9-Feb-17

Dr. David Dudgeon
Editor-in-Chief, *Freshwater Biology*

Dear Dr. Dudgeon

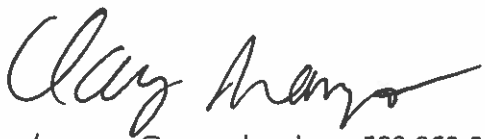
Attached, please find an original manuscript titled "Carbon limitation of heterotrophic respiration is linked to dissolved organic matter quality in urban streams" by CP Arango plus 8 other co-authors. This contribution describes the results of a study done in buried and open reaches of urban streams in Cincinnati, Ohio, USA to quantify how heterotrophic respiration responds to different carbon sources in different seasons using nutrient diffusing substrata. This work was done as part of a larger research effort to document how burial of urban streams affects nitrogen cycling on the stream-reach and at the watershed scale.

The attached research complements the current disciplinary interest of refining our understanding of how urban streams process and transport carbon with implications for better management practices. In buried and open reaches in three heavily urbanized streams we describe fluorescence characteristics of dissolved organic matter (DOM) quality in spring, summer, and autumn, and how seasonal DOM quality affects biofilm extracellular enzyme profiles and carbon limitation patterns measured with nutrient diffusing substrata. We found that fluorescence metrics indicated higher quality DOM in spring, coincident with vernal algal blooms which produce more labile carbon, compared to autumn when low quality terrestrial leaf inputs dominated. Microbes allocated more effort to acquire recalcitrant carbon in buried reaches compared to open reaches, possibly reflecting open reaches as a source of labile carbon production compared to buried reaches where light was extinguished. Finally, carbon limitation was much higher in autumn than spring, likely due to the pulse of low quality terrestrial organic matter in autumn. Collectively, we show that changes in the quality of the DOM pool has consequences for how microbes use those carbon sources, but regardless of seasonal DOM quality differences, open and buried stream reaches were always carbon limited. Carbon limitation in these urban streams suggests that increasing light to buried reaches could stimulate labile carbon production via increased photosynthesis with consequences for microbial carbon use and nitrogen and/or phosphorus spiraling.

Based on guidelines at the *Freshwater Biology* website, we confirm that these are original data that have not been published elsewhere, and all authors fully participated in and accept responsibility for the work. We do acknowledge that Figure 8 in this paper is based on a figure appearing in Beaulieu et al. 2014, but this is cited in the manuscript. Finally, we confirm that this work has been cleared for submission through internal approval at the US Environmental Protection Agency.

Thank you for considering this manuscript, and we look forward to hearing from you soon.

Sincerely,



Dr. Clay Arango (arangoc@cwu.edu, phone 509-963-3163, fax 509-963-2730)
Dr. Jake Beaulieu
Dr. Ken Fritz
Dr. Brian Hill

Dr. Colleen Elonen
Dr. Michael Pennino
Dr. Paul Mayer
Dr. Sujay Kaushal
David Balz, M.S.