

C-Biomass of Bacteria, Fungi, and Protozoan Communities in Arctic Tundra Soil, Including Some Trophic Relationships

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Abstract. The ecology of tundra terrestrial environments has gained increasing attention due to potential major changes resulting from global warming and climate change. However, the composition of terrestrial microbial communities and their role in the biogeochemical carbon cycle are less well studied. This is the first report of the C-biomass of bacteria, fungi, and representative protozoa (heterotrophic nanoflagellates, naked amoebae, and testate amoebae) in Alaskan tundra soil samples, and the effects of glucose solution enrichment in laboratory studies simulating release of soluble organic compounds as may occur during permafrost melt and increased plant root exudates due to global warming. The data for three moss-rich surface samples, two in spring and one in summer (2011), are reported for C-supplemented (8,000 μg glucose-C) and non-supplemented treatments in laboratory culture. Seven days after supplementation, fungal C-biomass in the glucose-treated and untreated samples were similar in the range of 5 to 11 mg g^{-1} soil dry weight, the highest values in the summer samples. The bacterial C-biomass was the next highest in the range of 20 to 120 $\mu\text{g g}^{-1}$ soil dry weight, followed by heterotrophic nanoflagellates (2 to 14 $\mu\text{g g}^{-1}$ soil dry weight). The naked amoebae (0.13 to 0.94 $\mu\text{g C g}^{-1}$ soil dry weight) and testate amoebae (2 to 20 ng C g^{-1} soil dry weight) contributed the least C-biomass. All of the bacterial and protozoan treatments showed increased biomass with glucose supplementation. Based on size, and C-biomass estimates, the phagotrophic protozoa appear to be organized in a classical bacterial-based trophic hierarchy (i.e. bacteria – nanoflagellates – naked amoebae – testate amoebae, in ascending order). Correlations of the C-biomass of bacteria to each of the protozoa, provided further evidence of a trophic pyramid; bacteria vs. nanoflagellates ($r = -0.986$), indicating top-down control by predatory flagellates, bacteria vs. naked amoebae ($r = -0.361$) and bacteria vs. testate amoebae ($r = -0.131$), each of decreasing magnitude as would be predicted for higher level consumers. Estimates of bacterial predation indicated strong predatory pressures on bacteria by the protozoa, greater with C-supplementation compared to the non-supplemented treatments.

Key words: Bacterial biomass, carbon biogeochemistry, climate change, fungal biomass, protozoan biomass, soil carbon enrichment.

INTRODUCTION

The role of microbial organisms in terrestrial ecology has been widely investigated in agricultural soils

(Clarholm 1984, Swift and Anderson 1993) and natural environments (Griffiths *et al.* 2001, Clarholm 2002), especially at lower latitudes. However, with increasing evidence of global warming, producing major changes in the climate and ecology globally, a substantial amount of research attention is being given to the role of soil microbial communities in less well-studied geographic regions. This is especially true for the Alaskan tundra where some of the most dramatic effects are be-

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ing documented (Anderson 2010a, Billings *et al.* 1983, Loya and Grogan 2004, Oechel *et al.* 1995). The moss-rich soil of the Alaskan tundra is a major reservoir of organic carbon that has accumulated over many centuries, sequestered in a frozen condition in the permafrost, and largely unavailable until now for microbial nutrition (Boddy *et al.* 2008, Heal *et al.* 1981). With increasing mean annual temperatures, and longer warm periods in summer, Alaskan permafrost is thawing to greater depths each year, releasing substantial amounts of carbon sources as nutrients for soil microbiota. Moreover, growing surface moss and roots of plants also release substantial amounts of carbon compounds, especially with increased atmospheric CO₂ concentrations, thus further enriching the pool of carbon sources for microbial metabolism (Anderson and Griffin 2001, Wilson and Coxson 1999). The effect of enriched organic nutrients on microbial community structure and function, and the fate of the metabolized carbon, is a topic of increasing investigation; especially what proportion of the metabolized C is conserved as microbial biomass and how much is lost as respiratory CO₂ to the atmosphere (Anderson 2012, Drigo *et al.* 2008, Raich and Schlessinger 1992).

Much attention has been given to the role of bacteria and fungi in high latitude environments in recent years (Campbell *et al.* 2010, Hansen *et al.* 2007, Lee *et al.* 2012, Rinnan and Bååth 2009), and also bacterial-based protozoan communities (e.g. Anderson 2012, Stapleton *et al.* 2005), but there is less documentation of the relationships among bacteria, fungi and major eukaryotic microbiota (protozoa) in tundra soil communities.

This paper builds on prior research on bacterial-protozoan C-budgets in tundra soil (Anderson 2012) by including estimates of the C-biomass of fungi as well as bacteria, heterotrophic nanoflagellates, naked amoebae and testate amoebae in glucose-supplemented and non-supplemented laboratory cultures. It is intended to contribute toward the goal of a complete account of the tundra terrestrial carbon biogeochemical cycle. This is one of the first reports of the C-biomass of bacteria, fungi, and soil protozoa in tundra soil samples, including some observations on relationships among trophic levels in the bacterial-based eukaryotic microbial food web of the tundra soils. The guiding hypothesis for this research was that pulsed application of soluble carbon nutrients will increase the biomass of bacteria in bacterial-based protozoan food webs yielding a concomitant increase in protozo-

an C-biomass and greater sequestration of C in the biota, thus simulating the effects of increased C soluble compounds resulting from thawing of the tundra permafrost during continued global warming. However, the proportion of total C sequestered by biota at each level in the hierarchy should decrease with increasing level in the trophic hierarchy due to inefficiencies in predation. Additionally, the study aimed to determine the relative effect of the soluble C nutrient on tundra fungal C-biomass in relation to that of the bacteria and protozoa, an aspect that has not been examined previously. The protozoa examined were heterotrophic nanoflagellates (HNF), naked amoebae, and testate amoebae. For conciseness, naked amoebae are referred to hereafter as “amoebae” and testate amoebae as “testates.” These are considered to be among the main protozoan predators of bacteria and fungi in soils (e.g. Adl 2003, Fenchel 1987).

MATERIALS AND METHODS

Sampling site and sample collection

Three tundra soil samples were obtained. Two were taken at an Alaskan tundra tussock in early spring (May 30, 2011) in northwestern Alaska (71°18'9.19"N, 156°40'8.97"W), one near the base of the tussock (Spring Sample 1) and the other with thicker surface moss growth taken near the top of the tussock (Spring Sample 2). Another (Sample 3) collected in summer (July 7, 2011) and largely composed of peat moss and some mineral soil, was obtained at an open tundra field site at Toolik Lake, AK (68°37'24.51"N, 149°35'46.26"W). All samples (~ 100 g) were taken in the upper five cm of moss-soil immediately above the permafrost layer. The two spring sampling sites were chosen in a tussock-rich region of northwestern Alaska where early spring studies were underway as part of the larger research project. The summer sample was taken at Toolik Lake, AK, where a long-term ecological research site was established to examine the effects of summer permafrost melt on the plant community and below-ground microbial communities (e.g. Anderson 2012). The samples were put in sealed plastic bags, placed in an insulated container with ice packs, and sent by overnight air shipment to the Lamont-Doherty Earth Observatory. Samples were stored at 5°C for up to three weeks prior to laboratory investigation. Moisture content was assayed gravimetrically by change in weight after drying at 106°C overnight. The moisture content was used to convert fresh weight of each soil sample to dry weight. The density of these soils is very close to 1 g cm⁻³. The moisture content of the two spring samples was 83% (w/w) and for the summer sample 33% (w/w). Organic content of the soil was determined by weight loss after combustion at 137°C for 12 h. Soil pH was measured (1g soil in 10 ml distilled H₂O) using an Accumet® model 15 pH meter (Fisher Scientific, Pittsburgh, PA). The pH is typically moderately acidic, pH 4.0–5.0. The organic content of the summer soil sample

was 20% (w/w). For the more moist and organic fibrous tussock soil samples taken in spring the organic content was in the range of 80% (w/w) and the pH 5.0 to 5.5 (e.g. Anderson 2010b).

Laboratory procedures

The laboratory procedures were identical for the three soil samples. One C-supplemented and one non-supplemented was used for each of the three samples, six preparations in total. One hundred g of soil were removed from the 5°C storage bags, and the moist, peaty-layer of soil was thoroughly mixed; all living roots (very sparse) were removed. Fifty g of the mixed soil were placed in 300-ml culture bottles (Nalgene®). The bottles were wrapped in aluminum foil to eliminate light, lightly capped and placed in a temperature-controlled incubator at 20°C for three days (similar to summer moderate daytime temperatures in Alaska) to become acclimated before the laboratory procedures were begun. One of the objectives of the study was to assess the status of the microbial community under conditions of warming that occur in early spring to summer. Given the rapid response of soil microbiota to temperature changes, we assumed that a three-day acclimation would provide sufficient time to simulate early seasonal warming. A remaining portion of the mixed soil was also analyzed gravimetrically for moisture content as described above.

On Day 1 of the investigation, the C-supplemented flask was amended with 2 ml of 0.5% (w/v) glucose solution (total = 1% w/v) using a transfer pipette, distributed as two applications of 1 ml each, and thoroughly mixed throughout the soil with a stainless steel spatula to ensure as complete distribution in the soil as possible. On day 2 an additional 2 ml of the glucose solution was added and thoroughly mixed into the soil as was done on Day 1. This pattern was chosen to more evenly distribute the pulsed addition of the glucose temporally, and to more thoroughly mix it into the soil. The weight of glucose in the aliquots added all-totaled on Days 1 and 2 was 0.02 g. The equivalent glucose-C was 8,000 µg. Glucose solution of 1% (w/v), ~ 50 mM, was chosen because it is one of the typical concentrations used in soil experimental studies at final concentrations that may be representative of the enriched carbon sources released in the soil from autotrophs (e.g. Jones and Murphy 2007, Wilson and Coxson 1999). The non-supplemented control flasks were prepared in the same way, except 2 ml of micropore-filtered (0.22 µm pore-size) deionized water were added instead of the 2 ml of glucose solution.

After seven days of incubation in a temperature-controlled incubator at 20°C, subsamples of the soil in the C-supplemented and in the non-supplemented flasks were removed for analysis. Seven days of incubation were used to adequately allow for growth of the slower growing naked amoebae and larger testate amoebae. One g of the soil was removed from each of the culture bottles and suspended thoroughly in 5 ml of micropore-filtered (0.45 µm pore size) pond water. A portion of the freshly prepared suspension was used for enumeration and size determination of living naked amoebae using the microscopic culture observation method (COM) routinely employed in our laboratory (Anderson 2002, 2008). Bacteria and flagellates were enumerated by fluorescent, microscopic methods (acridine orange stain) using an epifluorescence microscope with UV illumination (Anderson *et al.* 2001). Testate amoebae were concentrated by centrifugation (200 g) for 3 min. in a conical centrifuge tube, stained with Lugol's solution, brought to 1-ml volume, and exhaustively enumerated using 20-µl portions observed with

an inverted phase contrast Nikon® Diaphot compound microscope (Morrell Instruments Co., Melville, NY). Very few ciliates were observed in the Lugol's stained preparation, indicating that ciliates were largely encysted (cysts were observed in the preserved samples). Soil ciliates are likely active only during periods of substantial saturation of the soil with water sufficient to permit locomotion by ciliary action. During microscopic enumeration of bacteria and each group of protists, they were sized within 1 µm using an ocular reticule.

The C-content of each group of microbes was estimated using size-based regression equations as previously published (Anderson 2008, Pelegri *et al.* 1999). Biomass was expressed as µg g⁻¹ or ng g⁻¹ soil dry weight as appropriate to the particular microbiota. Grazing rates were estimated for HNF based on measurements made at 20°C. A grazing rate per each individual flagellate of 10 bacteria HNF⁻¹ h⁻¹, estimated from the approximate modal value of the predicted predation rate at 20°C plotted by Vaqué *et al.* (1994), was used in our research. Cell volume-based grazing rates of amoebae were obtained using the regression estimates of Rogerson *et al.* (1996).

Fungal and bacterial abundance was measured using phospholipid fatty acid analysis (PLFA, Frostegard and Bååth 1996). Total lipids were extracted from soil sub-samples (2 g) using a one-phase solution of chloroform, methanol, and citrate buffer (1:2:0.8 v/v/v) and silicic acid chromatography. PLFAs were converted to fatty acid methyl esters (FAMES) and analyzed on a gas chromatograph with a flame ionization detector (Agilent 6980N, Agilent Technologies, Santa Clara, CA). Unique bacterial and fungal biomarkers were identified using known standards and quantified using Agilent's ChemStation software. Total microbial biomass was calculated as the sum of all PLFAs and an internal standard of methyl nondecanoate (19:0) was added to each sample to calibrate lipid concentration. Bacterial biomass was quantified as the sum of a15:0, i14:0, i15:0, i16:0, i17:0, i17:1ω8c, 11:0, 12:0, 14:0, 15:0, 16:0, 16:1,9, 16:1ω6c, 16:1ω7t, 17:0, 17:0Δ, 18:0, 20:0, 10Me16:0, 10Me17:0, 18:1ω9t, 18:1ω7c/9t, 19:0Δ, 2-OH 10:0, 2-OH 12:0, 2-OH 14:0, 2-OH 16:0, 3-OH 12:0, and 3-OH 14:0. Biomarkers 18:2ω6,9 and 18:1ω9c were summed for total fungal PLFA biomass. C-biomass of the fungi was estimated using the formula of Klamer and Bååth (2004) to convert the concentration of PLFA 18:2ω6,9 to g fungal C-biomass (11.8 µmol 18:2ω6,9 = 1g total fungal C-biomass).

Statistical procedures

Representative C-biomass data are presented for each of the six treatment conditions. Pearson linear correlations were obtained using StatPlus® (AnalystSoft, Vancouver, BC, Canada). Six pairs of data used for each correlation. For the correlation analyses to examine possible trophic hierarchical relationships among the protists, C-biomass of biota for each sample was expressed as percent of total C within each sample to normalize it. This was done to control for possible confounding effects of variations in biomass between treatment conditions and variations across samples collected at different seasons. Linear regressions (n = 6 pairs of data for each analysis) were obtained using StatPlus® to examine the relationship between C-biomass of fungi and bacteria, C-biomass of naked amoebae and bacteria, and C-biomass of HNF and bacteria. An Excel x-y scatter plot was used to determine that the data for the correlation and regression analyses were sufficiently linear to meet the assumptions of the statistical methods.

RESULTS

C-biomass data

For brevity Samples 1 and 2 refer to the two spring samples, and Sample 3 refers to the summer sample. Experimental treatments containing glucose C-enrichment for the three samples are denoted by 1a, 2a, and 3a, respectively. Comparative data on biomass of fungi, bacteria, HNF, amoebae and testates in the C-supplemented and non-supplemented soil samples for the three sampling sites are presented in Table 1. The fungal C-biomass, in the range of mg g⁻¹ soil dry weight, was substantially higher than that of the other biota (µg g⁻¹ for bacteria, HNF and amoebae, and ng g⁻¹ for testates). Glucose C-enrichment (treatments 1A, 2A, and 3A) produced an increase in C-biomass for bacteria, HNF, amoebae and testates across all three samples compared to the non-enriched condition, but there was no appreciable increase for fungi. To better estimate the C distribution in the bacterial-based protozoan trophic hierarchy, the C-biomass of bacteria, HNF, amoebae and testates was expressed as percent of total in each of the groups of biota (Table 2). The percentage C-biomass estimates were quite consistent across the three pairs of treatment conditions for each of the groups of biota. The estimated percent C-biomass of bacteria was highest relative to all other biota, and consistently so across all six conditions. As may be expected for biota in a trophic hierarchy, the biomass of bacteria, and HNF, occupying lower levels of the trophic food web, accounted for more C-biomass than the amoebae and

testates that are in the upper tiers of the bacterial-based food web (e.g. Fig. 1). The percentage biomasses of HNF in the C-supplemented condition were higher than in the non-supplemented condition, but not appreciably different for all other biota, with possibly the exception of naked amoebae in Sample 1 and Sample 1A.

C-biomass and trophic relationships

Bacteria are major sources of nutrition for protozoa in bacterial-based food webs. Data on typical mean C-biomass of protozoa and bacteria within a bacterial-based trophic web, based on data from the tundra soil samples examined here. As expected in a trophic hierarchy, the biomass and size per organism increase markedly for biota at increasingly higher levels in the food chain. The size ranges for protozoan taxa observed in this research, corresponding to a hierarchy of increasing size, are: HNF (3–5 µm), naked amoebae (10–50 µm) and testates (20–100 µm).

Carbon-biomass, however, is inversely related to position in the hierarchy; the highest amounts (bacteria and HNF) are at the base. Further evidence of a bacterial-based hierarchical food chain is shown by the correlation of biomass of bacteria vs. each of the three main protozoan predators, HNF, amoebae, and testates. There was a strong negative correlation ($r = -0.986$, $p < 0.01$) of bacteria and their most immediate major predators (HNF), possibly indicating a top-down control of HNF on bacterial populations. The correlation of bacterial biomass versus higher-level protozoan predators was increasingly less, amoebae ($r = -0.361$, $p < 0.05$) and testates ($r = -0.131$, $p = 0.16$). The cor-

Table 1. C-biomass for each group of biota in the six samples from the treatment and control conditions.^a

Biota	Sample 1	Sample 1A	Sample 2	Sample 2A	Sample 3	Sample 3A
Fungi (mg g ⁻¹)	7.7	6.9	4.9	7.0	10.7	8.4
Bacteria (µg g ⁻¹)	22.7	28.3	51.1	74.7	57.7	124.2
HNF (µg g ⁻¹)	2.0	3.8	7.2	14.4	2.9	6.7
Amoebae (µg g ⁻¹)	0.36	0.94	0.22	0.24	0.13	0.17
Testates (ng g ⁻¹)	12.9	18.6	3.3	16.0	2.0	20.1

^a Samples 1 and 2 are from the spring sampling site and Sample 3 is from the summer sampling site. Column headings for samples are as follows: 1, 2, and 3 are controls, non-supplemented with glucose; samples 1A, 2A, and 3A are treatments, supplemented with glucose. Note the differences in weight units for each row entry (Fungi, mg g⁻¹), Bacteria, HNF and Amoebae (µg g⁻¹), and Testates (ng g⁻¹).

Table 2. Percentage of total C-biomass for each group of biota in the bacterial-based protozoan food chain and total C-biomass ($\mu\text{g g}^{-1}$) in the six treatment conditions.^a

Biota	Sample 1	Sample 1A	Sample 2	Sample 2A	Sample 3	Sample 3A
Bacteria	90.7	85.5	87.3	83.7	95.0	94.7
HNF	7.9	11.6	12.3	16.1	4.8	5.1
Amoebae	1.4	2.9	0.4	0.3	0.2	0.1
Testates	0.05	0.06	0.01	0.02	< 0.01	0.02
Total C-biomass	25.36	32.76	58.42	89.66	61.03	130.89

^a As in Table 1, Samples 1, 2, and 3 are non-supplemented with glucose; Samples 1A, 2A, and 3A are supplemented with glucose.

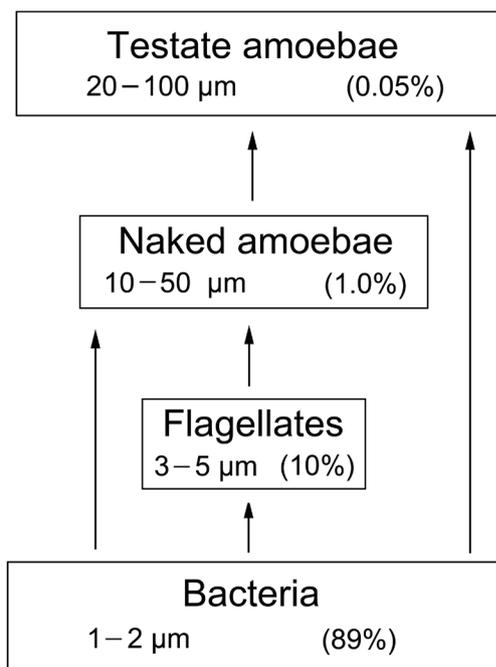


Fig. 1. Diagram of a tundra, bacterial-based protozoan trophic hierarchy showing the typical size range of each group of biota (μm), percent of total C-biomass in parentheses, and predator prey relationships (arrows) based on data in this paper. As in trophic webs of higher organisms, biota at increasingly higher levels of the hierarchy are larger, but account for increasingly less of the total C-biomass in the community of organisms. Bacterial-based protozoan trophic hierarchies are often more complex, because higher-order predators also prey on bacteria at the base of the food chain in some cases, in addition to preying on intermediate taxa in the hierarchy.

relation results, indicating a higher trophic level for amoebae and testates, are consistent with trophic data from other terrestrial environments at lower latitudes (e.g. Fenchel 1987).

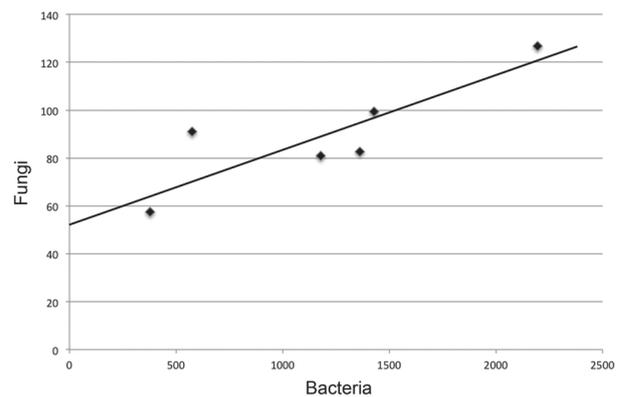


Fig. 2. Plot of the concentration of fungal PLFA (18:2 ω 6,9) compared to bacterial PLFAs, yielding a correlation of $r = 0.85$ ($r^2 = 0.72$).

To obtain additional evidence of biomass relationships, regression equations were calculated relating total protozoan C-biomass to bacterial C-biomass. $P_c = 3.411 + 0.052 B_c$ ($p = 0.39$), where P_c is total protozoan C-biomass (i.e. HNF, amoebae and testates). Biomass is expressed as $\mu\text{g cm}^{-3}$ fresh soil. To estimate the relationship between fungi and bacteria, the PLFA concentrations of fungi obtained in the six samples was regressed against the PLFA concentrations in bacteria. A strong positive correlation ($r = 0.85$, $p = 0.03$) was obtained (Fig. 2) and the regression equation was $F_c = 54.29 + 0.03 B_c$, where F_c = fungal PLFA concentration and B_c = bacterial PLFA concentration. Assuming that these relationships can be replicated at other tundra sites, such regression equations may be useful in estimating the biomass of fungi and protozoa based on estimates of bacterial biomass.

Grazing rates of protozoa on bacteria

Estimates of grazing rates of HNF and naked amoebae on bacteria (Table 3) for the C-supplemented and non-supplemented conditions show that HNF and naked amoebae are major predators on tundra soil bacteria. The total grazing rate for the total number of HNF is in the range of 10^6 to 10^7 bacteria $\text{h}^{-1} \text{g}^{-1}$ soil dry weight. The total amoebae consume 10^5 to 10^6 bacteria $\text{h}^{-1} \text{g}^{-1}$ soil dry weight. Given that bacterial densities are on the order of 10^8 to 10^9g^{-1} soil in many terrestrial environments, a substantial amount of the standing crop of bacteria can be consumed each hour by the predatory protozoa. HNF, largely in the size range of 2 to 3 μm , prey almost exclusively on bacteria (Fenchel 1987). Naked amoebae prey on bacteria and small flagellates (Anderson 1994, Bovee 1985). Testate amoebae consume bacteria and flagellates (Fenchel 1987), fungal hyphae (Couteaux and Devaux 1983) and solitary fungal cells including yeasts (Anderson 1989). It is also likely that they consume smaller naked amoebae. However, there are few published reports on feeding rates in testate amoebae, and given their lower abundances compared to HNF and naked amoebae their impact on soil microbial populations appears to be less substantial than the other two protozoa (e.g. Fenchel 1987).

DISCUSSION

Moss-rich ecosystems, including tundra and boreal forests, are estimated to occupy millions of square kilometers, circumpolar (O'Neill 2000, Walker and Walker 1996). Given the geographic size of these regions, and increasing evidence of the substantial role of tundra soil microbial communities in the biogeochemical carbon cycle and terrestrial productivity (e.g. Anderson

2010b, 2012), further research is needed to more fully document their role across broader geographic regions and with soil from more diverse tundra terrestrial sites (Anderson 2010a, Schmidt and Boelter 2002, Zak and Kling 2006). Prior studies examined the role of protozoa in tundra C-budgets (e.g. Anderson 2008, 2012). Others focused more particularly on fungal and bacterial contributions (e.g. Ananyeva *et al.* 2006, Eskelinen *et al.* 2009, Rinnan and Bååth 2009). The research reported here is one of the first to document the relative C-biomass of bacteria, fungi and protozoa in freshly collected tundra soil samples and to examine the changes in their C-biomass when the soil is enriched with water-soluble, low molecular weight carbon compounds. Prior research (e.g. Clemmensen *et al.* 2006) published some of the first data on living fungal biomass in organogenic arctic soils, and responses to long-term fertilization and warming. They reported their data, however, as biomass per square meter of soil surface and did not include any other soil eukaryotic microbes.

C-biomass estimates

As may be expected for organic-rich soil, the fungal C-biomass (mg g^{-1} soil dry weight) was substantially greater than bacterial C-biomass ($\mu\text{g g}^{-1}$ soil dry weight). The fungal C-biomass values reported here (5 to 10 mg g^{-1} soil dry weight) are within the range found for other organic rich environments. For example a mean of 17 mg g^{-1} soil organic matter (SOM) in the upper 10 cm of tussock tundra soil at Toolik Lake, AK was reported by Clemmensen *et al.* (2006). The values reported for our samples are substantially lower than those reported for the humus layer of forest soils (50–70 mg g^{-1} SOM) reported by Wallander *et al.* (2004). However, it should be noted that the latter two papers report fungal C-biomass per g SOM, while our data are reported per g of soil dry weight. Our data are closer

Table 3. Estimated bacterial grazing rates for heterotrophic nanoflagellates (HNF) and naked amoebae in C-supplemented and non-supplemented conditions for Samples 1, 2, and 3.^a

	C-Supplemented		Non-supplemented	
	HNF	Amoebae	HNF	Amoebae
Sample 1	4.1×10^6	1.2×10^6	2.1×10^6	0.5×10^6
Sample 2	15×10^6	0.4×10^6	7.7×10^6	0.3×10^6
Sample 3	7.1×10^6	0.2×10^6	3.1×10^6	0.1×10^6

^a Rates are expressed as number of bacteria consumed by total number of HNF and amoebae per hour per g dry weight of soil based on the number g^{-1} soil dry weight and the composite sizes of the HNF and amoebae.

to values of fungal C-biomass reported by Klamer and Bååth (2004) in early stages of organic-rich compost ($\sim 15 \text{ mg g}^{-1}$ soil organic matter).

Glucose-C supplementation in our study produced no appreciable change in fungal C-biomass between the treatment and control cultures. This may be attributed partially to the relatively low concentration of soluble organic matter supplied in the treatment condition compared to the total mass of fungal mycelium. Moreover, the metabolic state of fungi in soil environments is not fully understood, although PLFAs are reported to be a signature of active cells (e.g. Zelles 1999) and may provide a better estimate of arbuscular mycorrhizal and saprotrophic fungal biomass compared to use of hyphal length (Balser *et al.* 2005). Fungi may contribute 63–82% of substrate-induced respiration in diverse soils spanning tundra, temperate meadow, and forests (e.g. Ananyeva *et al.* 2006).

The conversion formula we have used (Klamer and Bååth 2004) is the only one available and employed in widely different environments (e.g. Bezemer *et al.* 2010, Luo *et al.* 2005, Seifert *et al.* 2011). It is likely to yield a somewhat higher estimate of C-biomass compared to fungal growth that is less robust under more limited nutrient conditions than in the compost sample used to derive the formula. However, we believe that their formula may be appropriate for our work given the high organic content of the moss-rich soil and also the glucose-C enrichment treatments that we used.

With respect to the bacterial-based protozoan community, the highest percent of C-biomass across all samples, and between the two treatment conditions, was for bacteria, followed in descending order by biomasses of HNF, amoebae and testates (Table 2), thus supporting the working hypothesis. The slower growing, and larger sized, naked amoebae that are higher up the food chain, while showing reasonable gains in percent C-biomass, may not have had sufficient time to reach their maximum potential gain within seven days. They also may be under top-down control by other predators such as nematodes (Anderson *et al.* 1977), thus suppressing potential maximum biomass gains. Overall, however, the data show that within one week of laboratory culture at 20°C, glucose enrichment produced substantial gains in total C-biomass compared to the non-enriched condition, increasingly across the three samples from the two spring samples to the summer sample. The major increase was contributed by bacteria (Table 1) as may be expected because bacteria are particularly efficient in assimila-

ting soluble organic compounds, and in some cases respond more rapidly in biomass production following a glucose pulsed supplementation compared to fungi that respond later (Stamatiadis *et al.* 1990). Soil fungi are clearly capable of utilizing glucose (e.g. Paterson *et al.* 2007, Rinnan and Bååth 2009), but in some cases less efficiently than bacteria (Thiet *et al.* 2006). It is not possible to make a complete analysis of how much of the glucose-C supplement was incorporated into the bacterial biomass because of several potential biological and soil structural contributory factors. These include the complex absorption kinetics of soluble carbon compounds on soil particles of different sizes resulting in differences in fungal and bacterial productivity (e.g. Chenu *et al.* 2001), localized regions of concentration of the soluble C compounds in the moss-rich soil structure resulting in hot spots of bacterial activity, and possible enhanced bacterial release of lytic enzymes during carbon nutrient supplementation, thus mobilizing additional C nutrients from the stored organic matter in the tundra soils. Also a substantial amount of C is lost due to respiratory activity and should be addressed in a complete accounting of the fate of added organic carbon (e.g. Anderson 2012).

Protozoan trophic relationships

Phagotrophic protozoa are capable of exerting heavy predation pressure on bacteria at the base of the food web in aquatic and terrestrial environments (e.g. Adl 2003, Anderson 2012, Fenchel 1987). The total bacterial grazing rates for individual HNFs in this study ranged from 2.1 to 7.7×10^6 bacteria HNF⁻¹ h⁻¹ in the non-supplemented condition and 4.1×10^6 to 15×10^6 bacteria HNF⁻¹ h⁻¹ in the C-supplemented condition. Naked amoeba predation (10^5 – 10^6 bacteria amoeba⁻¹ h⁻¹) was approximately one to two orders of magnitude less than that of the HNF, but higher than the rate of 0.02 to 0.03×10^6 bacteria amoeba⁻¹ h⁻¹ previously reported for an Alaskan high and low tussock site in early spring (Anderson 2010b). Prior data for a tundra site in summer (Anderson 2008) yielded a value of 0.82×10^6 bacteria amoeba⁻¹ h⁻¹, closer to the results in this study.

In general, the amoeba predation rates reported here can be considerable given that amoebae are particularly adapted as surface dwelling protists to exploit prey in the thin water films on soil particles and aquatic particles in suspension (e.g. Anderson 2011, Fenchel 1987). Moreover, based on ultrastructural evidence, amoebae produce long invasive pseudopodia that penetrate deep-

ly into microscopic pores of soil particles (Foster and Dormaar 1991). The amoebae may ingest bacterial prey that is not accessible to surface grazers such as HNF. Amoeba bacterial predation can reduce the numbers of bacteria by as much as 25 to 60% in arable soil (Bryant *et al.* 1982, Clarholm 1981), but less data are available for tundra, moss-rich soils. Protozoa are size selective when grazing on bacteria (e.g. Chrzanowski and Simek 1990, Hahn and Höfle 2001, Koton-Czarnecka and Chróst 2003, Jürgens *et al.* 1999) and may change the composition and structure of soil bacterial communities (Rønn *et al.* 2002).

Among the naked amoebae, fungal predation has been documented largely among the vampyrellids, e.g. *Arachnula* sp. (Old and Dabysshire 1978, 1980), although other mycophagous amoebae (e.g. *Arachnula impatiens*, *Cashia mycophaga*, *Leptomyxa reticulata*, *Thecamoeba granifera* and *Vampyrella lateritia*) have also been reported, but studies on their soil abundance are limited (e.g. Ducek 1983). Vampyrellids typically feed by attaching to the wall of fungal mycelia, where they secrete lytic enzymes that produce a hole permitting the amoeba to either suck out or ingest the fungal cytoplasm. The vampyrellids are also capable of ingesting bacteria, apparently required for vitality (Old and Darbyshire 1978), and the general consensus is that protozoan food webs are largely based on bacterial predation (e.g. Adl 2003, Fenchel 1987). Mycophagous flagellates are generally large (> 10 µm in length) compared to bacterial-feeding HNF, and their numbers in soil can be in the range of 10²–10³ g⁻¹ soil dry weight (Ekelund 1998). Clearly more information is needed on the predation pressure of larger mycophagous flagellates on soil fungi, especially at higher latitudes where fungi compose a significant proportion of soil eukaryotic microbiota.

HNF and amoebae are consistently found to be major predators in soil systems, including tundra soil (e.g. Stapleton *et al.* 2005), but there is little published data on their bacterial feeding rates in tundra. Moreover, other studies at lower latitudes often use different units to report the feeding rates in relation to soil parameters, including area (m⁻²), volume (cm⁻³), or mass (g). Therefore, it is difficult to make comparisons across published reports. More consistent measurement units have been used by researchers investigating protozoan grazing in aquatic and marine environments. Thus, most estimates of bacterial consumption rates come from measurements of protists feeding in plankton or on marine substrates, the lat-

ter is particularly applicable to soil particle-dwelling amoebae (e.g. Rogerson *et al.* 1996). In this study, we used a modal estimate of individual HNF grazing rate at ~ 10 bacteria HNF⁻¹ h⁻¹, based on temperature-related estimates at 20°C (Vaqué *et al.* 1994), the temperature used in our culture experiments. This estimate is consistent with other reports of HNF bacterial grazing in natural environment conditions and in laboratory studies. For example, Barcina *et al.* (1992), studying a coastal site in Spain (mean temperature 18.6°C), reported a mean ingestion rate of 14 bacteria flagellate⁻¹ h⁻¹. This is within range of other published moderate values for flagellate bacterial predation expressed as the number of bacteria consumed per individual flagellate per hour, e.g. 10 bacteria HNF⁻¹ h⁻¹ (Sherr *et al.* 1983), ~11 (Chrzanowski and Simek 1990), ~ 20 (Hahn and Höfle 1999), 25 (Daggett and Nerad 1982) and 27 bacteria HNF⁻¹ h⁻¹ (Fenchel 1982). As may be expected, however, the rate of ingestion depends on the abundance of available bacteria and is linearly related to the log₁₀ of the bacterial concentration (e.g. Davis and Sieburth 1984, Park and Cho 2002). Vaqué *et al.* (1994) noted that at 20°C, there is a marked variation of grazing rates culled from the literature, spanning a lower range of one bacterium HNF⁻¹ h⁻¹ to an extreme estimate near 100 HNF⁻¹ h⁻¹. These variations depend apparently on the HNF size and culture or environmental conditions. However, even at the lowest value of one bacterium h⁻¹, given the very large populations of HNF typically observed in soils, the predation pressure on bacteria can be substantial.

Unfortunately, there is very little data available on the predation rate of testate amoebae. Prey diversity, however, has been documented for some species. For example, *Nebela collaris* sensu lato collected from sphagnum in a peatland, most frequently ingested micro-algae (45% of the total predator-prey associations), and spores and mycelia of fungi (36%) as well as large ciliates, rotifers and small testate amoebae, the latter three mainly in summer (Gilbert *et al.* 2003). This is similar to other reports of predation by *Hyalosphena papillo* and *Nebela tinctoria* (Jassey *et al.* 2012). Bacterial consumption by *Arcella vulgaris* was shown to be relatively constant at ~ 6 × 10³ bacteria individual⁻¹ h⁻¹ over a temperature range of 10–25°C (Laybourn-parry and Whymant 1980). *Arcella vulgaris* (100–150 µm in size) is among the larger soil-dwelling testate amoebae, and its predation rate may be substantially higher than the rate of more moderately sized species (30–50 µm).

CONCLUSIONS

In general, our results support a view that bacterial and fungal C-biomasses are a major component of the total tundra soil microbial C-content among the microbiota studied here, at least in some Alaskan Tundra sites in spring and summer. Glucose-C pulsed supplementation of the soil samples produced an appreciable increase in bacterial and protozoan C-biomass compared to non-supplemented samples. However, no appreciable increase was observed for fungal C-biomass under the laboratory procedures used here. Larger concentrations of a soluble C source and for longer duration, however, may produce an effect and further research is needed to elucidate this possibility. Evidence of trophic relations among the protozoan bacterial-based food chain, as represented by the relative estimated biomasses and grazing rates of the three major protozoan groups (HNF, amoebae, and testates), indicates that the tundra moss-rich environment supports a heterotrophic eukaryotic microbial community that is basically similar in structure to that of other terrestrial environments (e.g. Adl 2003, pp. 137–200; Fenchel 1987). The proportion of total biomass C sequestered in the bacteria and each of the protozoa (Table 2) was consistent across the six conditions indicating that the results were largely replicable even though the samples came from different locales and at different seasons. Additional research is needed to determine formulae for converting fungal PLFA concentrations to fungal C-biomass, especially for different environmental and nutrient conditions. The conversion formula of Klamer and Bååth (2004) is probably suitable for organically rich environments, but additional studies are needed to determine conversion factors for fungi grown under varying nutrient sources and concentrations. Given the relatively sparse data on C-biomass for soil protozoa, and more importantly the lack of a consistent use of measurement units in published reports, more detailed research is needed using standardized techniques to document the carbon budgets of soil communities globally, especially at high latitudes where major changes are occurring due to global warming and climate change (e.g. Anderson 2010a, Billings *et al.* 1983, Loya and Grogan 2004).

Acknowledgments. This research was supported partially by funds from the National Science Foundation (NSF 0732664, ORA, Co-PI). We thank Caitlyn Gillikin for assistance with sample processing for PLFA. This is Lamont-Doherty Earth Observatory Contribution number 7687.

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Received on 15th December, 2013; revised on 26th March, 2013; accepted on 21st April, 2013