<u>Naked Amoebae</u>	($\times 10^3$)	(ng/g)	($\times 10^3$)	(ng/g)
Day 1	5.0 \pm 0.5	250 \pm 29	5.0 \pm 0.5	250 \pm 29
Day 8	11.1 \pm 1.7	1,532 \pm 242	6.8 \pm 0.1	356 \pm 86
Day 15	5.5 \pm 0.6	394 \pm 127	4.2 \pm 0.4	105 \pm 14

Table 5. Statistical analyses of microbiota means \pm s.e. for densities (number/g) and carbon content for bacteria, flagellates, and naked amoebae, on Day 1 and Days 8 and 15 for the Forest site experiment, Palisades, NY.

	Treatment		Control	
	Densities	Carbon	Densities	Carbon
<u>Bacteria</u>	($\times 10^9$)	($\mu\text{g/g}$)	($\times 10^9$)	($\mu\text{g/g}$)
Day 1	1.0 \pm 0.1	121.7 \pm 9.1	1.0 \pm 0.1	121.7 \pm 9.1
Day 8	1.2 \pm 0.1	162.3 \pm 16.7	2.6 \pm 0.3	346.8 \pm 42.7
Day 15	2.8 \pm 0.3	365.8 \pm 52.5	6.3 \pm 0.6	823.8 \pm 8.9
<u>Flagellates</u>	($\times 10^6$)	($\mu\text{g/g}$)	($\times 10^6$)	($\mu\text{g/g}$)
Day 1	0.9 \pm 0.1	13.3 \pm 2.1	0.9 \pm 0.1	13.3 \pm 2.1
Day 8	2.4 \pm 0.3	33.8 \pm 4.9	0.7 \pm 0.1	9.3 \pm 0.9
Day 15	2.1 \pm 0.3	29.0 \pm 3.5	1.3 \pm 0.1	18.6 \pm 0.8
<u>Naked Amoebae</u>	($\times 10^3$)	(ng/g)	($\times 10^3$)	(ng/g)
Day 1	6.6 \pm 0.8	496 \pm 248	6.6 \pm 0.8	496 \pm 298
Day 8	18.5 \pm 1.7	1,187 \pm 417	8.5 \pm 1.4	154 \pm 37
Day 15	8.3 \pm 1.1	794 \pm 240	6.1 \pm 0.7	332 \pm 92

IMPLICATIONS AND FUTURE OUTLOOK

Implications. Terrestrial carbon budgets and the role of soil-dwelling microbial communities in the biogeochemical carbon cycle are receiving increasing atten-

tion, not only in terms of the importance of microbes in maintaining soil fertility through mineralization processes, but also due to interest in the fate of soil organic carbon, particularly how much is sequestered in biotic and abiotic pools, and how much is lost to the atmo-

sphere as respiratory CO₂. Calculated on a global scale, terrestrial sources of respiratory CO₂ release carbon at a rate that is more than one order of magnitude larger than anthropogenic emissions (Luo and Zhou 2006, p. 24). There are particular concerns about possible major increases in respiratory CO₂ emissions from tundra and boreal forest terrestrial ecosystems with increased global warming, particularly given their large expanse globally of moss-rich terrestrial environments (O'Neill 2000, Walker and Walker 1996). Estimates of the respiratory CO₂ efflux from the moss-rich terrestrial communities can be substantial, for example, 1.0–3.0 μmol/m²/s (3.6–10.8 kmol/km²/h) (Oberbauer *et al.* 2007). Compared to other global ecosystems, the Arctic is undergoing climate change at an alarming rate, and particular attention to terrestrial functional ecology is needed to more fully understand the consequences of, and the feedback effects on, climate warming by the respiratory production of CO₂ by indwelling biota of high latitude soils (e.g. Anderson 2010a).

Findings reported here for Arctic and temperate sampling sites, as well as published reports for other locales (e.g. Jones and Murphy 2007, van Hees *et al.* 2005), indicate that pulsed release of low-molecular weight organics in soil produces only a limited and transitory spike in release of respiratory CO₂. Moreover, additional data presented here show that an appreciable portion of the organic C is accumulated in microbial biomass (including bacterial, nanoflagellate, and naked amoebae), information not previously reported in the literature. Thus, the immediate efflux of respiratory CO₂ to the atmosphere from pulses of organics, is likely to be minimal compared to other sources, and much of the organic-C can be sequestered in microbial biomass, thus contributing to increased stores of C to support trophic networks within the soil communities. The increased C-biomass in microbes within days after application of a pulse of glucose in the culture experiments, further indicates rapid throughput of C within soil microbial communities (e.g. Fitter *et al.* 2005, Jones *et al.* 2007, Rutherford and Juma 1992). The decline in bacterial abundance in the glucose-pulsed experiments, relative to the controls, as presented here is consistent with prior reports of bacterial declines when protists (e.g. *Acanthamoeba* sp.) are added to soil microcosms (Rutherford and Juma 1992), and undoubtedly reflects increased predation pressure on the bacteria.

Additional evidence is also reviewed here to show that intermittent pulses of precipitation, or other sour-

ces of soil moisture, also contribute to a transitory spike in respiratory CO₂ loss. This could be significant on an ecosystem scale, especially if changes in global climate increase the intermittent frequency of precipitation in previously more arid environments (e.g. Anderson 2011, Miller *et al.* 2005, Steenworth *et al.* 2005).

Although fairly robust estimates of soil respiration are possible using modern respirometry, we have less reliable ways of estimating the contribution of different soil microbial taxa to the total respiratory loss of CO₂. Some fairly well constrained regression estimates of respiration rate relative to cell biovolume are available for amoebae and other protists (e.g. Baldock *et al.* 1982, Crawford *et al.* 1994, Fenchel and Finlay 1983, Laybourn-Parry *et al.* 1980). However, the respiration rate is expressed as O₂ consumed, not CO₂ produced. Because we have limited evidence about the respiratory quotient ($RQ = \text{CO}_2 \text{ evolved} / \text{O}_2 \text{ consumed}$) for protists, especially when they are preying on bacteria in either soil or water, and under defined growth conditions, it is not possible to accurately convert O₂-based measurements of respiration rate to CO₂ units. The RQ for whole soil microbial respiration has been published for basal rates (typically ≤ 1) in terms of natural soil conditions (e.g. Dilly 2001, Mamilov and Dilly 2011) or in relation to variations in nutrient availability and other edaphic and physical contributory factors (e.g. Dilly 2003, 2004; Heinze *et al.* 2010; Mamilov and Dilly 2011; Xu *et al.* 2010). In most cases, the RQ is probably largely a response of the bacterial taxa that tend to be the most dominant. Therefore, given these limitations, more precise estimates of CO₂ respiratory loss by protists are further hindered by lack of good evidence of the RQ when they are in natural soils, or in experimentally altered states. Moreover, Dilly (2003) showed that RQ values in agricultural and forest soils typically < 1 , increased with increasing amounts of available C, varying between 0.44 and 1.35. Thus, for experiments where available soluble organic C is varied, even the total soil microbial RQ may be difficult to reliably predict, without direct measurements. Given the diversity of prey consumed by phagotrophic protists, especially amoebae, an RQ of 1 (typical for glucose metabolism) would unlikely be appropriate for exact assessments. However, as a means of making comparisons across major groups (where the magnitudes are likely to be much different than the error due to RQ estimates), use of an RQ of 1, is probably adequate to convert O₂ respiratory rates to CO₂ rates. Using this approximation,

some estimates of the contribution of the major microbial groups (bacteria, nanoflagellates, naked amoebae and testate amoebae) enumerated in tundra moss-rich soil have been published (e.g. Anderson 2008). The estimated respiration rates expressed as $\mu\text{mol CO}_2/\text{m}^2/\text{h}$ for bacteria, nanoflagellates and naked amoebae were respectively: 300, 213, and 5.5. It is clear that the naked amoebae contributed a much smaller amount of CO_2 to the total respiratory efflux than bacteria or nanoflagellates in this example. Although the relative contribution of amoebae to atmospheric respiratory CO_2 may be small, their predatory pressure on bacteria may be much more significant in terms of the functional ecology of the microbial community; and appropriate application of equations to estimate rate of bacterial consumption (e.g. Rogerson *et al.* 1996) can be a valuable additional source of evidence in evaluating the role of amoebae in microbial community dynamics (e.g. Lessen *et al.* 2010). Moreover, as documented in previous sections of this review, the sequestration of biomass carbon in the higher trophic levels of bacterial-based communities, including amoebae, can conserve the carbon within the microbial communities and thus moderate the amount of respiratory loss of CO_2 contributed by bacteria.

Future outlook. If modern genomic techniques can be applied to more reliably assess prokaryotic and eukaryotic microbial abundances and diversity in aquatic and terrestrial environments, especially if they include quantitative estimates of C-biomass, the accuracy and thoroughness of our assessment of microbial communities in natural environmental and experimental studies would be substantially enhanced. Presently, we have only scratched the surface in identifying the diversity of protists, especially naked amoebae, on a global scale. New species are discovered increasingly as more field observations and ecological studies are done. Application of single-cell genomic analyses, coupled with video and still microscopic documentation of each microorganism that is genomically analyzed, has substantially increased our ability to develop genomic markers for specific protist taxa. However, in the absence of comprehensive newer assay methods, we are left with laboratory-based culture observation methods, such as the COM method (e.g. Anderson 2000) for assaying amoeba abundances, diversity and biomass (applied successfully in many publications in our laboratory and elsewhere), and fluorescent counting and size assessment for flagellates and bacteria. The biovolume of naked amoebae can be estimated increasingly accu-

rately using regression equations relating C-biomass to cell volume, but the estimation of cell volume remains less than perfect, typically requiring microscopic measurements and geometric shape estimates of living or fixed individuals. For naked amoebae, some recent innovative approaches using estimates of rounded size based on appropriate measurements of motile length have helped to increase our efficiency and accuracy in estimating C-biomass for this group (Anderson 2006, 2007).

Given that soil microbial communities vary across seasons, and in relation to sporadic environmental forcing functions (e.g. precipitation patterns, short-term and longer-term climatic temperature variations, and sources and patterns of nutrient concentrations etc.), additional time series studies across seasons, combining simultaneous field-based and laboratory-designed experimental studies, may be needed to more fully explain the functional ecology of microbial communities in carbon flow and the biogeochemical carbon cycle. Such simultaneous studies in the field and laboratory may allow us to make more sophisticated interpretations of the events, and also provide more convincing evidence of how validly laboratory studies predict natural environmental processes in microbial communities.

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