ACTA
PROTOZOOLOGICA

Acta Protozool. (2016) 55: 189–196
www.ejournals.eu/Acta-Protozoologica
doi:10.4467/16890027AP.16.018.5750

Microbial Respiration of Organic Carbon in Freshwater Microcosms: The Potential for Improved Estimation of Microbial CO_2 Emission from Organically Enriched Freshwater Ecosystems

O. Roger ANDERSON

Biology, Lamont-Doherty Earth Observatory of Columbia University, Palisades, New York, USA

Abstract. Respiratory CO_2 emissions from laboratory freshwater microcosms enriched with organic C (glucose and amino acids) and kept in the dark at 24°C were compared to control microcosms without C enrichment for two different freshwater pond sources. The purpose was to estimate experimentally the rate of respiratory CO_2 emission from organically polluted freshwater ecosystems compared to non-enriched water. Experiment One, used pond water collected at the Lamont-Doherty Earth Observatory campus; and Experiment Two used pond water, inoculated with natural detritus, obtained from North Carolina. At peak respiration, the net efflux of CO_2 (enriched minus control) to the atmosphere was ~ 90 nmol min^{-1} L^{-1} (Day 7, Experiment One) and ~ 240 nmol min^{-1} L^{-1} (Day 3, Experiment Two). The corresponding net efflux of C to the atmosphere was 25 nmol C min^{-1} L^{-1} (Day 7, Experiment One) and 65 nmol C min^{-1} L^{-1} (Day 3, Experiment Two). Peak CO_2 emissions from the organic-enriched microcosms expressed as µmol m^{-2} s^{-1} (as more typically reported for natural bodies of water) were 0.20 for Experiment One, and 0.42 for Experiment Two, at a surface layer depth of approx. 20 cm, i.e. the microcosm depth. There was a relatively large correlation between respiratory CO_2 emission and bacterial densities in the organic-C enriched microcosms ($r = 0.76$), but a smaller correlation ($r = 0.32$) in the non-enriched, control microcosm. Further broad scale research, robustly sampling natural bodies of organically polluted water, is needed to confirm and better establish the results of the research reported here using microcosms.

Key words: Aquatic microbial CO_2 emissions, atmospheric CO_2, climate change, environmental change, organic aquatic pollution, protists

INTRODUCTION

Considerable attention has been given to increasing evidence that anthropogenic sources of atmospheric CO_2 are contributing to global climate change, especially global warming and consequent deleterious effects on our environment (e.g. Broecker 1975, Friedlingstein *et al*. 2010, Karl and Trenberth 2003, Solomon *et al*. 2009). Likewise, there is increasing evidence that nutrient pollution (particularly N and P sources) of natural water bodies can also lead to substantial ecological changes, including algal blooms and their decay that release organics, contributing to increased microbial respiratory activity and additional sources of CO_2 emissions to the atmosphere (e.g. Casper *et al*. 2000, Smith *et al*. 1999, Schrier-Uijl et *al*. 2011). In addition to impacts on the natural envi-

Address for correspondence: O. Roger Anderson, Biology, Lamont-Doherty Earth Observatory of Columbia University, 61 Route 9W, Palisades, N. Y. 10964, USA; Tel.: +01 845 365 8452; Fax: +01 845 365 8150; E-mail ora@LDEO.columbia.edu

ronment there are also serious consequences for human endeavors (e.g. Carpenter *et al*. 2011), including indirect evidence of elevated CO_2 efflux from turbid, usually organically-enriched, urban estuaries (e.g. Anderson 2016, Frankignoulle *et al*. 1998) and from shallow lakes and inland aquatic systems (e.g. Cole *et al*. 2007, Davidson *et al*. 2015, Richey *et al*. 2002, Wilkinson *et al*. 2016), thus potentially contributing to global warming. Considerable attention has been given to bacterial production and organic C transformation in aquatic ecosystems (e.g. Cole and Kinne 2013, Cole *et al*. 1988, Pollard and Ducklow 2011), including evidence that respiration rates in bacteria can exceed phytoplankton production in some freshwater lakes (e.g. del Giorgio *et al*. 1997), and that night CO_2 effluxes can exceed daytime primary fixation (Liu *et al*. 2016). However, additional experimental research is needed to assess the effects of organic C pollution (e.g. raw sewage effluent, runoff from animal husbandry, food processing wastes, etc.) on aquatic CO_2 exchange with the atmosphere. This is a report of a laboratory experimental study to assess the effects of exogenous organic C enrichment on freshwater heterotrophic microbial communities (particularly bacteria and bacterivorous ciliates) and consequent respiratory CO_2 emission to the atmosphere based on controlled experiments in laboratory microcosms. This provides some of the first experimental evidence for the effects of organic C-enrichment on freshwater microbial communities coupled with measurements of atmospheric respiratory CO_2 emissions that may provide insights into anthropogenic organic pollution of freshwater ecosystems and its effects on the biogeochemical C cycle.

Research questions

1. What are the effects of organic C-enrichment (glucose + amino acids) on rates of respiratory CO_2 emissions from pond freshwater microcosms compared to non-enriched freshwater microcosms?
2. What are the effects of the organic C-enrichment on the bacterial and ciliate densities in freshwater microcosms compared to non C-enriched microcosms?
3. If there are differences in the respiratory CO_2 emission rates for microcosms prepared with freshwater from different pond sources, to what extent can any differences in the densities of bacteria and ciliates provide evidence to at least partially explain the differences in respiratory CO_2 emission rates?

MATERIALS AND METHODS

Pond water samples

Two sources of pond water were used for the Experiments. For Experiment One, water was collected from a freshwater shallow pond on the campus of the Lamont-Doherty Earth Observatory on October 27, 2015 and stored in a polyethylene carboy at 24°C in a walk-in temperature controlled culture room at our laboratory until the experiment began on October 28, 2015. For Experiment Two, microfiltered pond water collected at a freshwater source in North Carolina, especially prepared for laboratory culture work, was obtained from Carolina Biological Supply Company (Burlington, N.C.) and inoculated with dried pond detritus (2.5 g L^{-1}) supplied by Carolina Biological to provide an inoculum of microbes in the microfiltered pond water. This was used to broaden the geographic source of pond water used in the experimental study. The prepared pond water was also stored in a polyethylene carboy in the walk-in controlled temperature room until Experiment Two was begun within one week on Dec. 14, 2015.

Experimental procedures

Culture preparations. Nalgene 2,500 ml bottles (Cat. No. 84-2000) were used for the microcosm experiments maintained in a temperature-controlled, walk-in culture room at 24°C. Each Nalgene bottle was wrapped in aluminum foil to exclude light and promote heterotrophic growth of the microbial communities. The total pond water added per bottle was two liters, leaving a head space of 280 cm^3. The surface area of the pond water in the microcosm bottle was 106 cm^2, with a surface to volume ratio of 0.052 cm^2/cm^3, and a depth of ca. 20 cm. The microcosm bottles were lightly capped during incubation between intervals when respiration measurements were made and water samples were taken for bacterial and ciliate counts. All experiments were run in triplicate with a total of 12 microcosms. C-enriched microcosms were amended by adding 0.2 g dry reagent grade glucose and 2 ml of a sterile amino acid mixture (MEM, Sigma Aldrich, St. Louis, Mo.) containing 12 amino acids with equivalent total weight of 0.03 g/ml. This mixture of glucose and amino acids was used to provide a broad source of C from carbohydrate and nitrogen-containing compounds. The pond water was thoroughly mixed by swirling after addition of the organic nutrients. Thus, the total C-equivalents added per L of pond water were 40 mg from the glucose, and 14 mg from the MEM amino acid solution. Control microcosms were identical to the experimental microcosms, but only 2 ml of deionized water were added to be equivalent to the volume of MEM solution added in the experimental microcosoms.

Respiration measurements. Respiration was measured at least three times successively at each sampling time (to estimate method error) using an infrared CO_2 gas analyzer (Vernier, Beaverton, OR) with the sealed probe inserted into the screw cap opening of the microcosm bottle. All measurements were made at the culture room temperature of 24°C. Respiration measurements with each microcosm were made for a period of one week on Days 1, 2, 3, 5 and 7, following Day 0 when the experimental microcosms were initially amended with the glucose and amino acid nutrients, for a total of 60 respiration measurements. Each respiration measurement was made for three to five min. for each of the replicate

measurements, or until the slope of the respiration rate on the data logger was linearly stable (typically 10 to 15 min.). Respiration rates were expressed as nmol CO_2 min^{-1} L^{-1}, and for comparison to published data from field measurements, peak respiration rates were converted to µmol CO_2 m^{-2} s^{-1}. More measurements were made initially (Days 1–3) to obtain a better estimate of the initial respiratory response of the microbial community, followed by further measurements at longer intervals on Days 5 and 7, when the daily changes in rates were less.

Bacteria and ciliate enumeration. After each respiration measurement on days 1, 5 and 7, three ml of thoroughly mixed water was removed from each microcosm and placed in a screw cap five-ml vial, fixed by addition of ultrapure, TEM-grade glutaraldehyde with a final concentration of 2.5% (w/v) and refrigerated until analyzed. Bacteria were counted using acridine orange stained preparations, collected on 0.22 µm pore-size opaque filters, and examined with a Leitz Laborlux 11 epifluorescent UV microscope using an oil immersion objective lens (e.g. Hobbie *et al.* 1977, Anderson *et al.* 2001). Bacterial densities were expressed as number per ml (typically in the range of 10^8 or 10^9 ml^{-1}). Bacterial counts were made on Days 1, 3 and 7 to more closely bracket the measurements to the initial respiratory rate measurement on Day 1, and at the peak respiration rate measurements (Days 3 and 7) during the seven-day experiments. Ciliates were counted by concentrating one ml of Lugol's iodine-stained sample to 0.1 ml using centrifugation, followed by repeatedly taking 20 µl aliquots that were scanned and counted exhaustively. The total ciliate counts were expressed as number L^{-1} after making conversions for the initial concentration step. Ciliates were sampled on the final day of the experiment (Day 7) to estimate predation pressure on the bacteria. Ciliate sizes were largely in the range of 30 µm, typical for bacterivorous ciliates. There was no evidence of other prey in the vacuoles of the fixed cells.

Statistical analyses. Descriptive statistics were obtained using Excel spreadsheets. Correlation estimates of respiration rate vs. bacterial densities were made using StatPlus: Mac 2009 (AnalystSoft); one large outlying point was removed from the data set relating respiration to bacterial density for the experimental microcosms apparently due to a possible bacterial counting error.

RESULTS

Research question 1. The effects of organic C enrichment on respiratory CO_2 emission from the pond water microcosms for Experiments One and Two are presented in Table 1, including the densities of bacteria on Days 1, 3, and 7. The net difference in respiration rates (nmol CO_2 min^{-1} L^{-1}) between C-enriched and control preparations for Experiment One, Days 1 to 7 were: 30, 32, 49, 77, and 94, respectively; and likewise for Days 1 to 7 in Experiment Two: 97, 175, 242, 208, and 178, respectively. At the peak respiration within seven days, the net efflux of CO_2 to the atmosphere was ~ 90 nmol min^{-1} L^{-1} (Day 7, Experiment One) and ~ 240 nmol min^{-1} L^{-1} (Day 3, Experiment Two). The corresponding net efflux of C to the atmosphere was 25 nmol C min^{-1} L^{-1} (Day 7, Experiment One) and 65 nmol C min^{-1} L^{-1} (Day 3, Experiment Two). Peak CO_2 emissions from the organic-enriched microcosms expressed as µmol m^{-2} s^{-1} (as more typically reported for natural bodies of water) were 0.20 for Experiment One, and 0.42 for Experiment Two, at a surface layer depth of approx. 20 cm, i.e. the microcosm depth. In general, the rate of respiratory CO_2 emission was more substantial during the first three days after organic C emendment, and either reached a peak value or increased less rapidly, approaching a peak thereafter (Table 1). The larger density of bacteria in Experiment Two may have contributed to an earlier peak in the respiration (Day 3), compared to Experiment One, because the organic C enrichment apparently was utilized more rapidly leading to a subsequent decline in respiration rates as organic C became more limiting. Given the surface area of the pond water in each microcosm was 106 cm^2, the mean net surface transference of CO_2 from the bulk, two-L aqueous phase to the gas phase in each microcosm at peak respiration rate was calculated to be 1.8 nmol cm^{-2} min^{-1} for Experiment One, and 4.5 nmol cm^{-2} min^{-1} for Experiment Two. Overall, there is a positive correlation between respiratory CO_2 release and bacterial density ($r = 0.76$, $p < 0.001$) based on the combined data for the experimental microcosm treatments, One and Two. The correlation of respiratory CO_2 release and bacterial density was much lower for the data from the two control microcosms ($r = 0.32$, n.s.).

Research question 2. The effects of the organic C enrichment on the bacterial and ciliate densities compared to the non-enriched controls are shown in Fig. 1. The mean densities of ciliates at Day 7 were higher in Experiment One, and correspondingly the mean densities of bacteria in Experiment One were lower compared to Experiment Two. Mean densities of ciliates in the C-enriched microcosm of Experiment One and Experiment Two were $7.3 \pm 1.4 \times 10^4$ L^{-1} and $1.1 \pm 0.5 \times 10^4$ L^{-1}, respectively. The mean bacteria density on Day 7 for Experiment One was ~ 15×10^8 ml^{-1}, and for Experiment Two ~ 26×10^8 ml^{-1} for the C-enrichment microcosms as shown in Table 1. The control preparations also showed a similar pattern of ciliate densities, i.e. 1.8 ± 1.2 for Experiment One and 0.3 ± 0.1 for Experiment Two. However, the mean bacterial density for the control in Experiment Two (~ 31×10^8 ml^{-1}) was higher than the mean bacterial density in the control for Experiment One (~ 6×10^8 ml^{-1}), as examined more fully in the next subsection.

Table 1. Mean respiration rates ± S.E (nmol min^{-1} L^{-1}) on Days 1, 2, 3, 5 and 7, and bacterial densities (N x 10^8 ml^{-1}) on Days 1, 3, and 7 for Experiments One and Two

	Day 1	Day 2	Day 3	Day 5	Day 7
Experiment One					
C-enriched respiration	58 ± 10	74 ± 12	82 ± 13	111 ± 8	122 ± 1
Bacteria densities	6.6 ± 1.2		13.6 ± 3.8		14.9 ± 3.5
Control respiration	28 ± 8	42 ± 14	33 ± 7	34 ± 5	28 ± 4
Bacteria densities	4.6 ± 0.3		6.6 ± 0.6		6.3 ± 0.5
Experiment Two					
C-enriched respiration	114 ± 19	200 ± 39	265 ± 42	223 ± 26	200 ± 41
Bacteria densities	16.7 ± 5.5		35.7 ± 24.5		26.3 ± 16.2
Control respiration	17 ± 7	25 ± 5	23 ± 5	15 ± 5	22 ± 2
Bacteria densities	19.0 ± 5.6		22.7 ± 5.7		30.7 ± 15.4

Research question 3. The larger respiratory CO_2 emissions for the C-enriched treatments in Experiment Two compared to Experiment One may be partially explained by the larger densities of bacteria in Experiment Two, especially given the correlation evidence that respiratory CO_2 rates are positively correlated with bacterial densities. Ciliate densities are greater in Experiment One microcosms, and may account in part for the lower densities, possibly due to top down predatory control by the bacterivorous ciliates. Because these microcosms were maintained under dark conditions, there was no potential algal prey for the ciliates. There was a moderate negative correlation of ciliate densities and the bacterial densities in the experimental microcosms at Day 7 (r = –0.31, n.s.).

DISCUSSION

While it is clearly evident that the burning of fossil fuels contributes substantially to atmospheric CO_2 pollution, increasing organic pollution of natural bodies of water leading to eutrophication may also have major effects on the aquatic biota (especially promoting heterotrophic respiration) and also potentially leading to increased release of respiratory CO_2. Anthropogenic sources of organic pollution that are likely to have major effects on aquatic microbial community composition and consequent release of metabolic respiratory CO_2 include raw sewage, run-off of waste from livestock farms, food processing industry, etc. (e.g. Edwards and Daniel 1992, Islam *et al*. 2004, Kahn and Wayman 1964, Painter and Viney 1959). Amino acids and glucose were used for the organic enrichment because they have been shown to be present in raw sewage and other anthropogenic sources of organic pollution (e.g. Confer *et al*. 1995, Painter and Viney 1959, Subrahmanyam *et al.* 1960), but are not so commonly found in treated municipal wastewater (e.g. Scully, Jr. *et al.* 1988). Amino acids and glucose are commonly used in microcosm experiments examining the effects of exogenous organic C enrichment on aquatic microbial communities and metabolic respiration (e.g. Alonzo-Sáez *et al*. 2012, Crawford *et al*. 1974, Hanson and Snyder 1980, Meon and Amon 2004, Shiah and Ducklow 1994, Vallieres *et al*. 2008). Moreover, pollution due to excessive terrestrial run-off of fertilizers can lead to intense algal blooms that subsequently collapse and decay releasing low molecular weight organics, including glucose and amino compounds, that fuel bacterial heterotrophic respiratory activity (e.g. Chróst *et al*. 1989). A small fraction of primary produced organic matter is also released during active growth of algal blooms (e.g. Baines and Pace 1991).

Substantial evidence has been gathered to show that polluted estuaries (often with heavy allochthonous sources of organic input) exhibit major changes in microbial communities and trophic relations (e.g. Chen *et al.* 2009), leading to increased heterotrophic respiration and substantial release of CO_2 to the atmosphere, with fluxes as high as hundreds of mmol CO_2 m^{-2} day^{-1} for some of the most heavily organically enriched sites (e.g. Anderson 2016, Daniel *et al*. 2013, Frankignoulle

et al. 1998, Jiang *et al*. 2008, Liu *et al*. 2016). Organic pollution of freshwater systems, including ponds, lakes and waterways results in major changes in biotic communities, shifting from largely autotrophic to heterotrophic regimes and greater CO_2 emissions (e.g. Dai *et al*. 2016, Qin *et al*. 2013, Richey *et al*. 2002), while some wetlands with increasing mineral eutrophication (e.g. phosphorus), may exhibit less CO_2 respiratory loss to the atmosphere due to enhanced plant growth and greater net phototrophic fixation of CO_2 (e.g. Grasset *et al*. 2016).

Given the relative paucity of experimental evidence of the effects of organic C pollution on freshwater microbial heterotrophic microplankton communities and the resultant respiratory CO_2 emission to the atmosphere, the study reported here is one of the first to present some controlled experimental evidence of these effects. Natural sources of pond water were used in the microcosms to make the study more relevant to the natural environment, an approach that is commonly used in aquatic microcosm research (e.g. Crawford *et al*. 1974, Pollard and Ducklow 2011, Shiah and Ducklow, 1994). The goal of this research was to evaluate the net increase in CO_2 efflux due to enrichment with organic C compounds, relative to the controls. The initial DOC and water quality were not assessed. While, this approach used natural pond water, it may be more difficult to fully reproduce the microcosm experiments compared to an approach that used water with a known chemical composition; however, the option of using natural pond water sources was chosen with the aim of improving implications for natural settings.

Overall as may be expected, organic C enrichment contributed to a substantial increase in respiratory CO_2 emissions compared to the non-enriched control microcosms, with a peak net yield (compared to the control) in Experiment One of ~ 90 nmol CO_2 min^{-1} L^{-1} (25 nmol C min^{-1} L^{-1}), and in Experiment Two of ~ 240 nmol CO_2 min^{-1} L^{-1} (65 nmol C min^{-1} L^{-1}). The peak increase in atmospheric respiratory CO_2 released in the experimental microcosms compared to the controls was four-fold for Experiment One, and twelve-fold for Experiment Two. Higher densities of bacteria were found in the experimental, C-enriched microcosms compared to the controls. Although total density counts of bacteria do not differentiate between active and dormant bacteria (e.g. Zimmermann *et al*. 1978), there is evidence that respiratory CO_2 release is correlated with bacterial densities, especially in organically enriched aquatic environments as presented here; and elsewhere in organically-enriched terrestrial environments (e.g. Anderson 2014, Iriarte *et al*. 1996, Smith 1998, Williams 1981). Therefore, some of the differences in the amount of respiratory CO_2 released between the experimental and control microcosms may be attributed to differences in the densities of bacteria as well as other microheterotrophs.

While it is not possible to definitively determine what accounts for the differences in densities of bacteria in the microcosms of Experiment One compared to Experiment Two, the greater density of the relatively small (ca. 30 µm) predatory ciliates in Experiment One may explain the lower density of bacteria, especially given the evidence that both the experimental and control preparations for Experimental One have higher ciliate densities than in the corresponding microcosms of Experiment Two. The predatory pressure of the substantially higher densities of ciliates in C-enriched microcosms of Experiment One, compared to the C-enriched microcosms of Experiment Two, may explain the differences in bacterial densities. The control of bacterioplankton composition and productivity by protists (including ciliates and nanoflagellates) has been documented in numerous other aquatic systems (e.g. Beaver and Crisman 1989, Berninger *et al.* 1991, Bjørnsen *et al.* 1988, Bloem and Bär-Gilissen 1989, Bong and Lee 2011, Caron 1987, del Giorgio *et al*. 1996, Kuuppo-Leinikki 1990). The evidence presented here that respiratory CO_2 emission may be related to protist controls of bacterial densities, suggests that predicting CO_2 emissions and/or modeling of emissions may require much more careful attention to the microbial community composition and trophic relations in the freshwater site to ensure a complete representation of the events.

This is one of the first attempts to use microcosms to study possible effects of anthropogenic organic pollutants from sources such as raw sewage, agriculture and food processing (Confer *et al*. 1995, Edwards and Daniel 1992, Islam *et al*. 2004, Subrahmanyam *et al*. 1960) on microbial communities and their release of respiratory CO_2. The respiratory data are reported as nmol min^{-1} L^{-1}, because microcosm cultures are based on a known volume of water. Most studies of CO_2 emission from natural bodies of freshwater, however, use units expressed as respiratory rate per unit area (e.g. mmol m^{-2} day^{-1} or µmol m^{-2} s^{-1}) because measurements are made in the open atmosphere, and the total volume of water is not accurately known. While caution is necessary in scaling up microcosm respiration results to landscape levels, as also is problematic broadly for aggregating such

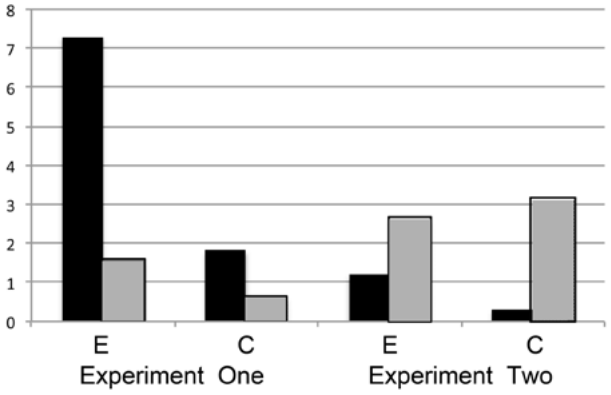

Fig. 1. Densities (ordinate) of ciliates ($N \times 10^4$ L^{-1}) black bars, and densities of bacteria ($N \times 10^9$ ml^{-1}) grey bars; for Experiment One and Experiment Two with carbon-enriched (E) and control (C) preparations (abscissa).

data across natural settings (e.g. Seekell *et al*. 2014), the peak respiratory data obtained here was converted to µmol m^{-2} s^{-1} to provide a more useful comparison to published data from the natural environment. The values obtained for the peak respiration for Experiments One and Two were 0.20 and 0.40 µmol m^{-2} s^{-1}, respectively. This is within the range reported for some natural bodies of water, particularly estuaries, e.g. 0.14–0.54 µmol m^{-2} s^{-1} (Cavalho and Duarte 2013, Jiang et al. 2008, Sarma *et al*. 2012); although other estuaries are higher ranging up to 5.16 µmol m^{-2} s^{-1} (e.g. Anderson 2016). However, given the shallow depth of the microcosm water (ca. 20 cm) compared to the larger depths of natural bodies of water, a substantially higher predicted emission of CO_2 per unit area based on the data from this study would be likely if integrated across the entire depth of a water column in natural bodies of water, assuming they had similar organic input and density of biota.

While the current study using microcosms provides laboratory-based experimental evidence for the effects of organic C-enrichment on freshwater microbial communities and the resultant CO_2 emissions, further broad scale sampling studies across wide geographic locales and geological settings at freshwater sites varying in amount of organic pollutants are needed to more clearly evaluate the environmental applicability of this experimental evidence based on data from natural settings.

Acknowledgments. This is Lamont-Doherty Contribution Number 8061.

REFERENCES

Alonzo-Sáez L., Sánchez O., Gasol J. M. (2012) Bacterial uptake of low molecular weight organics in the subtropical Atlantic: Are major phylogenetic groups different? *Limnol. Oceanogr.* **57:** 798–808

Anderson O. R. (2014) Bacterial and heterotrophic nanoflagellate densities and C-biomass estimates along an Alaskan tundra transect with prediction of respiratory CO_2 efflux. *J. Eukaryot. Microbiol.* **61:** 11–16

Anderson O. R. (2016) The role of heterotrophic microbial communities in estuarine C budgets and the biogeochemical C cycle with implications for global warming: research opportunities and challenges. *J. Eukaryot. Microbiol*. **63:** 394–409

Anderson O. R., Gorrell T., Bergen A., Kruzansky R., Levandowsky M. (2001) Naked amoebae and bacteria in an oil-impacted salt marsh community. *Microb. Ecol.* **42:** 474–481

Baines S. B., Pace M. L. (1991) The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater ecosystems. *Limnol. Oceanogr.* **36:** 1078–1090

Beaver J. R., Crisman T. L. (1989) The role of ciliated protozoa in pelagic freshwater ecosystems. *Microb. Ecol*. **17:** 111–136

Berninger U.-G., Finlay B. J., Kuuppo-Leinikki P. (1991) Protozoan control of bacterial abundances in freshwater. *Limnol. Oceanogr*. **36:** 139–147

Bjørnsen P. K., Riemann B., Horsted S. J., Nielsen T. G., Pock-Sten J. (1988) Trophic interactions between hteterotrophic nanoflagellates and bacterioplankton in manipulated seawater enclosures. *Limnol. Oceanogr*. **33:** 409–420

Bloem J., Bär-Gilissen M.-J. B. (1989) Bacterial activity and protozoan grazing potential in a stratified lake. *Limnol. Oceanogr*. **34:** 297–309

Bong C. W., Lee C. W. (2011) The contribution of heterotrophic nanoflagellate grazing towards bacterial mortality in tropical waters: comparing estuaries and coastal ecosystems. *Mar. Freshwater Res*. **62:** 414–420

Broecker W. S. (1975) Climate change: are we on the brink of a pronounced global warming? *Science* **189:** 460

Caron D. (1987) Grazing of attached bacteria by heterotrophic microflagellates. *Microb. Ecol.* **13:** 203–218

Carpenter S. R., Stanley E. H., Vander Zanden M. J. (2011) State of the World's freshwater ecosystems: physical, chemical, and biological changes. *Annu. Rev. Environ. Resour*. **36:** 75–99

Carvalho R., Duarte P. (2013) Carbon fluxes in a coastal area of northern Portugal. *Limnetica*, **32:** 229–244

Casper P., Maberly S. C., Hall G. H., Finlay B. J. (2000) Fluxes of methane and carbon dioxide from a small productive lake to the atmosphere. *Biogeochem*. **49:** 1–19

Chen B., Liu H., Landry M. R., Chen M., Sun J., Shek L., Chen X., Harrison P. J. (2009) Estuarine nutrient loading affects phytoplankton growth and microzooplankton grazing at two contrasting sites in Hong Kong coastal waters. *Mar. Ecol. Prog. Ser*. **379:** 77–90

Chróst R. J., Münster U., Rai H., Albrecht D., Witzel P. K., Overbeck J. (1989) Photosynthetic production and exoenzymatic degradation of organic matter in the euphotic zone of a eutrophic lake. *J. Plankton Res*. **11:** 223–242.

Cole J. J., Kinne O. (2013) *Freshwater Ecosystems and the Carbon Cycle*. Olendorf/Luhe: International Ecology Institute

Cole J. J., Findlay S., Pace M. L. (1988) Bacterial production in fresh and saltwater ecosystems: A cross-system overview. *Mar. Ecol. Prog. Ser*. **43:** 1–10.

Cole J. J., Prairie Y. T., Caraco N. F, McDowell W. H., Tranvik L. J., Striegl R. G., Duarte C. M., Kortelainen P., Downing J. A., Middelburg J. J., Melack J. (2007) Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* **10:** 171–164

Confer D. R., Logan B. E., Aiken B. S., Kirchman D. L. (1995) Measurement of dissolved free and combined amino acids in unconcentrated wastewaters using high performance liquid chromatography. *Water Environ. Res*. **67:** 118–125.

Crawford C. C., Hobbie J. E., Webb K. L. (1974) The utilization of dissolved free amino acids by estuarine microorganisms. Ecology **55:** 551–563

Dai Y., Yang Y., Wu Z., Feng Q., Xie S., Liu Y. (2016) Spatiotemporal variation in planktonic and sediment bacterial assemblages in two plateau freshwater lakes at different trophic levels. *Appl. Microbiol. Biotech*. **100:** 4161–4175

Daniel I., DeGrandpre M., Farias, L. (2013) Greenhouse gas emissions from the Tubul-Raqui estuary (central Chile). *Estuar. Coast. Shelf Sci*. **134:** 31–44

Davidson T. A., Audit J., Svenning J.-C., Lauridsen T. L., Søndergaard M., Landkildehus F., Larsen S. E., Jeppesen E. (2015) Eutrophication effects on greenhouse gas fluxes from shallow-lake mesocosms override those of climate warming. *Global Change Biol*. **21:** 4449–4463

del Giorgio P. A., Cole J. J., Cimbleris A. 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* **385:** 148–151

del Giorgio P. A., Gasol J. I. M., Vaque D., Mura P., Agusti S., Duarte C. M. (1996) Bacterioplankton community structure: protists control net production and the proportion of active bacteria in a coastal marine community. *Limnol. Oceanogr*. **41:** 1169–1179

Edwards D. R., Daniel T. C. 1992. Environmental impacts of on-farm poultry waste disposal – A review. *Bioresource Technol*. **41:** 9–33

Frankignoulle M., Abril G., Borges A., Bourge I., Canon C., DeLille B., Libert E., Theate J. M. (1998) Carbon dioxide emission from European estuaries. *Science* **282:** 434–436

Friedlingstein P., Houghton R. A., Marland G., Hackler J., Boden T. A., Conway T. J., Canadell J. G., Raupach M. R., Ciais P., Le Quéré C. (2010) Update on CO_2 emissions. *Nature Geoscience* **3:** 811–812

Grasset C., Abril G., Guillard L., Delolme C., Bornette G. (2016) Carbon emission along a eutrophication gradient in temperate riverine wetlands: effect of primary productivity and plant community composition. *Freshwater Biol.* doi:10.1111/fwb.12780

Hanson R. B., Snyder J. (1980) Glucose exchanges in a salt marsh-estuary: Biological activity and chemical measurements. Limnol. Oceanogr. **25:** 633–642.

Hobbie J. E., Daley R. J., Jasper S. (1977) Use of nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Microbiol*. **33:** 1225–1228

Iriarte A., de Madariaga I., Diez-Garagarza F., Revilla M., Orive E. (1996) Primary production, respiration and nitrification in a shallow temperate estuary during summer. *J. Exp. Mar. Biol. Ecol.* **208:** 127–151

Islam Md. I., Khan S., Tanaka M. 2004. Waste loading in shrimp and fish processing effluents: potential source of hazards to the coastal and nearshore environments. *Mar. Pollut. Bull.* **49:** 103–110

Jiang L. Q., Cai W. J., Wang Y. C. (2008) A comparative study of carbon dioxide degassing in river – and marine dominated estuaries. *Limnol. Oceanogr*. **53:** 2603–2615

Karl T. R., Trenberth K. E. (2003) Modern global climate change. *Science* **302:** 1719–1723

Kahn L., Wayman C. (1964) Amino acids in raw sewage and sewage effluents. *Water Pollut. Control* **36:** 1368–1371

Kuuppo-Leinikki P. (1990) Protozoan grazing on planktonic bacteria and its impact on bacterial population. *Mar. Ecol. Prog. Ser*. **63:** 227–238

Liu H., Zhang Q., Katul G. G., Cole J. J., Chapin III, F. S., MacIntyre S. (2016) Large CO_2 effluxes at night and during synoptic weather events significantly contribute to CO_2 emissions from a reservoir. *Environ. Res. Lett*. **11:** 064001

Meon B., Amon R. M. W. Heterotrophic bacterial activity and fluxes of dissolved free amino acids and glucose in the Arctic rivers Ob, Yenisei and the adjacent Kara Sea. *Aquat. Microb. Ecol.* **37:** 121–135

Qin B. Q., Gao G., Zhu G. W., Zhang Y. L., Song Y. Z., Tang X. M., Deng J. M. (2013) Lake eutrophication and its ecosystem response. *Chinese Sci. Bull*. **58:** 961–970

Painter H. A., Viney M. (1959) Composition of a domestic sewage. *J. Biochem. Microbiol*. **1:** 143–162

Pollard P. C., Ducklow H. (2011) Ultrahigh bacterial production in a eutrophic subtropical Australian river: Does viral lysis short-circuit the microbial loop? *Limnol. Oceanogr*. **56:** 1115–1129

Richey J. E., Melack J. M., Aufdenkampe A. K., Ballester V. M., Hess L. L. (2002) Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO_2. *Nature* **416:** 617–620

Sarma V., Viswanadham R., Rao G. D., Prasad V. R., Kumar B. S. K., Naidu S. A., Kumar N. A., Rao D. B., Sridevi T., Krishna M. S., Reddy N. P. C., Sadhuram Y., Murty T. V. R. (2012) Carbon dioxide emissions from Indian monsoonal estuaries, *Geophys. Res. Lett*. **39:** 1–5

Schrier-Uijl A. P., Veraart A. J., Leffelaar P. A., Berendse F., Veenendaal E. M. (2011) Release of CO_2 and CH_4 from lakes and drainage ditches in temperate wetlands. *Biogeochem*. **102:** 265–279

Scully Jr., F. E., Howell G. D., Penn H. H., Mazina K., Johnson J. D. (1988) Small molecular weight organic amino nitrogen compounds in treated municipal waste water. *Environ. Sci. Technol.* **22:** 1186–1190

Seekell D. A., Carr J. A., Gudasz C., Karlsson J. (2014) Upscaling carbon dioxide emissions from lakes. *Geophys. Res. Lett*. **41:** 7555–7559

Shiah F.-K., Ducklow H. W. (1994) Temperature and substrate regulation of bacterial abundance, production and specific growth rate in Chesapeake Bay, USA. *Mar. Ecol. Prog. Ser.* **103:** 297–308.

Smith E. M. (1998) Coherence of microbial respiration rate and cell-specific bacterial activity in a coastal planktonic community. *Aquat. Microb. Ecol*. **16:** 27–35

Smith V. H., Tilman G. D., Nekola J. C. (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ. Pollut*. **100:** 179–196

Solomon S., Plattner G.-K., Knuti R., Friedlingstein P. (2009) Irreversible climate change due to carbon dioxide emissions. *Nat. Acad. Sci. Proc*. **106:** 1704–1709

Subrahmanyam P. V. R., Sastry A., Prabhakara R., Pillai C. (1960) Amino acids in sewage sludges. *Water Pollut. Control* **32:** 344–350

Valliéres C, Retamal L., Ramlal P., Osburn C. L. Vincent W. F. (2008) Bacterial production and microbial food web structure in a large

arctic river and the coastal Arctic Ocean. *J. Marine Syst.* **74:** 756–773

Wilkinson G. M., Buelo C. D., Cole J. J., Pace M. L. (2016) Exogenously produced CO_2 doubles the CO_2 efflux from three north temperate lakes. *Geophys. Res. Lett*. **43:** 1996–2003

Williams P. J. leB. (1981) Microbial contribution to overall marine plankton metabolism: direct measures of respiration. *Oceanol. Acta* **4:** 359–364

Zimmermann R., Iturriaga R., Becker-Birck J. (1978) Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration. *Appl. Environ. Microbiol.* **36:** 926–935

Received on 30th June, 2016; revised on 25th September, 2016; accepted on 3rd October, 2016