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# Pollution-tolerant invertebrates enhance greenhouse gas flux in urban wetlands

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*Abstract.* One of the goals of urban ecology is to link community structure to ecosystem function in urban habitats. Pollution-tolerant wetland invertebrates have been shown to enhance greenhouse gas (GHG) flux in controlled laboratory experiments, suggesting that they may influence urban wetland roles as sources or sinks of GHG. However, it is unclear if their effects can be detected in highly variable conditions in a field setting. Here we use an extensive data set on carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$) flux in sediment cores ($n = 103$) collected from 10 urban wetlands in Melbourne, Australia during summer and winter in order to test for invertebrate enhancement of GHG flux. We detected significant multiplicative enhancement effects of temperature, sediment carbon content, and invertebrate density on $CH_4$ and $CO_2$ flux. Each doubling in density of oligochaete worms or large benthic invertebrates (oligochaete worms and midge larvae) corresponded to ~42% and ~15% increases in average $CH_4$ and $CO_2$ flux, respectively. However, despite exceptionally high densities, invertebrates did not appear to enhance $N_2O$ flux. This was likely due to fairly high organic carbon content in sediments (range 2.1–12.6%), and relatively low nitrate availability (median 1.96 µmol/L $NO_3^-$-N), which highlights the context-dependent nature of community structural effects on ecosystem function. The invertebrates enhancing GHG flux in this study are ubiquitous, and frequently dominate faunal communities in impaired aquatic ecosystems. Therefore, invertebrate effects on $CO_2$ and $CH_4$ flux may be common in wetlands impacted by urbanization, and urban wetlands may make greater contributions to the total GHG budgets of cities if the negative impacts of urbanization on wetlands are left unchecked.

*Key words: carbon dioxide; Chironomidae; climate change; constructed wetland; golf course; methane; Naididae; nitrous oxide; Oligochaeta; Tubificidae; Tubificinae; urbanization.*

## Introduction

One of the objectives of urban ecology is to search for generalizable processes and community structures that influence ecosystem function within urban habitats (McPhearson et al. 2016). Faunal community structure in aquatic ecosystems can be altered by several environmental impacts associated with urbanization, such as increased mobility of metals, nutrients, and pesticides (Carpenter et al. 1998, Walsh et al. 2005, Kaye et al. 2006, Kaushal et al. 2008, Allinson et al. 2015), introduction of exotic predators (Hamer and Parris 2013), and increased prevalence of hypoxia (Jenny et al. 2016). Urbanization is a major factor contributing to the global loss and degradation of natural wetlands (van Asselen et al. 2013), but urbanization also spurs the construction of new wetlands for storage of water supplies, for recreation (such as golf

course ponds), and for stormwater and wastewater treatment (Davidson 2014). Although urban wetlands may contribute to biodiversity at the landscape scale (Hill et al. 2016), they can seldom be considered analogs of natural wetlands as far as community structure is concerned (Hassall 2014). Because urban wetlands intercept heavy metals and other toxic substances that often become associated with sediments, pollutant accumulation and associated negative impacts may be greatest for benthic fauna, an important functional component of the faunal community (Covich et al. 1999). Furthermore, because treatment wetlands are designed to intercept and treat large inputs of carbon (C) and nitrogen (N), one of the primary concerns associated with their increased adoption is the potential for enhanced greenhouse gas (GHG) emissions (Verhoeven et al. 2006, Burgin et al. 2013, Mander et al. 2014). Wetland roles as sinks or sources of GHG are strongly controlled by environmental drivers such as temperature and the availability of C and N, which can stimulate emission of nitrous oxide ($N_2O$), methane ($CH_4$), and carbon dioxide ($CO_2$) (Cao et al. 1996, Burgin et al. 2013, Nisbet et al. 2014, Butman et al. 2016, Deemer et al.

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2016). Human-made wetlands in natural and urban environments are important contributors to global aquatic emissions of $N_2O$ and $CH_4$ (Deemer et al. 2016), which have been rapidly increasing in recent years (Syakila and Kroeze 2011, Nisbet et al. 2014). An understanding of controls on GHG emissions in these built environments is critical to the assessment of urban wetland contributions to global GHG budgets (Burgin et al. 2013, Mitsch et al. 2013, Mander et al. 2014).

Pollution-tolerant oligochaete and midge species are able to reach high densities in severely impacted aquatic environments where other taxa may be excluded (Datry et al. 2003, Pigneret et al. 2016). They have also been shown to enhance nutrient, metal, and GHG flux in controlled laboratory experiments (Lagauzère et al. 2009, Stief et al. 2009, Nogaro and Burgin 2014, Hölker et al. 2015), and therefore may exert strong influence on multiple ecosystem functions in urban wetlands. However, addressing the effects of invertebrates on GHG flux can be challenging for multi-species assemblages. Previous studies demonstrating enhancement of GHG flux by oligochaetes and midge larvae have done so under highly controlled conditions in laboratories, primarily with single species, and often with elevated nutrient concentrations (Stief et al. 2009, 2010, Nogaro and Burgin 2014, Poulsen et al. 2014). While controlled conditions have allowed for effective isolation of invertebrate effects, an assessment of the effects of mixed assemblages under variable conditions is important to our understanding of faunal influence on GHG fluxes in aquatic ecosystems. Sediment-dwelling taxa may have synergistic or antagonistic effects when present in combination, due to their different modes of bioturbation. For example, midge larvae bioirrigate sediments through construction and ventilation of U-shaped tubes, but oligochaetes redistribute sediments to a greater degree and at greater depths than midge larvae, via "conveyor-belt feeding" (Fig. 1; Lagauzère et al. 2009). Furthermore, given the variable environmental conditions present in field settings, the degree to which invertebrate effects can be detected relative to other drivers of GHG flux amidst the "noise" in urban environments requires further investigation.

Our objectives were to (1) compare benthic fluxes of $N_2O$, $CH_4$, and $CO_2$ in golf course ponds and constructed stormwater treatment wetlands, (2) characterize the representation and densities of dominant invertebrates with potential to alter GHG fluxes, and (3) determine if benthic invertebrate density can help to predict GHG flux in field-collected samples. We hypothesized that (1) GHG flux would be higher in constructed wetlands than in golf course ponds, due to their design goals of intercepting and treating stormwater pollutants, and (2) both oligochaetes and chironomids would be present in high densities due to their abilities to tolerate conditions in degraded aquatic environments, and (3) the density of aquatic worms and midge larvae would be positively correlated to GHG flux, and would substantially improve the predictive ability GHG flux models.
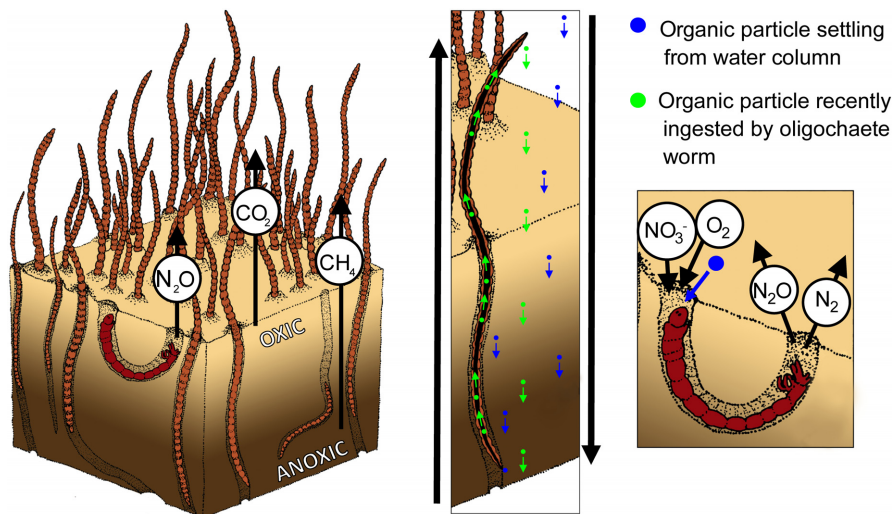


FIG. 1. Wetland sediment inhabited by a midge larva (Diptera: Chironomidae) in its U-shaped burrow, and oligochaete worms (Oligochaeta: Tubificinae) feeding head-down in sediments. Oligochaete consumption of organic particles from deeper sediment layers and subsequent translocation and accumulation of particles at the sediment–water interface causes a downward movement of sediment, the rate of which is determined by oligochaete size and density. Particles move downward until they reach the zone of oligochaete feeding, where they are ingested and transported rapidly above the sediment–water interface. This process, which may be repeated several times until particles pass below the zone of oligochaete feeding, results in enhanced transport and "conveyor belt" cycling of organic particles between oxic and anoxic layers of sediments. Meanwhile, midge larvae ventilate their U-shaped burrows in shallower layers of sediment, alternately drawing nitrate and oxygen as well as organic particles into burrows, thereby enhancing oxygenation of sediments, nitrification, and denitrification, and the production of $N_2$ and $N_2O$ in shallow sediments. [Color figure can be viewed at wileyonlinelibrary.com]

## MATERIALS AND METHODS

### Study sites

We collected 104 sediment cores (four per site) from 10 urban wetlands (five stormwater treatment wetlands and five golf course ponds; hereafter referred to as treatment wetlands and gc ponds) in the suburbs of Melbourne, Victoria, Australia during summer (29 January–4 March) and winter/early spring (5 August–16 September, hereafter referred to as "winter") 2014 (see Appendix S1: Table S1 for site information). In three of the five treatment wetland sites, both inlets and outlets were sampled for comparison. To measure GHG flux and benthic invertebrate density, sediment cores ($27.5 \times 6.6$ cm acrylic cylinders) were collected from four spatially separated locations haphazardly chosen in each site, in areas without emergent vegetation. After gently inserting a core to roughly half its length into the sediment, cores were capped with rubber stoppers, carefully removed to prevent shaking and disturbance of sediments, and returned to the laboratory at Monash University Water Studies Center. Surface water samples were also collected for analysis of chlorophyll and nutrients, and other environmental parameters (oxygen, temperature, pH) were measured with Horiba probes (Appendix S1: Table S2).

### Lab methods

*Core incubations.*—To link invertebrate densities to benthic GHG emissions, four sediment cores from each site were incubated following methods described by Roberts et al. (2012). Briefly, intact cores were placed in a water bath controlled to within $1.3 \pm 0.3°C$ (mean $\pm$ 95% CI) of in situ temperature, and equilibrated overnight while core water was gently aerated. On the following day, aeration was ceased, cores were capped, and dissolved oxygen (DO) measurements were taken through a sealable port in the cap with an optical DO probe (HQ40d Portable Meter, LDO101 Rugged Optical Dissolved Oxygen Probe, HACH Company, Loveland, CO, USA). Water overlying sediment in each core was stirred gently and continuously throughout the incubation by a suspended magnetic bar, with a stirring rate below levels that would cause sediment disturbance. 12.5 mL of overlying water were removed through a port in the cap every few hours, placed into 12.5-mL Exetainers (Labco, Lampeter, UK) with gas-tight septa, and preserved with $HgCl_2$ until analysis for $CO_2$, $CH_4$, and $N_2O$ concentrations with a gas chromatograph (GC) (Shimadzu GC17A, Shimadzu Corp., Kyoto, Japan) equipped with a Methanizer (SRI Instruments, Torrance, CA, USA). Changes in GHG concentration over time were used to calculate the flux of the three GHGs. Aliquots of water removed during incubation were replaced with an equal volume of water collected from the same site, and care was taken during all procedures to prevent the creation of headspace or entrapment of air bubbles during incubation periods. The water volume and surface area of the sediment were taken into consideration when calculating fluxes, along with correction for the addition or dilution of constituents by the replacement of water (Roberts et al. 2012).

*Water quality parameters.*—Water samples were analyzed for total N and P, $PO_4^{3-}$, $NO_3^-$, $NH_4^+$ in the Water Studies Center of Monash University. Nutrient samples ($NO_x$, $NO_2^-$, and $NH_4^+$) were analyzed via flow injection analysis (FIA; Lachat Quickchem 8000 Flow injection Analyzer, spectrophotometric detector, Lachat Instruments, Milwaukee, WI), following standard procedures (APHA 2012). Chlorophyll was measured on precombusted glass fiber filters using spectrophotometric methods described by Lorenzen (1967).

*Core processing.*—Immediately following each incubation, overlying water was carefully removed (with syringe) from above the sediment–water interface. To estimate sediment porosity, a subsample (~3 $cm^3$) of surface sediments was pulled into a syringe, volume recorded, then sediments were placed into a conical bottom tube, wet mass was recorded, sediment was dried at 60°C for 4 d, and finally dry mass was recorded. Sediment porosity (mL pore volume per mL sample volume) was calculated as water volume (sample wet mass − dry mass) divided by sample volume. Dried sediments were later ground until they were capable of passing through a 250-μm sieve and analyzed for elemental organic C ($C_{org}$), organic N ($N_{org}$), $\delta^{13}C$ (‰), and $\delta^{15}N$ (‰) using an ANCA GSL2 elemental analyzer interfaced to a Hydra 20–22 continuous-flow isotope ratio mass-spectrometer (Sercon Ltd., Crewe, UK) at the Water Studies Centre, Monash University. This was repeated for sediments at depths >10 cm within the core as well. Remaining core material was rinsed over a 300-μm sieve. To evaluate trophic position and the contribution of methanotrophic bacteria to invertebrate diets, midge larvae and oligochaetes were analyzed for $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰). A few specimens were removed from each core, allowed to clear their guts for 24 h, and dried at 60°C for 48 h. These were weighed to the nearest μg, and then analyzed for $C_{org}$ and $N_{org}$ content and stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) using the methods already described. Data are provided in the supporting information (Appendix S1: Fig. S1).

*Faunal density.*—To sort and enumerate invertebrates, remaining core material was washed through a 300-μm mesh sieve, and retained material was preserved in 10% phosphate-buffered formalin and stored until invertebrates could be sorted from sediments using a binocular dissecting microscope. When a sample was sorted, formalin was decanted off the top, and sediments were resuspended in water. Sediments were then rinsed over nested 1-mm and 300-μm sieves to sort invertebrates into large (>1 mm) and small (0.3–1 mm) size classes.

*Statistical analysis*

To determine the relative importance of invertebrate density among a set of factors best explaining variability in benthic fluxes of $CH_4$, $N_2O$, and $CO_2$, we compared multiple linear regression models using Akaike's Information Criterion (AIC) and an information theoretic approach (Burnham and Anderson 2002). For all parameters, values from individual sediment cores (four per site) were averaged together in a given season, and treated as a single replicate. Parameters initially selected for possible inclusion in multiple regression models were invertebrate density, which included density of oligochaetes and midge larvae (Tanypodinae and/or non-Tanypodinae), in both large (>1 mm) and small (0.3–1 mm) size classes. In addition, temperature, water column chlorophyll, sediment %C, and dissolved oxygen saturation (DO%) were included as environmental factors that may influence GHG flux. DO% was included both for the inhibitory effects that oxygen availability has on denitrification and $CH_4$ production, and also as an indicator of the presence of benthic microalgae, which may compete with $N_2O$-producing bacteria for $NO_3^-$, and would not be detected with water column chlorophyll measurements. For $N_2O$ flux models, water column $NO_x$ (nitrate + nitrite) concentration was included as a model parameter, but water column nutrients were excluded from other models after initial examination failed to show statistically significant correlations with response variables.

Explanatory variables were tested for multicollinearity with Pearson's correlation coefficient ($r$) matrices and variance inflation factors (VIF), and any two variables with $r \geq 0.60$ were not included in the same model. The predictive abilities of models were compared by using leave-one-out cross-validation (LOOCV; James et al. 2013). Predicted GHG flux values were regressed against observed GHG fluxes, and root-mean-squared errors (RMSE)

were calculated for each model, with lower RMSE indicating improved predictive power. All analyses were conducted in R software (R Development Core Team 2008).

Average $CO_2$, $CH_4$, and $N_2O$ fluxes were predicted for increasing densities of benthic invertebrates using parameter estimates from the model with lowest RMSE for each GHG. For $CO_2$ and $CH_4$, the average flux was reported for four different scenarios: (1) low temperature and low sediment C concentration ($C_{org}$), (2) low temperature and high $C_{org}$, (3) high temperature and low $C_{org}$, and (4) high temperature and high $C_{org}$. Low and high values for temp and $C_{org}$ were set at the first and third quartile of field-collected data: 13° and 25°C, and 4% and 8% $C_{org}$ in sediments. For $N_2O$, average flux was predicted for high and low temperatures, for first and third quartiles of $NO_x$-N availability (0.7 and 40 μmol/L), and also for 10th and 90th percentiles (48% and 151%) of dissolved oxygen saturation.

## RESULTS

### Benthic greenhouse gas flux

Benthic fluxes of $CO_2$ and $CH_4$ were higher in summer than in winter (Fig. 2, $F_{1,22} = 23.89$ and 5.10, $P < 0.001$ and <0.05, respectively), and seasonal differences were strongest in stormwater treatment wetlands (hereafter referred to as treatment wetlands), where benthic fluxes were higher in inlets during the summer than in outlets for $CH_4$ (season × inlet $F_{1,8} = 21.06$, $P < 0.005$) and $CO_2$ (season × inlet $F_{1,8} = 16.35$, $P < 0.005$). While overall fluxes of GHG were not significantly different between wetland types, inlet fluxes in treatment wetlands were higher than fluxes in golf course ponds (hereafter referred to as gc ponds) during the summer for $CH_4$ (season × site $F_{2,16} = 11.26$, $P < 0.001$) and $CO_2$ (season × site $F_{2,16} = 11.04$, $P < 0.001$, Fig. 2). There
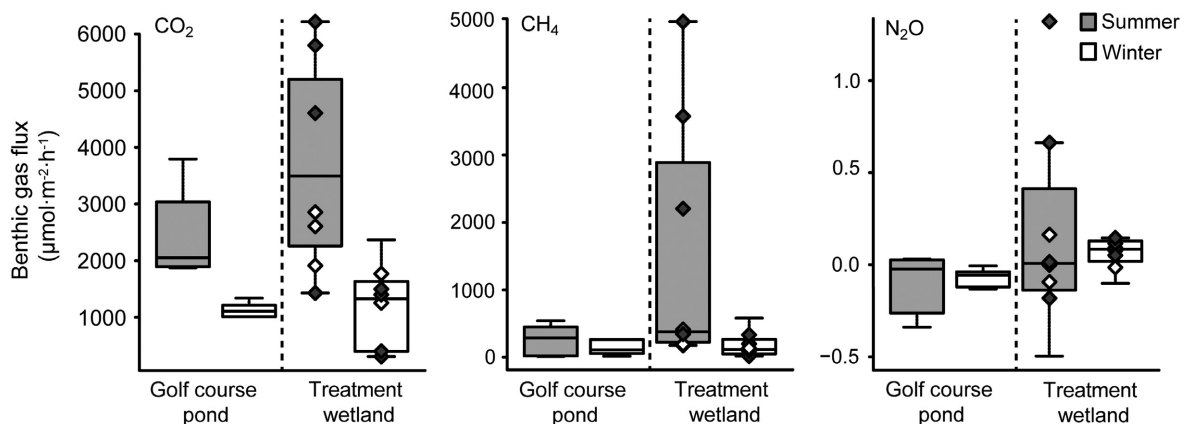


FIG. 2. Benthic fluxes of $CO_2$, $CH_4$, and $N_2O$ in golf course ponds ($n = 40$) and constructed treatment wetlands ($n = 63$) sampled January–March (summer) and August–September (winter/spring) in Melbourne, Australia. For treatment wetlands, solid diamonds overlaying box plots indicate average flux measurements in wetland inlets, while hollow diamonds indicate average flux measurements in wetland outlets. Each diamond is the average of four greenhouse gas (GHG) flux measurements per site. Box plot whiskers show the minimum and maximum flux measurements that are within 1.5 interquartile ranges of the first and third quartiles, respectively. Note differences in *y*-axis scales among panels.

were no significant differences in benthic $N_2O$ fluxes either seasonally, among site types, or among locations within sites.

### Benthic invertebrates

Invertebrate communities in all sites were dominated by oligochaetes and midge larvae. Oligochaetes accounted for ~82% ± 10% and 60% ± 20% (mean ± 95% CI) of average total invertebrate density in treatment wetlands and gc ponds, respectively (Fig. 3, Appendix S1: Table S3). Tubificinae (family Naididae) was the most abundant subfamily of oligochaetes, accounting for 78% ± 9% and 85% ± 14% of all oligochaetes encountered in treatment wetlands and gc ponds (Appendix S1: Table S4). Oligochaete density did not differ significantly between treatment wetlands and gc ponds ($F_{1,22} = 2.15$, $P = 0.16$), or between inlets and outlets within treatment wetlands ($F_{1,8} = 2.36$, $P = 0.16$). However, oligochaete density was significantly higher in inlets of treatment wetlands than in gc ponds ($F_{1,12} = 5.73$, $P < 0.05$), with densities as high as 508,886 individuals/m² (Table S4). Although oligochaete density did not undergo statistically significant seasonal changes, it was reduced by 97% between summer and winter in the inlet of the Huntingdale Road treatment wetland, following the removal of upper layers of sediment during wetland maintenance (reduced from 478,634 ± 24,580 to 11,472 ± 4,231 individuals/m² [mean ± 95% CI]).

Midge larvae accounted for 16% ± 7% and 34% ± 19% of average total invertebrate density in treatment wetlands and gc ponds, respectively (Appendix S1:

Tables S3, S5). Midge larval density did not differ significantly between seasons or site types, and there was no significant difference in midge larval density between the families Tanypodinae and Chironominae (paired $t$ test, $t_{25} = 2.06$, $P = 0.50$, Appendix S1: Table S5). Almost all Tanypodinae present in our sites were *Procladius* spp., and stable isotope values suggest a non-predatory diet, as $\delta^{15}N$ of Tanypodinae (7.68 ± 1.62, range −0.52 to 10.50) were not significantly different from those of Chironominae (8.37 ± 0.53, range −0.18 to 13.56) or oligochaetes (7.25 ± 0.65, range 4.16–18.43).

### Drivers of greenhouse gas flux and the importance of invertebrates

Regression models with the best predictive power (lowest RMSE) explained 84%, 64%, and 62% of the variability in benthic flux of $CO_2$, $CH_4$, and $N_2O$, respectively (Table 1). Top-ranked multiple regression models ($\Delta i < 2$) for all three GHGs included oligochaete and chironomid density (Table 1, Figs. 4–6), but chironomid density never appeared as the sole invertebrate parameter; it appeared in models either together with oligochaete density or via the combined parameter "all invertebrates."

The predictive abilities of $CO_2$ and $CH_4$ models were substantially improved with the inclusion of invertebrates (Table 1, Figs. 4, 5). Each doubling in density of large benthic invertebrates or oligochaetes corresponded to a ~15% and ~42% average increase in $CO_2$ and $CH_4$ flux, respectively. The RMSE of the best model excluding invertebrates was 17% higher (poorer predictive power)
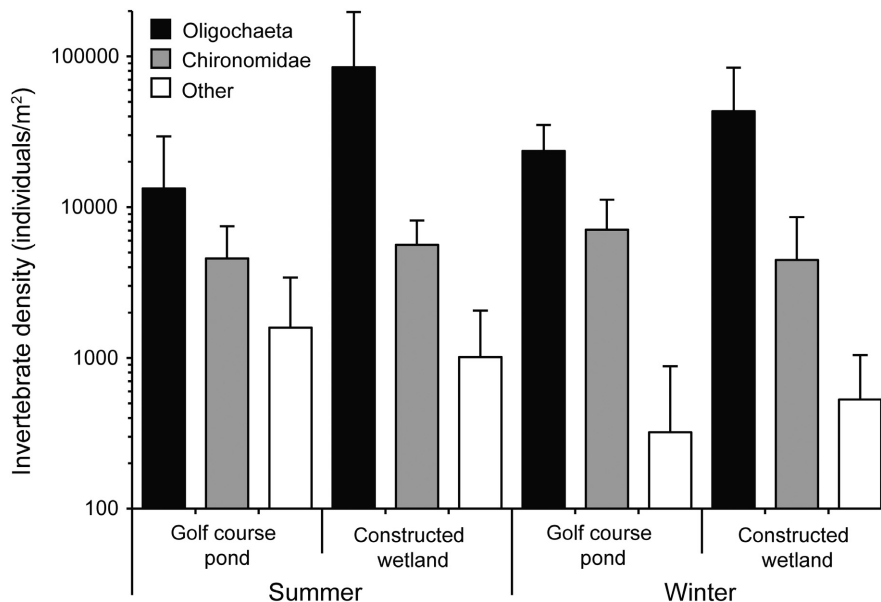


FIG. 3. Average density of oligochaete worms (Oligochaeta), chironomid midge larvae (Chironomidae), and all other invertebrates (Other) in sediment cores collected from golf course ponds ($n = 40$) and stormwater treatment wetlands ($n = 63$) in summer and winter. Error bars indicate 95% confidence intervals.

TABLE 1.  Comparison of candidate multiple regression models explaining variation in benthic $CO_2$, $CH_4$, and $N_2O$ flux.

| GHG (response) | Environmental parameters | Invertebrate parameters | $C_p$ | $R^2$ | $AIC_c$ | $\Delta_i$ | RMSE |
|---|---|---|---|---|---|---|---|
| $CO_2$ | temp, %C | Invert (large) | 3.66 | 0.84 | 27.7 | | **0.3981** |
| | | | 5.78 | 0.73 | 38.6 | 10.9 | 0.4653 |
| $CH_4$ | temp, %C | Oligo (all) | 30.61 | 0.64 | 83.0 | | **1.0357** |
| | | | 48.12 | 0.39 | 93.7 | 10.7 | 1.3354 |
| $N_2O$ | temp, $NO_X$, DO, $NO_X \times$ DO | Oligo (small) | 1.70 | 0.62 | 10.9 | | **0.2877** |
| | | | 1.92 | 0.53 | 12.4 | 1.5 | 0.2994 |

*Notes:* For each greenhouse gas (GHG), the top model as selected by Akaike's information criterion, the model with the best predictive power (lowest RMSE, in boldface type) and the top model excluding invertebrates are provided. $C_p$ is Mallows' $C_p$ (a measurement of model error), $AIC_c$ is Akaike's information criterion corrected for small sample size, $\Delta_i$ is the difference between the candidate and best model's $AIC_c$, and RMSE is the root-mean-square error (model predictive ability). Model components are temp, temperature; %C , sediment C content (upper 3 cm); Invert, Oligochaeta + Chironomidae density; Oligo, Oligochaeta density (all, >0.3 mm; large, >1 mm; small, 0.3–1 mm), $NO_X$, total initial $NO_3^- + NO_2^-$ (mg) present in overlying water of sediment core; DO, field measurement of percent saturation of dissolved $O_2$ in water column; $NO_X \times$ DO%, interaction between $NO_X$ and DO%.

than that of the top-performing $CO_2$ flux model, which included temperature, density of all large oligochaetes and chironomids, and sediment $C_{org}$ content (Table 1, Fig. 4). The improvement of $CH_4$ flux prediction with the inclusion of benthic invertebrate density in models was even greater, with the RMSE of the best model excluding invertebrates being 29% higher (poorer predictive ability) than that of the top-performing $CH_4$ flux model, which included temperature, density of large oligochaetes, and sediment $C_{org}$ content (Table 1, Fig. 5).

In the case of $N_2O$ flux, inclusion of invertebrate density did not substantially improve the predictive ability of models. All of the highest-ranked models ($\Delta i < 2$) models included temperature, nitrate + nitrite ($NO_X$), oxygen % saturation (DO) and an interaction term ($NO_X \times$ DO), but the RMSE of models excluding or including invertebrates were similar (within 4.5%) in predictive ability (Table 1, Fig. 6).

## DISCUSSION

Animals may play critical roles in the function of urban ecosystems, and there is mounting evidence that sediment-dwelling invertebrates influence GHG flux in aquatic ecosystems (Stief et al. 2010, Bonaglia et al. 2014, Nogaro and Burgin 2014, Poulsen et al. 2014, Welsh et al. 2015). The invertebrates dominating faunal communities in the current study frequently reach high densities in degraded wetlands and lakes, due to their abilities to withstand hypoxia, eutrophication, and heavy metal contamination (Wiederholm 1980, Datry et al. 2003, Nogaro and Mermillod-Blondin 2009, Pigneret et al. 2016). Using a unique data set, our analysis shows that $CO_2$ and $CH_4$ flux in urban wetlands is positively correlated to the densities of these ubiquitous, pollution-tolerant invertebrates, suggesting faunal enhancement of GHG flux. Each doubling in density of large benthic
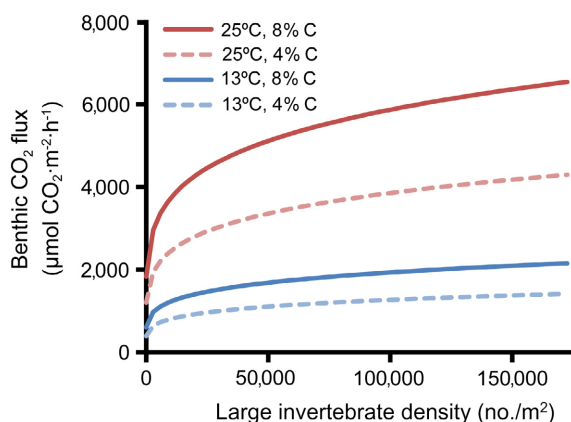


FIG. 4.  Estimated effects of large benthic invertebrates (>1 mm) on benthic $CO_2$ flux at varying temperatures and sediment carbon (C) concentrations in urban wetlands. Low and high values for temperature and C are set at the first and third quartile of field-collected data: 13° and 25°C, and 4% and 8% C in sediments. [Color figure can be viewed at wileyonlinelibrary.com]
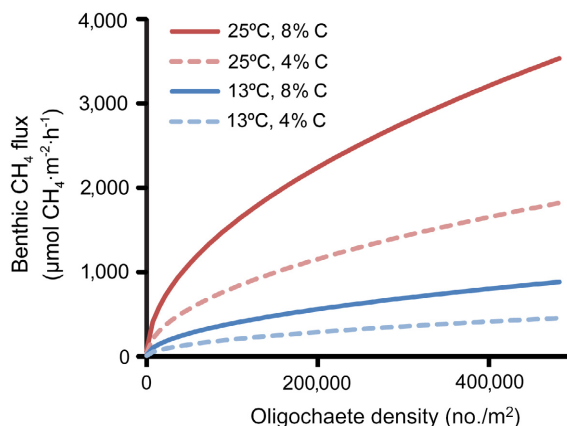


FIG. 5.  Estimated effects of oligochaetes on benthic $CH_4$ flux at varying temperatures and sediment carbon (C) concentrations in urban wetlands. Low and high values for temperature and C are set at the first and third quartiles of field-collected data: 13° and 25°C, and 4% and 8% C in sediments. [Color figure can be viewed at wileyonlinelibrary.com]
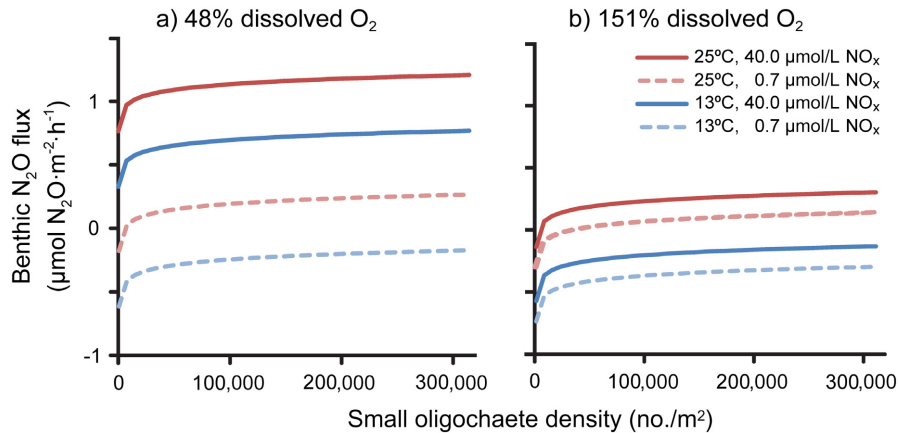
FIG. 6. Estimated effects of small oligochaetes (0.3–1.0 mm) on benthic $N_2O$ flux at (a) low and (b) high dissolved oxygen percent saturation, with varying temperature and water column nitrate + nitrite ($NO_x$) concentrations in urban wetlands. Low and high values for temperature and $NO_x$ are set at the first and third quartiles of field-collected data: 13° and 25°C and 0.7 and 40.0 μmol/L $NO_x$-N. Dissolved oxygen is set at 10th and 90th percentiles (48% and 151% saturation). [Color figure can be viewed at wileyonlinelibrary.com]

invertebrates (Tubificid oligochaetes + midge larvae) or oligochaetes alone corresponded to a ~15% and ~42% average increase in $CO_2$ and $CH_4$ flux, respectively. Thus, urbanization, by causing shifts in benthic fauna communities, may alter GHG budgets of cities. As conditions in urban aquatic ecosystems often favor the development of faunal communities dominated by Tubificid oligochaetes (Palmer 1968, Paul and Meyer 2001), stimulatory effects on both $CO_2$ and $CH_4$ emissions may occur as a result. This suggests a functional link between faunal community structure and GHG flux enhancement that may be globally widespread among urban aquatic ecosystems.

The substantial stimulation of $CO_2$ and $CH_4$ flux with increasing invertebrate density in the present study (Figs. 4, 5) is likely due to several factors, including (1) enhanced upward gas transport and diffusion through invertebrate burrows, (2) rapid cycling of organic matter between oxic and anoxic layers of sediment, due to conveyor-belt feeding by oligochaetes (Fig. 1), and (3) simultaneous enhancement of oxygenation and aerobic microbial respiration in upper sediment layers due to increased porosity, and enhanced anoxia (and therefore methane production) in lower layers of sediment due to microbial and oligochaete respiration. While it has been suggested that invertebrates grazing on methanotrophic bacteria have the potential to enhance $CH_4$ fluxes in aquatic ecosystems (Kankaala et al. 2007), this seems unlikely in the current study. The $\delta^{13}C$ values as low as −38.4 ‰ suggest the possibility that midge larvae may have consumed methanotrophic bacteria at a few of our study sites (Appendix S1: Fig. S1; Jones et al. 2008), but this was rare and not observed for oligochaetes, which were the only taxa with densities significantly correlated to $CH_4$ flux.

Oligochaete bioturbation enhances sediment porosity and diffusion (Wang and Matisoff 1997, Mermillod-Blondin

et al. 2003), which could facilitate upward transport of $CO_2$ and $CH_4$ through sediments. Oligochaete and chironomid burrows also allow for increased penetration of oxygen from overlying water into sediments, which enhances aerobic respiration and flux of $CO_2$ (Nogaro and Burgin 2014). However, while increased sediment porosity may enhance $CH_4$ *transport*, increased movement of sediments via conveyor-belt feeding (Matisoff et al. 1999) may be the most plausible mechanism by which oligochaetes contribute to enhanced $CH_4$ *production*. Through feeding head-down in sediments, and defecating above the sediment surface, oligochaetes rapidly transport reduced organic matter from deeper sediments upward to the oxic sediment-water interface. As fecal pellets accumulate in successive layers at the sediment surface while organic particles are consumed in deeper sediments by oligochaete feeding, this causes an enhanced movement of sediments from surface to deeper anoxic sediment layers. This downward transport of particles can be rapid (Fisher et al. 1980), with sediment velocities as high as 3.66 cm/d 100,000 individuals/m$^2$ measured for larger oligochaetes (Matisoff et al. 1999). The rate of sediment turnover caused by oligochaetes often exceeds sedimentation rates in their absence by more than an order of magnitude (Fisher et al. 1980, Robbins et al. 1989). Presumably, during this process labile organic particles (such as sinking phytoplankton, oligochaete fecal pellets, or guano) spend a great amount of time in deeper anoxic layers of sediment, allowing for enhanced $CH_4$ production, while in the absence of bioturbation, a greater proportion of labile organic particles are respired at the oxic sediment-water interface, resulting in C being respired primarily as $CO_2$. Oligochaete density was not correlated to the quantity of C in sediments, but may have varied among sites in response to differences in sediment C *quality*. The disentangling of the relative importance of these drivers has the potential

to vastly improve our understanding of the drivers of $CH_4$ flux in wetlands, as well as our ability to forecast changes in wetland GHG flux in response to urbanization and shifts in faunal community structure.

Why was stronger invertebrate enhancement of $N_2O$ emission not detected in the current study, even though oligochaete and midge larval densities were high? This is likely due to the combined effects of $NO_3^-$ availability, temperature, and sediment organic matter content. Benthic invertebrates can enhance the flux of $N_2O$ by creating alternating oxic and anoxic conditions within sediments during burrow ventilation, thereby enhancing coupled nitrification–denitrification (Stief et al. 2009, Hölker et al. 2015). Furthermore, the ingestion of denitrifying bacteria briefly exposes them to anoxic conditions within invertebrate guts, which enhances production of $N_2O$ via incomplete denitrification (Stief et al. 2009). However, the activity of denitrifying microbes (and invertebrate enhancement of their activity) is enhanced by temperature and the availability of $NO_3^-$ (Stief and Schramm 2010). Compared with previous laboratory studies, wetlands in the current study had low concentrations of $NO_3^-$ in water overlying sediments (median = 1.96 µmol $NO_3^-$ N/L, range: below detection to 77.96 µmol N/L). Several studies have found enhanced $N_2O$ emission by invertebrates to be dependent upon temperature and $NO_3^-$ thresholds (Stief and Schramm 2010, Stief et al. 2010). For example, Stief et al. (2010) showed that $N_2O$ emissions from midge larvae were positively stimulated by temperature at $NO_3^-$ concentrations of 250 and 500 µmol N/L, but not at concentrations of 10 and 50 µmol N/L. Likewise, larval $N_2O$ emissions were stimulated by $NO_3^-$ availability, but only above a temperature threshold somewhere around 4–10°C. In the current study, water column temperature and $NO_3^-$ availability were negatively correlated ($R^2 = 0.32$, $t_{1,24} = -3.37$, $P < 0.005$). During the winter, when $NO_3^-$ concentrations were elevated (median = 36.27, range = 0.57–77.96 µmol N/L), temperatures were approaching the threshold below which the stimulatory effects of $NO_3^-$ on larval $N_2O$ emission might be reduced (median = 11.9, range = 8.6–14.8°C). During the summer, when temperatures were high (median = 23.5, range = 18.8–27.3°C), $NO_3^-$ concentrations were low (median = 0.21, range = below detection–61.97 µmol N/L).

While benthic invertebrates can enhance $N_2O$ production within the walls of their burrows or emit $N_2O$ directly from their guts (Stief et al. 2009, Heisterkamp et al. 2010, Bonaglia et al. 2014), the $N_2O$ flux that ultimately escapes to the water column depends upon redox conditions within the surrounding sediments. Sediment $N_2O$ emissions are highest at intermediate redox (de Bie et al. 2002, Seo and DeLaune 2010), and high organic matter content in sediments will lead to more strongly reducing conditions, which consume $N_2O$ and result in higher fluxes of $N_2$. Other studies have observed invertebrate enhancement of $N_2O$ flux at

low temperatures and low concentrations of $NH_4^+$ and $NO_3^-$, but these were either conducted in sites with substantially lower (<0.3%) sediment organic C content (Bonaglia et al. 2014, Welsh et al. 2015) than those in the current study (2.1–12.6% C, median = 5.5% C), or measured emission of $N_2O$ from invertebrates incubated without surrounding sediments (Heisterkamp et al. 2010). Organic matter in surrounding sediments may enhance $N_2$ emissions relative to $N_2O$, as was likely the case in studies by Stief and Schramm (2010) and Welsh et al. (2015), where $N_2O$ fluxes from animals incubated without sediments were substantially higher than fluxes measured from invertebrates in situ (within sediments). Likewise, Nogaro and Burgin (2014) measured significant stimulation of $N_2$ flux at higher densities of oligochaetes, but differences in $N_2O$ flux among worm density treatments were only moderately significant ($P = 0.064$). Although their incubations were run with $NO_3^-$ concentrations ranging from 100 to 470 µmol/L N (~51–240 × higher than the median value in the current study, 1.96 µmol/L N), the sediment utilized in their core incubations contained substantially more C (~15% organic C) than in the current study (median 5.5%C), which likely resulted in the conversion of $NO_3^-$ to $N_2$ rather than $N_2O$ prior to escaping the sediment. These results underscore the need to consider seasonality (i.e., variability in $NO_3^-$ availability and temperature) and the context-dependent nature (e.g., incubation conditions, sediment C content) of invertebrate effects on GHG fluxes.

The apparent multiplicative enhancement effects of temperature, sediment C content, and benthic invertebrate abundance on $CO_2$ and $CH_4$ flux in the current study (Figs. 4, 5) suggest a deleterious positive feedback with continuing climate change and urbanization. Shifts toward oligochaete-dominated invertebrate communities are a common consequence of eutrophication, pesticide, and heavy metal pollution in aquatic ecosystems (Palmer 1968, Wiederholm 1980, Winner et al. 1980, Paul and Meyer 2001, Devine and Vanni 2002, Verdonschot 2006). As regions of the world become increasingly urbanized, if the impacts of increased metal mobility, hypoxia, and nutrient loading on wetlands are not kept in check, shifts in aquatic invertebrate communities toward oligochaete dominance may become more widespread. As wetland $CO_2$ and $CH_4$ fluxes increase due to higher temperatures and oligochaete densities, additional atmospheric warming may result, causing greater enhancement of wetland GHG flux. This is one of the first field studies of the influence of invertebrate communities on urban wetland GHG emissions. Further research is needed in order to confirm whether the mechanisms suggested by our results are broadly applicable, but our findings open a new line of inquiry in the investigation of community structure effects on urban ecosystem function and global wetland GHG budgets.

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## Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1572/full

## Data Availability

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.f352f