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ORIGINAL PAPER

Effects of organic carbon enrichment on respiration rates, phosphatase activities, and abundance of heterotrophic bacteria and protists in organic-rich Arctic and mineral-rich temperate soil samples

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Abstract The release of respiratory CO_2 from polar tundra soils depends on the interactions between soil organic carbon and microbial communities, a topic of increasing importance due to global climate change. We experimentally amended samples of organic-rich tundra soil and mineral-rich temperate soil with dissolved organic carbon (DOC) and examined the following (at the same temperature): (1) the effects of DOC enrichment on bacterial and eukaryotic microbial biomasses, respiration, and acid phosphatase activities in both soil types, (2) whether relationships between microbial biomass, respiration, and acid phosphatase activities were similar across soil types, and (3) to what extent DOC enrichment altered the microbial food web in both soil types. Both soil types showed immediate, temporary increases in respiration following C enrichment, though the C source (amino acids, citric acid, or glucose) was an important source of variation. Additionally, a consistent relationship between respiration rates and acid phosphatase activities suggested that C and P cycling were linked in similar ways in the two soil types. The relationships of respiration rate and acid phosphatase activity to bacterial biomass were also consistent across soil types, excepting some amended tundra soil samples, perhaps due to greater protist bacterivory in those samples. In the tundra samples only, changes in predator biomass were accompanied by elevation of bacterial biomass-specific respiration rates. Our findings highlight the potential importance of protist bacterivory on microbial processes in tundra soils, but further research is needed to assess whether microbial food webs differ consistently among soils that vary in organic content.

Keywords Atmospheric CO_2 · C cycle · C and P cycle coupling · Global warming · Microbial ecology · P cycle · Soluble organic soil C

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Introduction

Increased atmospheric CO_2 and global warming are having major effects on ecosystems worldwide, especially in the Arctic, including climate, plant communities, and soil microbiota (Shaver and Jonasson 1999; Lloyd 2005; Buckeridge and Grogan 2008; Anderson 2008, 2010a; Swann et al. 2010; Heskel et al. 2014; Shi et al. 2015). Recent thawing of the permafrost to greater depths makes historically-fixed sources of C available for metabolism by soil microbial communities (Bockheim et al. 1999; Natali et al. 2014), thus potentially increasing respiratory flux of CO_2 to the atmosphere (e.g., Loya and Grogan 2004). Additionally, with milder climate, increased plant cover potentially increases the atmospheric CO_2 sink (Sweet et al. 2015), but can also provide additional labile organic C in the form of root exudates (e.g., glucose, amino acids, and organic acids) that may enhance soil microbial metabolism and net CO_2 efflux (Anderson and Griffin 2001; Loya and Grogan 2004; Bais et al. 2006; Anderson 2010b; Jin et al. 2014). While organic enrichment of tundra soil through plant root exudates would be expected to increase respiratory CO_2 loss to the atmosphere (Loya and Grogan 2004; Nguyen 2009), the effect could be at least partly offset by concomitant increases in nutrient availability to plants, such as enhanced phosphorus remineralization due to elevated microbial phosphatase activity (Spohn et al.

2013). In addition, the potential role of protist bacterivores on soil respiration rates needs to be considered. Available evidence indicates that while protist predation can dramatically decrease bacterial concentrations in soils (Clarholm 1981; Kuikman et al. 1990; Murase et al. 2006), bacterial cell-specific, or biomass-specific, respiration rates can increase when protists are present (Alphei et al. 1996; Bonkowski et al. 2000; Tarao et al. 2008).

The research reported here focuses on the effects of the addition, individually, of glucose, amino acids, and citric acid on soil respiratory CO_2 production and levels of acid phosphatase activity in organic-rich soil samples from the Alaskan tundra, and from mineral-rich temperate forest soil collected in the Northeastern USA for comparative analyses and to provide context. Furthermore, we examined how these added sources of organic C impacted the abundances of bacteria, heterotrophic nanoflagellates (HNF), and naked amoebae. Comparing the effects of different forms of organic enrichment on the soil microbial community is based on fundamental biochemical differences between the chosen organic amendments. Glucose is more likely to enter directly into the glycolytic respiratory pathway than amino acids or carboxylic acids, but biological effects of different added organic substrates can be complex, depending on soil composition, biogeographic locale, and plant–microbe interactions (e.g., Schutter and Dick 2001; Stevenson et al. 2004; Koranda et al. 2014), and also due to priming effects that mobilize existing sources of soil metabolizable organics (Fontaine et al. 2003; Dijkstra et al. 2013). Substantial research has been published on the effects of added organics in temperate soil, but similar observations for Arctic soils are sparse (Clarholm 2005; Anderson 2010a, 2012a).

Overall, our goal is to provide experimental evidence on likely effects of dissolved organic C (DOC) enrichment on microbial communities in organic-rich tundra soil and make comparisons to more mineral-rich temperate soil as a contextual reference to discover similar or different response patterns. While the laboratory experimental results are reported for tundra samples collected from a single geographic locale, the results are part of a larger research program on tundra soil microbial communities and their role in the C biogeochemical cycle and are therefore integrated into a larger dataset obtained from other tundra sites examined in prior studies in this multi-year research program (Anderson 2008, 2010a, b, 2012a, b, 2014; Anderson and McGuire 2013; Anderson et al. 2016), increasing the potential generality of the findings.

Research questions

1. What are the effects of DOC enrichment on bacterial and eukaryotic microbial biomasses, respiration, and acid phosphatase activities in both soil types?
2. Are the relationships between microbial biomass and respiration or acid phosphatase activity comparable in both soil types?
3. Does DOC enrichment alter the microbial food web in both soil types?

Materials and methods

Sampling sites and sample collection

Organic-rich tundra samples were collected in June (2014) at a site southeast of Utqiagvik (formerly Barrow), AK during the late spring melt (Fig. 1). The vegetation at the sampling sites was mainly moss with sparse growth of sedge plants. Each sample was obtained using a shovel and ice pick to remove approximately the upper 5 cm of soil overlying the frozen base in a roughly 15-cm radius. Three samples were taken from the top, middle, and base of three different hummocks that were within approximately 30 m of each other (nine samples in total). Each sample was enclosed in a separate plastic bag, placed in an insulated container with ice packs, returned to the Lamont–Doherty Earth Observatory by overnight air express, and stored briefly in a walk-in cold temperature room (3 °C) before the experiments began. We chose samples from hummocks because the distribution of soil from the top to the bottom of the hummock represented a range of tundra soil from drier to more moist conditions. Samples corresponding to the same elevation on a hummock (top, middle or base) from each of the hummocks sampled were combined and thoroughly mixed to provide three composite Arctic samples for use in the amendment experiments, one from each elevation on the hummocks (A_{top}, A_{mid}, A_{base}, respectively). Pooling across hummocks was necessary because it would have been logistically unfeasible to conduct the entire suite of analyses on each soil sample individually. The mean moisture content for the tundra samples was 78%, as is characteristic for water-rich peat soil in early spring. Total organic content based on high-temperature combustion (375 °C) for tundra tussock soil is typically in the range of 80% (w/w), but can be lower (~20%) for open tundra moss-rich soil (e.g., Anderson 2012a). When peat-rich tundra soil is compressed, a dark-brown, organic-rich soil suspension is expelled. Bockheim et al. (1999) estimated that the mean DOC for sites near Barrow, AK for the entire 64-km^2 terrestrial area that they studied was ~50 kg m^{-3}. After arriving at the laboratory, samples were placed at 20 °C, approximating summer surface temperatures of the soil in the tundra at locations more broadly on the North Slope, before the experiments were begun, as also described below for the temperate soil.

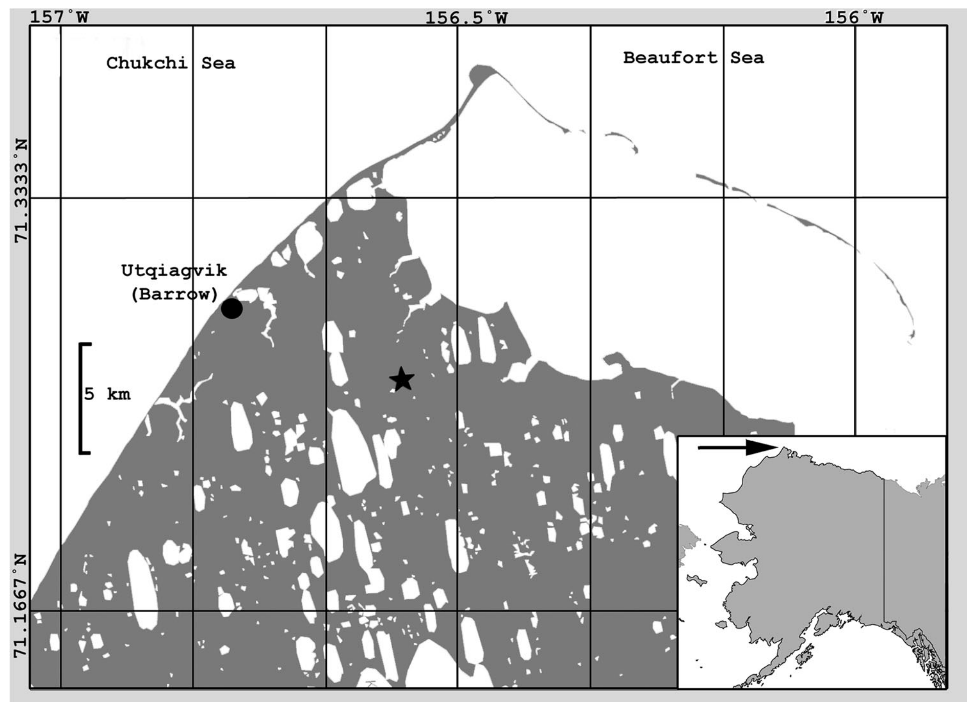

Fig. 1 Map showing the tundra soil sampling location (sampling centered around 71.26006, −156.57152, indicated by the *star*) relative to the town of Utqiagvik (formerly Barrow), AK, USA. The *arrow* on the *inset* outline of Alaska highlights the part of Alaska shown in detail on the map

For comparison and context, nine mineral-rich temperate soil samples were collected on the Lamont–Doherty Earth Observatory campus in the vicinity of a deciduous tree stand in a shallow hollow (41.00405, −73.909). The samples were collected in late autumn before the first frost, when the soil was chilled but not frozen, thus reducing the number of active small rootlets that could contribute to plant-derived respiration measurements in the core sample. The nine samples were obtained from surface soil in the understory of deciduous trees (largely maples, pin oak and white oak, with scattered small saplings) along an elevation gradient representing soil from drier conditions at the top of the slope to more moist conditions at the base of the slope. A 2.5-cm diameter Lamotte sampler (model EP) was used to collect small cores to a depth of 5 cm, including a thin organic-rich surface layer and a more mineral-rich underlying layer. At each of three elevations (top, midpoint, and base), three soil samples (approximately 2 m apart) were taken and then combined to yield three composite samples (T_{top}, T_{mid}, $T_{\mathrm{base,}}$) for use in amendment experiments, representing the three sampling elevations, respectively, consistent with the sampling regime at the Arctic sites.

Each mineral-rich temperate soil sample was preconditioned before experimental treatment by removing all visible small rootlets and any evidence of invertebrates. The soils were not sieved. The samples were placed in plastic bags and incubated without light in a temperature-controlled cabinet for three to four days to bring them slowly to the experimental assay temperature of 20 °C, more typical of the growing season. Also, the incubation pretreatment allowed sufficient time for any potentially-remaining rootlets in the soil to become less metabolically active. The mean moisture content for the temperate site samples was 35%, which is within the usual range that we find for near-surface soil collected during the summer or autumn at this location. Total organic content, based on high-temperature combustion (375 °C), was in the range of 15% (w/w).

The temperate soil samples were taken as examples of representative mineral-rich soil to provide context for the experiments with the tundra soil. The purpose was not to represent the same season as the spring samples from the tundra, because our study did not address seasonality, but rather to examine the effects of added DOC under controlled temperature in a laboratory experimental study. Moreover, because the laboratory methods for controlled microcosm culturing, microbial biomass analyses, and respiration were time consuming (especially the amoeba enumeration), we completed the tundra soil study immediately after collection and began the temperate soil study later in the autumn when we could devote full attention to the analyses. However, the sampling design and laboratory procedures were identical to those used for the tundra soil samples.

Experimental design

The experimental objective was to examine the effects of organic enrichments on the two soil types. Thus, treatments

represent different organic amendments, and replication within each treatment was based on repeating the treatment using different composite soil samples. The design of the experiments was the same for the tundra soil and for the mineral-rich temperate soil. Subsamples (from each one of the composite Arctic and temperate soil samples, A_{top}, A_{mid}, A_{base}, and T_{top}, T_{mid}, T_{base}, respectively) were used to prepare control (non-supplemented) and organic-supplemented treatments. Thus, the Arctic soil controls consisted of three replicates (one each from A_{top}, A_{mid}, A_{base}), and each of the three organic amendments (glucose, amino acids, and citrate) included three replicates (one each from A_{top}, A_{mid}, A_{base}). The design was identical for the temperate soil experiments. Therefore, a total of 24 soil subsamples were assayed (12 for the Arctic tundra samples and 12 for the temperate samples) with $n = 3$ (one replicate for each elevation) within each treatment. Experiments with both soil types were conducted at 20 °C using a constant temperature culture chamber. Respiration assays were also made at 20 °C as explained more fully below.

Enrichment with organic compounds

For each replicate, a 60-g soil subsample (from either A_{top}, A_{mid}, A_{base}, or T_{top}, T_{mid}, T_{base}) was placed into a 250-ml Nalgene® square bottle. The initial baseline respiration rates were assessed, as described below, before the experimental amendments were begun. After the initial baseline respiration rate was measured, each Nalgene bottle containing a soil subsample was amended by adding 4 ml of one of the following: glucose solution (2% w/v), citric acid monohydrate solution (2% w/v), or amino acids (MEM tissue culture solution, Sigma-Aldrich Chemical Co., St. Louis, MO), representing low-molecular weight carbohydrates (glucose), organic acids (citric acid), and amino acids (MEM solution), respectively. The control subsamples received 4 ml of 0.22-μm-filtered deionized water only. The citric acid and glucose solutions were prepared in 0.22-μm-filtered deionized water. The amino acid MEM solution was 2.8% (w/v) all totaled for the twelve amino acids in sterile water as supplied by the vendor. The C content of the organic solutions was as follows: glucose (8 mg ml^{-1}), citric acid (7 mg ml^{-1}), and amino acids (13 mg ml^{-1}). The concentration of glucose solution used was within the range of ~50–100 mM typically used in soil respiration studies (e.g., Jones and Murphy 2007) and produced a concentration in the soil that may be representative of carbon sources exuded by plant roots in the rhizosphere (e.g., Anderson 2012a). The amino acid and citric acid concentrations used were chosen to be within the same range as the glucose solution. The effects of these organic compounds were examined individually, rather than combined, to better understand the effects of different forms of DOC on soil microbial communities.

Estimation of bacteria, HNF, and naked amoebae densities and conversion to biomasses

Concentrations and C-biomass of bacteria, HNF, and amoebae were measured on Day 7 following amendment. A 1-g subsample from each Nalgene bottle was suspended in 5 ml of 0.22-μm-filtered, deionized water, and subsamples were then removed for amoeba enumeration using our standard microscopic culture observation method (COM) (e.g., Anderson 2008). The remaining soil suspension was fixed with 2% TEM-grade glutaraldehyde and refrigerated until bacteria and HNF were counted using acridine orange fluorescent counting techniques (e.g., Anderson et al. 2001). Three replicate counts each of amoebas, HNF, and bacteria were conducted on each soil subsample. Thus, given 24 bottles (12 each for the Arctic and temperate soil) and triplicate measurements, 72 counts were made for each of the three groups of microbiota (i.e., amoebae, bacteria, and HNF). Along with cell numbers, the sizes of each group were also determined to estimate C-biomasses using size-based regression equations, as previously published (Pelegri et al. 1999; Anderson 2006, 2008, 2012a). Bacteria, HNF, and amoeba biomasses were expressed as μg C g^{-1} soil dry weight.

Respiration assays

As mentioned above, baseline respiration rate measurements were taken before addition of DOC, to confirm that there were no differences between subsamples from the same elevation in either the tundra or temperate experiments. Following these baseline measurements, the organic enrichments (or deionized water for controls) were added and respiration was immediately assessed again. These post-amendment data are designated as respiration rates for Day 1, and comparisons of the amended respiration rates with those of the controls indicate the magnitude of the gain in respiration resulting from added DOC. After 6 days of incubation at 20 °C, respiration rate was measured again on Day 7. At each time point, the respiration rate of each 60-g soil sample was measured using an infrared gas analyzer (Vernier® Software Technology, Beaverton, OR) inserted in the Nalgene, soil-containing respiration bottle, maintained at 20 °C in a constant temperature bath (Isotemp model® 3006D; Fisher Scientific, Pittsburgh, PA) as published previously (Anderson 2008, 2010b). At each time point, respiration rate was measured at least three times successively for each bottle to estimate method error. Thus, all totaled, the number of respiration rate measurements made, including at least triplicate measurements on

each of the 24 experimental bottles, was 216 (72 for the Day 1 baseline measurements, another 72 post DOC addition on Day 1, and 72 Day 7 measurements). The experimental temperature of 20 °C was chosen as a representative summer surface temperature found in the temperate USA and also during the warmest periods in Alaska at locations broadly across the North Slope. The timing of respiration measurements was based on evidence from prior soil organic enrichment studies that microbial community respiration response to added organics is rapid (within minutes), but then tends to reach a steady state 5–7 days after enrichment (e.g., Jones and Murphy 2007; Anderson 2012a). Respiration rate was expressed as nmol CO_2 min^{-1} g^{-1} soil dry weight.

Acid phosphatase assays

For both tundra and temperate soils, subsamples from each of the glucose-amended and control Nalgene bottles were taken following completion of the respiration measurements at seven days and suspended in deionized water. Acid phosphatase activities were obtained using a Sigma Chemical Co. (St. Louis, MO) assay kit (CS0740), using citric acid buffer (pH 5.0) with paranitrophenyl phosphate substrate, according to the supplier's directions. Only the glucose experimental treatments were compared to the controls for this portion of the research, because glucose amendment produced the highest bacterial concentrations in the tundra experiments by Day 7. Acid phosphatase activity was expressed as µmol P $min^{-1}g^{-1}$ soil dw.

Statistical analyses

Analyses of respiration rates and microbial biomasses were based on a randomized block ANOVA with the blocks corresponding to the three elevation levels in the tundra and temperate sampling. Using a randomized block design, the control and treatment samples originating from a given composite soil sample (A_{top}, A_{mid}, A_{base}, or T_{top}, T_{mid}, T_{base}) were matched, controlling for any inherent differences related to elevation that would otherwise obscure treatment effects. Post hoc testing, based on the Holm–Sidak's test, compared treatments to their respective controls only, rather than also comparing treatments to each other, to increase power. Significant differences in the post hoc analyses were set at $\alpha = 0.05$. All t tests conducted were two-tailed. Bivariate relationships of soil respiration rates, acid acryl phosphatase activities, and microbial biomasses were based on least-squares linear regression. Prior to analyses, distributions were examined and found to be sufficiently normally distributed to use parametric methods. All analyses were conducted using a statistical package, Prism 6 (GraphPad software).

Results

Research question 1: DOC enrichment effects on bacterial and eukaryotic microbial biomasses, soil respiration rates, and acid phosphatase activity

Microbial biomass

Bacterial numbers and biomasses in the Arctic tundra soil declined significantly in all amended treatments relative to their respective controls by Day 7 (randomized block ANOVA, $F_{(3,6)} = 7.53$, $p = 0.02$, Fig. 2a). Meanwhile, the combined predator biomass of HNF and amoebae increased relative to controls (Fig. 2b). Pooling amended treatments, the average increase in bacterivore biomass in amended tundra samples was more than double the mean control value, a significant increase (t test, $t_{10} = 2.55$, $p = 0.03$). In contrast to the tundra samples, bacterial biomass of amended temperate soil treatments increased significantly relative to their respective controls by Day 7 (randomized block ANOVA, $F_{(3,6)} = 16.7$, $p = 0.003$, Fig. 2c). In addition, microbial bacterivore biomass in the amended temperate samples also increased significantly in all treatments (randomized block ANOVA, $F_{(3,6)} = 4.92$, $p = 0.05$, Fig. 2d). The mean bacterivore biomass in amended temperate soil treatments was approximately two-thirds higher than the mean of the control samples, a highly significant increase (t test, $t_{10} = 3.69$, $p = 0.004$).

Respiration rates

Mean respiration rates (at 20 °C) in the tundra samples increased significantly relative to the respective controls on Day 1 immediately following organic additions (randomized block ANOVA, $F_{(3,\ 6)} = 7.79$, $p = 0.02$, Fig. 3a). However, the increase was temporary. By Day 7, although average respiration rates in the amended samples remained slightly elevated, the differences in each treatment were no longer significant relative to the controls (randomized block anova, $F_{(3,\ 6)} = 2.43$, $p = 0.16$, Fig. 3b). In the temperate soil samples, respiration rates were consistently lower than those in the tundra samples when all treatments were pooled (t test, $t_{22} = 5.44$, $p < 0.0001$), and also when paired by treatment (paired t test, $t_{11} = 5.70$, $p < 0.0001$). However, it is important to note that the respiratory rates are expressed on a soil dry weight basis. These are the standard units used in our research program (and those of others), but differences in soil moisture content between the tundra and temperate samples are partially responsible for the higher rates observed in the tundra samples when expressed per unit of dry weight.

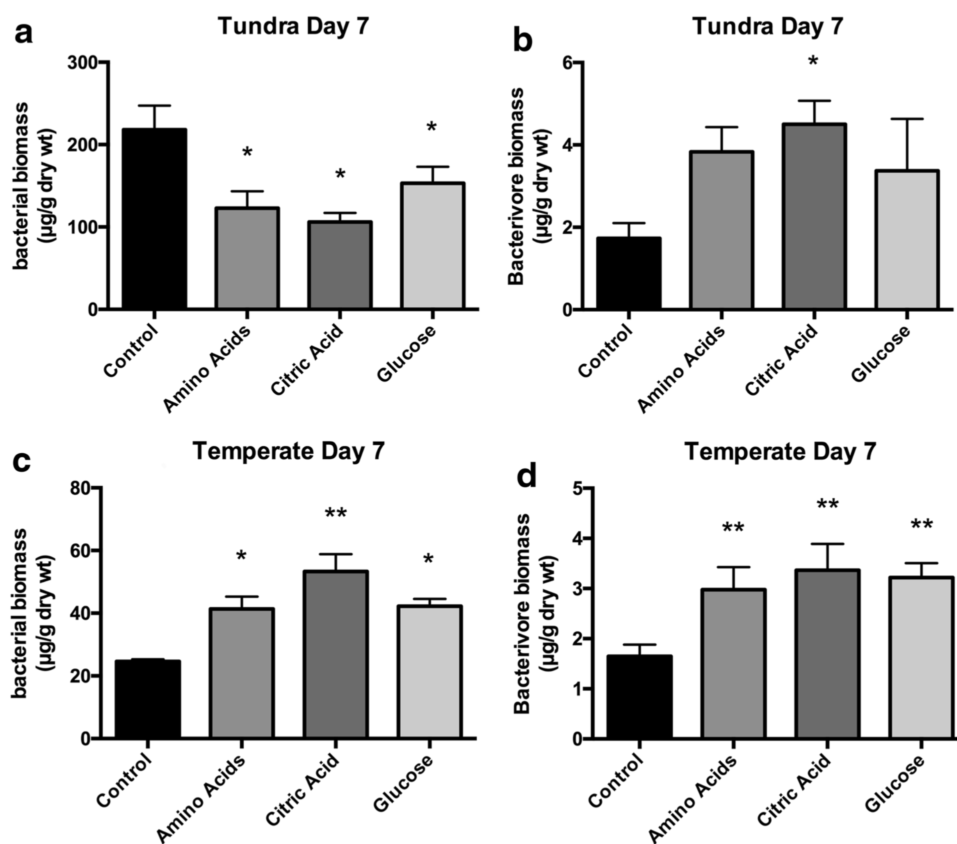

Fig. 2 Mean C-biomass of heterotrophic bacteria and bacterivorous protists (amoebas and HNF) in tundra (**a**, **b**) and temperate (**c**, **d**) soil samples on Day 7 after amendments. The mean shown by each *bar* is based on averaging counts from three different soil samples ($n = 3$), *error bars* = 1 SE. *Single asterisks* indicate significant differences ($p < 0.05$) and *double asterisks* highly significant differences ($p < 0.01$) from the respective controls, based on post hoc comparisons

As observed in the tundra samples, organic amendment of temperate samples resulted in significantly increased respiration rates (randomized block ANOVA, $F_{(3,\ 6)} = 10.6$, $p = 0.008$, Fig. 4a), though post hoc tests indicated that only the glucose and citric acid additions were significantly elevated relative to the controls. Respiration rates in all temperate soil treatments then declined to become more similar to the controls by Day 7, though the averages remained slightly elevated (Fig. 4b), also qualitatively consistent to the tundra samples. Despite the decline from Day 1, significant differences in respiration rates of amended samples relative to the respective controls were found on Day 7 (randomized block ANOVA, $F_{(3,\ 6)} = 4.92$, $p = 0.05$), though post hoc tests revealed that this was only for the citric acid additions. In both soil types, respiration rates of the Day 1 controls were similar to Day 7, indicating that changes in respiration rates through time were much smaller than the changes associated with organic additions. Thus, there were similarities in the response patterns shared across both soil types.

The respiration rates expressed on the basis of mg organic C added per g soil dw, i.e., normalized by dividing the rate by the equivalent C in mg added for each of the organic compounds, are presented in Table 1, including the normalized values for the change in respiration rate expressed as the difference between the treatment and control preparations. The normalized rates were consistently higher for glucose and citric acid compared to amino acid additions. In general, the rates on Day 1 were higher than on Day 7, consistent with the data presented in Figs. 3 and 4.

Acid arylphosphatase enzyme activity

Similar to the respiration rate comparison between systems, acid phosphatase activity was higher (on a dry weight basis) in the tundra samples (Table 2), and for both systems, phosphatase activity six days following glucose addition was similar to the respective controls. Although Day 7 phosphatase activity did not change with respect to amendment, when data from both environments were pooled, there was a strong, positive relationship between phosphatase activity and respiration rate (Fig. 5).

Research question 2: relationships between microbial biomass and bulk microbial respiration and acid phosphatase activities in the two soil types

The general relationship between respiration rate (at 20 °C) and bacterial biomass for Alaskan North Slope tundra samples was compiled from data in this study and several

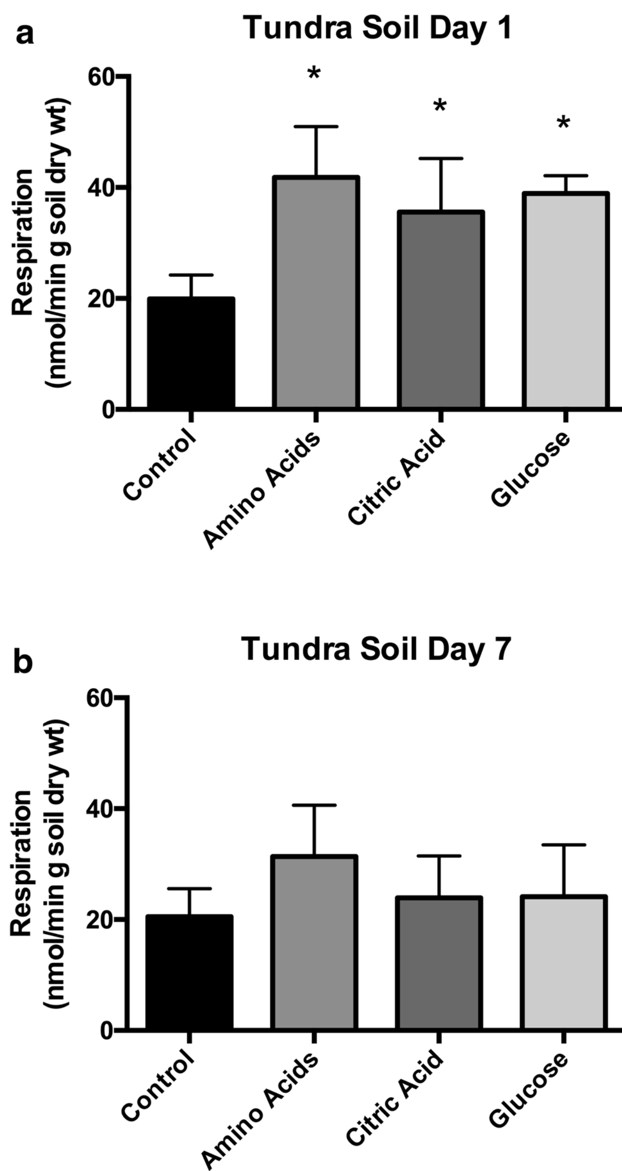

Fig. 3 Mean respiration rates of control and amended tundra soil, on Days 1 (**a**) and 7 (**b**), following amendment. The mean shown by each *bar* is based on averaging counts from three different soil samples ($n = 3$), *error bars* = 1 SE. *Asterisks* indicate statistical significance as described for Fig. 2

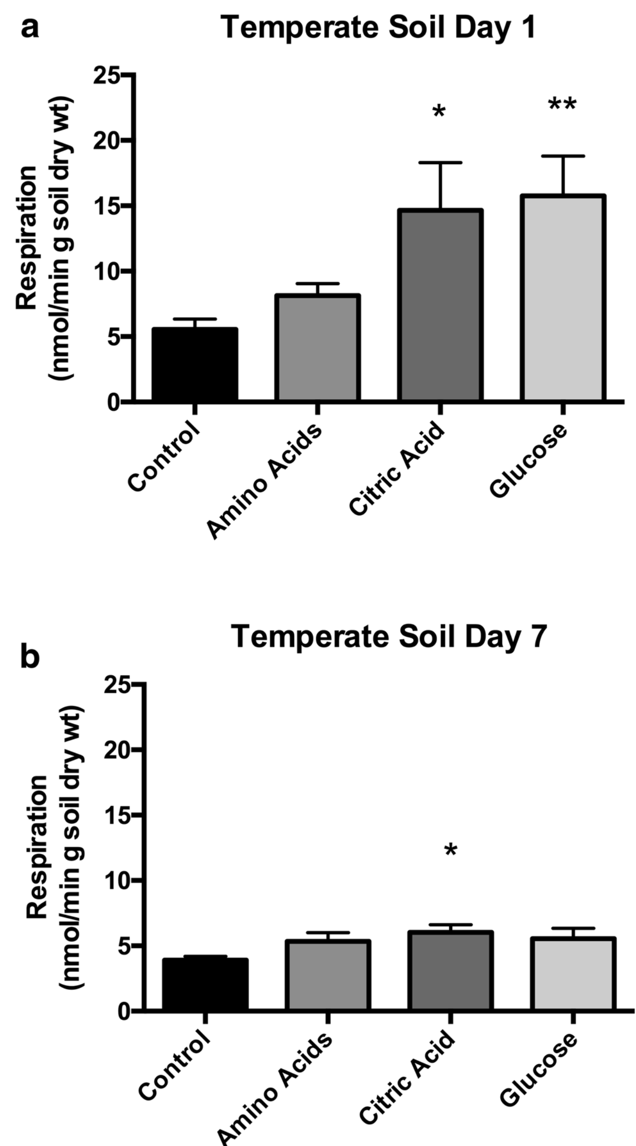

Fig. 4 Mean respiration rates of control and amended temperate soil on Days 1 (**a**) and 7 (**b**) following amendment. The mean shown by each *bar* is based on averaging counts from three different soil samples ($n = 3$), *error bars* = 1 SE. *Asterisks* indicate statistical significance as described for Fig. 2

earlier studies in this multi-year research program (Anderson 2012a, 2014; Anderson and McGuire 2013). Although tundra soil is rich in fungal biomass (e.g., Anderson et al. 2016), previous research has shown that bacterial densities are a good proxy for predicting microbial respiratory CO_2 emission in tundra soil (Anderson 2014).

As shown in Fig. 6a, tundra soil respiration rate compiled from previous studies increased linearly with bacterial biomass, with a regression slope of 0.11. Figure 6b shows respiration and bacterial biomass data from this study only, plotted as in Fig. 6a, compared to a line with a slope of 0.11. All of the temperate soil observations in this study, as well as data from the control tundra soil samples (open circles), fell close to the plotted line of the general bacterial biomass-to-respiration rate relationship shown in Fig. 6a (though note different scales because of the more limited range of values reported in this study). However, the amended tundra samples did not all follow the general trend (see next paragraph). Given the strong correlation between respiration rate and phosphatase activity (see above), it is not surprising that there was a positive

Table 1 Mean respiration data (respiration data for each experiment are the mean values of three replicate soil samples ($n = 3$) normalized for the amount of C equivalent grams added per g of soil dw) per mg C added (nmol CO_2 min^{-1} mg C^{-1}) and mean difference in respiration rate between treatment and controls per mg C added (Δ nmol CO_2 min^{-1} mg C^{-1}) in brackets on Days 1 and 7 for the organic enrichment treatments (amino acids, citrate, and glucose) for samples taken from the tundra tussocks and temperate, Northeastern USA site

Sample	Amino acids	Citric acid	Glucose
Day 1			
Tundra	10.9 [5.7]	17.3 [7.5]	16.4 [8.0]
Temperate	6.7 [2.1]	23.0 [14.0]	21.4 [7.4]
Day 7			
Tundra	8.1 [2.8]	11.5 [1.6]	10.1 [1.5]
Temperate	4.5 [1.2]	9.2 [3.2]	7.5 [2.2]

Table 2 Mean acid arylphosphatase activity (μmol min^{-1} g^{-1} dry wt.) and the relative activity expressed per nmol respiration rate (μmol nmol CO_2^{-1}) for the control and glucose enrichment treatments for soil samples from the northeast temperate site and the arctic tundra tussocks measured after 7 days of incubation (20 °C)

	Temperate site		Arctic tundra	
	Control	Treatment	Control	Treatment
Activity	0.81 ± 0.4	1.19 ± 0.4	10.9 ± 4.7	10.7 ± 3.9
Relative	0.06 ± 0.03	0.06 ± 0.02	0.12 ± 0.03	0.11 ± 0.03

relationship between bacterial biomass and phosphatase activity across environments (not shown, $y = 0.061x - 0.77$, $r^2 = 0.61$, $F_{(1,10)} = 15.5$, $p = 0.003$), mirroring the respiration vs. bacterial biomass relationship. The linear relationship found between bacterial biomass and respiration implies a consistent respiration rate per unit bacterial biomass. This consistent rate appeared to apply to the control tundra samples and all temperate soil samples (Fig. 6b). However, the deviation of some of the amended tundra samples from the general linear relationship indicated changes in respiration per unit bacterial biomass in amended tundra samples.

Research question 3: DOC enrichment effects on microbial food webs in both soil types

The decline in bacterial biomass in all amended tundra soil treatments, coincident with increases in amoeba and HNF biomasses, suggested strong predation pressure on bacteria in the tundra samples, potentially affecting bacterial respiration rates. Figure 6c shows the strong linear increase in respiration rate per unit bacterial biomass in tundra soil samples as a function of total predator biomass (amoebas plus HNF). Given that predatory protists made up no more than ~3% of the bacterial biomass on average, their direct contribution to total respiration would be minor. The data therefore suggest an indirect effect of predation on bacterial activity in tundra soils. For the temperate soil samples, by contrast, there was no significant relationship between predator biomass and bacterial biomass-specific respiration rate (not shown, $r^2 = 0.03$, $F_{(1,\ 10)} = 0.31$, $p = 0.59$).

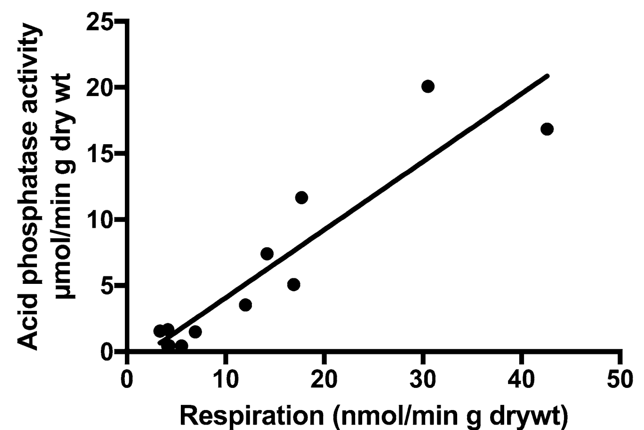

Fig. 5 Relationship between mean soil respiration rate and mean acid acryl phosphatase activity in temperate and tundra soils measured on Day 7 after amendment. Phosphatase activities were only measured in the control and glucose treatments ($n = 12$). The *solid line* represents the least-squares best linear fit to all data ($y = 0.052x - 1.1$, $r^2 = 0.86$, $F_{(1,\ 10)} = 58.8$, $p < 0.0001$)

Discussion

Changes in microbial community biomasses

In general, the soil microbial trophodynamics reported here are consistent with established principles of predator–prey relations. As the number of prey increases, there is a higher potential for the predators to gain access to the prey, increasing the source of nutrition, and thus enhancing the population of predators. Prior research with tundra soil microbial communities has provided evidence of classical trophic hierarchies, with smaller heterotrophic protists preying directly on bacteria, and the larger ones preying on a mixture of bacteria and small protists (e.g., Anderson and McGuire 2013).

In this study, organic amendment of the temperate soil led to increases in both bacterial and bacterivore biomasses, while in the tundra soil, organic amendments resulted in decreased bacterial biomass and even larger proportional increases in bacterivore biomass. There are

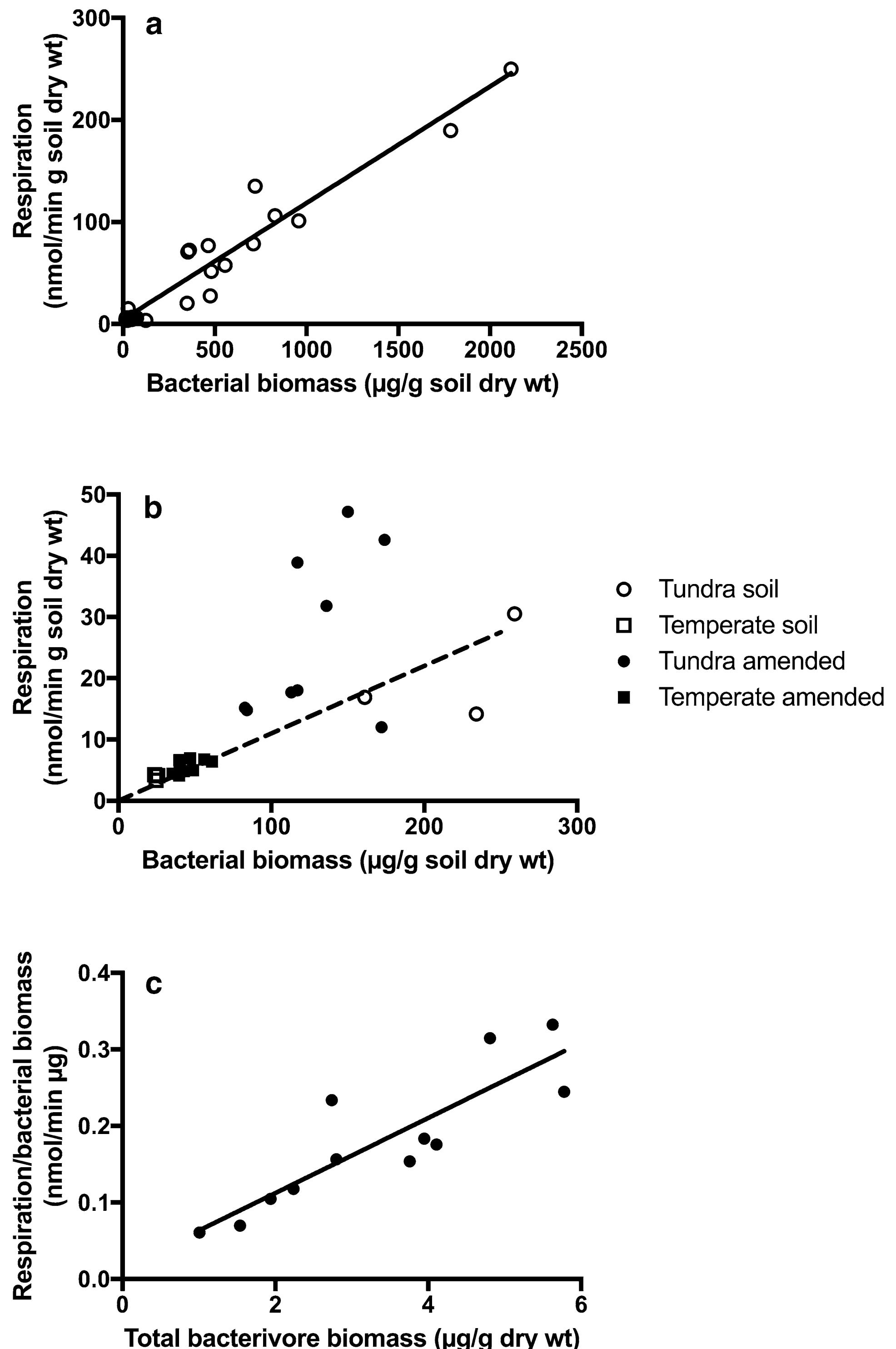

Fig. 6 Soil respiration-to-biomass relationships. **a** Tundra soil respiration rates as a function of bacterial biomass (compiled from Anderson 2012a, 2014; Anderson and McGuire 2013). Each *point* represents the mean respiration rate and bacterial biomass from a single soil sample ($n = 28$). *Vertical* and *horizontal error bars* associated with analytical error would not be visible for many points and are not shown. *Solid line* represents the least-squares linear best fit ($y = 0.11x + 4.4$, $r^2 = 0.93$, $F_{(1, 26)} = 331.5$, $p < 0.0001$). The y-intercept of the regression is not significantly different from zero. **b** Tundra and temperate soil respiration rates as a function of bacterial biomass in this study ($n = 24$, note different axis scales compared to Fig. 6a). For comparison, the *dashed line* shows a relationship with slope = 0.11 and y-intercept = 0, derived from Fig. 6a. **c** Bacterial biomass-specific respiration rate for tundra soil samples only ($n = 12$), as a function of total bacterivore biomass (HNF + amoebae). *Solid line* represents the least-squares linear best fit ($y = 0.05x + 0.01$, $r^2 = 0.76$, $F_{(1, 10)} = 31.4$, $p < 0.0002$)

several possible explanations for an increased impact of predation in the tundra compared to temperate soil samples, none of which are mutually exclusive. First, the increase in bacterivore biomass following organic additions was proportionally larger in the tundra samples than in the temperate soil samples. This difference was especially apparent in the amoeba biomasses. Greater bacterivore biomass, derived from increased available bacterial prey, likely indicates higher predation pressure in general (e.g., Lesen et al. 2010), and amoebae may be particularly effective bacterivores (Clarholm 1981; Butler and Rogerson 1995; Zubkov and Sleigh 1999). The greater

amoeba growth response in the organically enriched tundra samples, compared to the temperate soil samples, could be explained partially by the higher moisture content in the tundra soil, providing a more optimal environment to support amoeba exploitation of bacterial prey.

As a second point, the control tundra bacterivore biomasses may have been unusually low in this study. Anderson and McGuire (2013) found that bacterivore biomass comprised at least 5–10% of bacterial biomass in tundra samples, similar to the temperate samples examined in this study. However, in the tundra samples examined here, control bacterivore biomasses were only around 1% of bacterial biomasses. The low relative bacterivore biomass in the tundra controls suggests that initial bacterivory was very low, which may have led to a bacterial community that was unusually susceptible to increased predation pressure. For example, different sizes and types of bacteria can experience different predation pressure (Hahn and Höfle 1999; Boenigk et al. 2004; Corno and Jürgens 2008; Tarao et al. 2009).

The third possibility is that the difference in predation impacts between temperate and tundra soils was related to the degree of nutrient limitation of bacterial growth. When bacteria are nutrient limited, predation can increase biomass-specific respiration rates, or other measures of bacterial activity, as apparently occurred in the tundra samples, because predation increases nutrient cycling by liberating nutrients sequestered in bacterial biomass (Clarholm 1985; Bonkowski et al. 2000; Ekelund et al. 2009). However, this enhancement of specific activity would not be expected if bacterial growth were not nutrient limited (Mamilov et al. 2001). These three possibilities are not mutually exclusive, and other potential explanations could also revolve around changes in bacterial or bacterivore species composition, or other complex interactions within the soil microbial community. In any case, while this example highlights the potential importance of protist predators on microbial activity in tundra soils, it does not necessarily indicate a consistent feature of tundra soils. For example, Anderson and McGuire (2013) found that glucose addition to tundra soils increased both bacterial and protist biomasses, more similar to the observed changes in the temperate samples of this study.

Respiration rates

As demonstrated in Fig. 6a, b, the tundra respiration rates measured in this study overlapped with the values measured in previous work, though at the lower end of the potential range. Although the range of Alaskan tundra data summarized in Fig. 6a is quite broad (4–250 nmol CO_2 min^{-1} g^{-1}), it is comparable to the range of respiration rates measured across nine European and Siberian tundra sites (~30–200 nmol CO_2 min^{-1} g^{-1} at 15 °C, Christensen et al. 1999). Previous respiration rate measurements for the temperate forest soil site sampled for this study averaged approximately 5 nmol CO_2 min^{-1} g^{-1} (Anderson 2012b), very comparable to the current values. Cross-system comparisons of respiration rates comparable to this study are relatively rare, but Bekku et al. (2003) directly compared the respiration rates of Arctic and temperate soils in a range of temperatures, reporting the rates at 20 °C of approximately 20–35 nmol CO_2 min^{-1} g^{-1} for Arctic, and approximately 4–13 nmol CO_2 min^{-1} g^{-1} for temperate soil samples, both very similar to the values reported in this study.

All three sources of C enrichment (amino acids, citric acid, and glucose) showed statistically significant increases in respiration rate for the tundra soil. For the temperate soil, although the mean respiration rate also increased following amino acid addition, only the citric acid and glucose showed significant increases compared to the control. Overall, these data add further evidence that increasing available DOC from a variety of sources accompanying global warming can increase respiratory CO_2 loss to the atmosphere from different soil types. However, the composition of the DOC added made a difference in the respiratory C loss. In this respect, it is also important to acknowledge that some of the changes observed in respiration rates after the addition of organic supplements could be due to a priming effect (as noted in the Introduction). Priming increases microbial access to existing organic matter that would otherwise be refractory, not readily metabolized in the absence of other sources of DOC. The design of our experiment did not provide evidence of the source of C in the respiratory CO_2, and some of it could have been from enhanced decomposition of existing soil organic matter. Thus, in analyzing soil C budgets and accounting for changing pools of soil organic C, distinguishing the sources of the organic C that result in respiratory CO_2 production can be important. Moreover, from the perspective of tundra soil respiration, future research will have to consider the form of DOC that will be released into tundra soils due to climate warming. For example, it seems likely that plant exudates would have a different organic composition than older organic material released from thawing permafrost, or DOC derived from a priming effect, which would feed back differently to the respiratory losses from the soil. However, the rapid response to organic additions reported here suggests that the microbial response to plant exudates in the rhizosphere is likely to be almost immediate, and that the exudates themselves would be one of the primary C sources for the respiratory CO_2 released.

Comparing rates and bacterial biomasses

While the respiration rate per unit bacterial biomass of the temperate soil remained consistent after organic enrichment with the general relationship shown in Fig. 6a, the bacterial biomass-specific respiration rate rose in many of the amended tundra soil samples. Moreover, the bacterial biomass-specific respiration rates of the tundra samples were strongly related to increasing bacterivore biomass. A similar relationship between biomass-specific respiration rate and bacterivore biomass was not observed in the temperate soil samples. Together, these observations are consistent with the above description of a greater impact of bacterivory in the tundra samples.

The general relationship between respiration rates (aggregated for the entire microbial community) and bacterial biomass for tundra soils (at 20 °C, combining data from previous studies) provides a reasonable fit for both the control tundra observations and all temperate soil observations made during this study. Similarly, a single acid phosphatase activity-to-bacterial biomass relationship can be fit to data from both soil types. Although these particular tundra-to-temperate comparisons are unique, other studies have similarly found strong relationships between measures of aggregate microbial activity and bacterial biomass across different soil types (e.g., Cookson et al. 2007; Xu et al. 2015), indicating that much of the variability in microbial rates (especially when measured or normalized to a constant temperature) is associated with variability in bacterial biomass. This does not imply that rates per unit biomass should be interpreted as constants. For example, respiration per unit microbial biomass is often observed to vary by a factor of 2–3 across different soil types or conditions (e.g., Dilly and Munch 1998). However, as shown in Fig. 6a, bacterial biomasses span a much wider range and consequently have a larger effect on spatial, or cross-system, variability in aggregate rates.

Despite the strong relationships between microbial activities and bacterial biomass, it is important to recognize that bacteria may not actually be the dominant source of soil respiratory activity. In tundra soils in particular, fungi can have a large contribution to microbial biomass (Anderson and McGuire 2013; Buckeridge et al. 2013; Anderson et al. 2016). Available evidence suggests that tundra fungi are active and respond to environmental perturbations (Clemmensen et al. 2006; Buckeridge et al. 2013; Anderson et al. 2016), although their contribution to bulk respiration rate, and other microbial activities, has not been separately quantified. Nevertheless, bacterial biomass is a good predictor of soil respiration and may also be a good proxy for soil microbial biomass based on correlations in other tundra soil studies (Anderson and McGuire 2013; Anderson 2014). Quantifying the relative contribution of bacteria and fungi to tundra soil microbial activities is clearly an area that deserves further research attention.

Linking carbon and phosphorus cycles

Although acid phosphatase activity in the Arctic tundra samples was approximately an order of magnitude greater (per unit dry weight) than in the temperate soil, the strong relationship shared across soil types between respiration rate, a major component of the soil C cycle, and phosphatase activity, an important process in soil P cycling, suggests that C and P cycling in both soil types are linked in similar ways. This has implications for understanding potential CO_2 losses from tundra soils as the Arctic climate warms.

As reported here, and noted by Spohn et al. (2013), increased microbial respiration in tundra soils should lead to increased release of acid phosphohydrolases, or other sources of phosphate remineralization in the surrounding soil, a process documented in other soil ecosystems (e.g., Gressel et al. 1996; Allison and Vitousek 2005; Hou et al. 2015; Xu et al. 2015). Moreover, there is evidence that the addition of easily-available organic C sources could have promoted fast-growing microorganisms that have a high phosphorus demand (e.g., Elser et al. 2003). In many soil types, mobilization of free phosphate can increase plant productivity and enhance soil fertility (e.g., Richardson and Simpson 2011). While warmer temperatures and greater availability of DOC related to climate change should increase tundra soil respiration (as described above), the concomitant enhancement of P availability may also create a more favorable environment for plant growth (assuming other nutrients are sufficiently available), contributing to invasion of the tundra habitat by higher plants. Increased photosynthetic uptake of CO_2 by higher plants could counterbalance the production of respiratory CO_2 by soil microbes, though the quantitative importance of this potential feedback remains unknown. A diagrammatic depiction of these links between soil C and P cycling is shown in Fig. 7.

While microbial activities link C and P cycles in diverse soil types, more research is needed to quantify the stoichiometry of these connections. For example, in the current study, at the temperate site, $\sim$0.06 μmol of P was remineralized for each nmol respiratory CO_2 released from the soil. For the tundra samples, the values averaged $\sim$0.11 μmol per nmol CO_2. While that is a substantial difference, it is much smaller than the differences in total phosphatase activity between the two soil types. Future research should address whether the composition of the C source affects the amount of P remineralized per unit respiratory activity, and how environmental factors such as

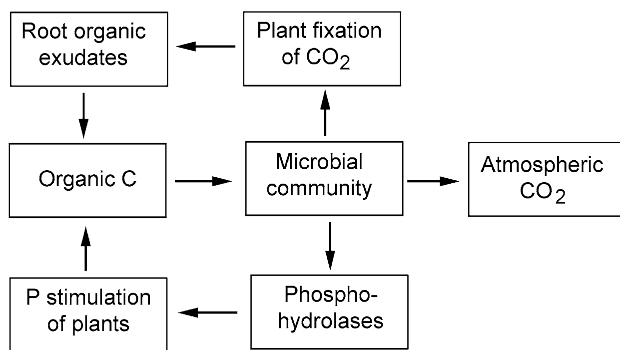

Fig. 7 Diagram of C and P biogeochemical cycle coupling. Organic C, assimilated by microbes, is respired partially as CO_2 to the atmosphere. Part of the photosynthetically fixed C may be secreted by roots as organic C; assimilated, and partially sequestered, by microbes in biological matter, or used metabolically to secrete enzymes, including phosphohydrolases. The latter promote remineralization of free P as potential nutrients for enhanced plant growth. This may lead to additional root exudation of organic C or other sources of C including decaying plant matter. Elevated plant productivity also may be a sink for photosynthetically fixed CO_2

temperature, moisture, and chemical composition of the soil influence this coupling between C and P cycles. Moreover, it is well established that nitrogen is often a limiting nutrient for soil microbes and plant productivity, especially in the tundra, and also some temperate soils (Hobbie et al. 2002; Sistla et al. 2012). Under conditions where phosphate remineralization is sufficient to promote plant growth, but where nitrogen is limiting, there would likely be little gain in plant productivity. Although we have focused on the linkage between the C and P cycles, as an example of the mediating role of soil microbes in linking the two cycles, further research is needed to address nitrogen remineralization driven by microbial activity (e.g., Clarholm 1985), as well as an examination of nitrogen-fixing microbes, to more fully explore N sources and dynamics in tundra soils.

Conclusions

Climate warming in the Arctic is predicted to increase the availability of DOC in tundra soils, through melting of permafrost and through the expansion of vascular plants potentially secreting DOC from roots. In this study, DOC additions to tundra soils increased microbial respiration rates, in parallel with observations of temperate soil samples. However, because microbial respiration and phosphatase activities appear to be linked in tundra soils, in a similar way as in temperate environments, increased P cycling concomitant with increased respiration could at least partly oppose the net release of soil C because of enhanced plant productivity driven by the increase in bioreactive phosphate. The composition of the organic matter added affected the fraction of the addition that was respired. Bacterial biomass explained most of the variability in respiration rates across both tundra and temperate soil samples, even though bacteria, per se, are only one component of the microbial community. Nevertheless, data from the tundra soil highlight that protist predation can have large impacts on bacterial biomass and biomass-specific activity rates.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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