

UC Irvine

UC Irvine Previously Published Works

Title

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

Permalink

<https://escholarship.org/uc/item/8bt3f1dc>

Journal

Ecological applications : a publication of the Ecological Society of America, 27(6)

ISSN

1051-0761

Authors

Mehring, Andrew S
Cook, Perran LM
Evrard, Victor
[et al.](#)

Publication Date

2017-09-01

DOI

10.1002/eap.1572

Peer reviewed

1 **Running head:** Invertebrates enhance wetland gas flux

2 **Pollution-tolerant invertebrates enhance greenhouse gas flux in urban wetlands**

3

4

5 Andrew S. Mehring^{1*}, Perran L. M. Cook², Victor Evrard³, Stanley B. Grant⁴, Lisa A. Levin^{1,5}

6

7 ¹Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA, 92093-
8 0218, USA

9 ²Water Studies Center, School of Chemistry, Monash University, Clayton, Victoria, 3800,
10 Australia

11 ³Department of Environmental Sciences, University of Basel, Schönbeinstrasse 6, CH-4056
12 Basel, Switzerland

13 ⁴Department of Civil and Environmental Engineering, Henry Samueli School of Engineering,
14 University of California, Irvine, Irvine, CA, 92697, USA

15 ⁵Center for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, La Jolla,
16 CA 92093-0218

17

18

19 ***Correspondance:** Email: amehring@ucsd.edu, Phone : 717-398-9162, Fax : 858-822-0562

20

21 **Abstract**

22 One of the goals of urban ecology is to link community structure to ecosystem function in
23 urban habitats. Pollution-tolerant wetland invertebrates have been shown to enhance greenhouse
24 gas (GHG) flux in controlled laboratory experiments, suggesting that they may influence urban
25 wetland roles as sources or sinks of GHG. However, it is unclear if their effects can be detected
26 in highly variable conditions in a field setting. Here we use an extensive dataset on carbon
27 dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) flux in sediment cores (n = 103)
28 collected from ten urban wetlands in Melbourne, Australia during summer and winter in order to
29 test for invertebrate enhancement of GHG flux. We detected significant multiplicative
30 enhancement effects of temperature, sediment carbon content, and invertebrate density on CH₄
31 and CO₂ flux. Each doubling in density of oligochaete worms or large benthic invertebrates
32 (oligochaete worms and midge larvae) corresponded to ~42% and ~15% increases in average
33 CH₄ and CO₂ flux, respectively. However, despite exceptionally high densities, invertebrates did
34 not appear to enhance N₂O flux. This was likely due to fairly high organic carbon content in
35 sediments (range: 2.1-12.6%), and relatively low nitrate availability (median 1.96 μM NO₃⁻-N),
36 which highlights the context-dependent nature of community structural effects on ecosystem
37 function. The invertebrates enhancing GHG flux in this study are ubiquitous, and frequently
38 dominate faunal communities in impaired aquatic ecosystems. Therefore, invertebrate effects on
39 CO₂ and CH₄ flux may be common in wetlands impacted by urbanization, and urban wetlands
40 may make greater contributions to the total GHG budgets of cities if the negative impacts of
41 urbanization on wetlands are left unchecked.

42

43 **Key Words:** carbon dioxide, Chironomidae, climate change, constructed wetland, golf course,
44 methane, Naididae, nitrous oxide, Oligochaeta, Tubificidae, Tubificinae, urbanization

45 **Introduction**

46 One of the objectives of urban ecology is to search for generalizable processes and
47 community structures that influence ecosystem function within urban habitats (McPhearson et al.
48 2016). Faunal community structure in aquatic ecosystems can be altered by several
49 environmental impacts associated with urbanization, such as increased mobility of metals,
50 nutrients, and pesticides (Carpenter et al. 1998, Walsh et al. 2005, Kaye et al. 2006, Kaushal et
51 al. 2008, Allinson et al. 2015), introduction of exotic predators (Hamer and Parris 2013), and
52 increased prevalence of hypoxia (Jenny et al. 2016). Urbanization is a major factor contributing
53 to the global loss and degradation of natural wetlands (van Asselen et al. 2013), but urbanization
54 also spurs the construction of new wetlands for storage of water supplies, for recreation (such as
55 golf course ponds), and for stormwater and wastewater treatment (Davidson 2014). Although
56 urban wetlands may contribute to biodiversity at the landscape scale (Hill et al. 2016), they can
57 seldom be considered analogs of natural wetlands as far as community structure is concerned
58 (Hassall 2014). Because urban wetlands intercept heavy metals and other toxic substances that
59 often become associated with sediments, pollutant accumulation and associated negative impacts
60 may be greatest for benthic fauna, an important functional component of the faunal community
61 (Covich et al. 1999). Furthermore, because treatment wetlands are designed to intercept and treat
62 large inputs of carbon (C) and nitrogen (N), one of the primary concerns associated with their
63 increased adoption is the potential for enhanced greenhouse gas (GHG) emissions (Verhoeven et
64 al. 2006, Burgin et al. 2013, Mander et al. 2014). Wetland roles as sinks or sources of GHG are
65 strongly controlled by environmental drivers such as temperature and the availability of C and N,

66 which can stimulate emission of nitrous oxide (N₂O), methane (CH₄), and carbon dioxide (CO₂)
67 (Cao et al. 1996, Burgin et al. 2013, Nisbet et al. 2014, Butman et al. 2016, Deemer et al. 2016).
68 Human-made wetlands in natural and urban environments are important contributors to global
69 aquatic emissions of N₂O and CH₄ (Deemer et al. 2016), which have been rapidly increasing in
70 recent years (Syakila and Kroeze 2011, Nisbet et al. 2014). An understanding of controls on
71 GHG emissions in these built environments is critical to the assessment of urban wetland
72 contributions to global GHG budgets (Burgin et al. 2013, Mitsch et al. 2013, Mander et al. 2014).

73 Pollution-tolerant oligochaete and midge species are able to reach high densities in
74 severely impacted aquatic environments where other taxa may be excluded (Datry et al. 2003,
75 Pigneret et al. 2016). They have also been shown to enhance nutrient, metal, and GHG flux in
76 controlled laboratory experiments (Lagauzère et al. 2009, Stief et al. 2009, Nogaro and Burgin
77 2014, Hölker et al. 2015), and therefore may exert strong influence on multiple ecosystem
78 functions in urban wetlands. However, addressing the effects of invertebrates on GHG flux can
79 be challenging for multi-species assemblages. Previous studies demonstrating enhancement of
80 GHG flux by oligochaetes and midge larvae have done so under highly-controlled conditions in
81 laboratories, primarily with single species, and often with elevated nutrient concentrations (Stief
82 et al. 2009, Stief et al. 2010, Nogaro and Burgin 2014, Poulsen et al. 2014). While controlled
83 conditions have allowed for effective isolation of invertebrate effects, an assessment of the
84 effects of mixed assemblages under variable conditions is important to our understanding of
85 faunal influence on GHG fluxes in aquatic ecosystems. Sediment-dwelling taxa may have
86 synergistic or antagonistic effects when present in combination, due to their different modes of
87 bioturbation. For example, midge larvae bioirrigate sediments through construction and
88 ventilation of U-shaped tubes, but oligochaetes redistribute sediments to a greater degree and at

89 greater depths than midge larvae, via “conveyor-belt feeding” (Fig. 1) (Lagauzère et al. 2009).
90 Furthermore, given the variable environmental conditions present in field settings, the degree to
91 which invertebrate effects can be detected relative to other drivers of GHG flux amidst the
92 “noise” in urban environments requires further investigation.

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

103 **Materials and Methods**

104 *Study sites* – We collected 104 sediment cores (four per site) from ten urban wetlands
105 (five stormwater treatment wetlands and five golf course ponds [hereafter referred to as
106 treatment wetlands and gc ponds]) in the suburbs of Melbourne, Victoria, Australia during
107 summer (January 29 – March 4) and winter/early spring (August 5 – September 16, hereafter
108 referred to as “winter”) 2014 (see Appendix S1: Table S1 for site information). In three of the
109 five treatment wetland sites, both inlets and outlets were sampled for comparison. To measure
110 GHG flux and benthic invertebrate density, sediment cores (27.5×6.6 cm acrylic cylinders)
111 were collected from four spatially-separated locations haphazardly chosen in each site, in areas

112 without emergent vegetation. After gently inserting a core to roughly half its length into the
113 sediment, cores were capped with rubber stoppers, carefully removed to prevent shaking and
114 disturbance of sediments, and returned to the laboratory at Monash University Water Studies
115 Center. Surface water samples were also collected for analysis of chlorophyll and nutrients, and
116 other environmental parameters (oxygen, temperature, pH) were measured with Horiba probes
117 (Appendix S1: Table S2).

118 *Lab methods*

119 *Core incubations* - To link invertebrate densities to benthic GHG emissions, four
120 sediment cores from each site were incubated following methods described by Roberts et al.
121 (2012). Briefly, intact cores were placed in a water bath controlled to within $1.3 \pm 0.3^\circ\text{C}$ (mean \pm
122 95% C.I.) of *in situ* temperature, and equilibrated overnight while core water was gently aerated.
123 On the following day, aeration was ceased, cores were capped, and dissolved oxygen (DO)
124 measurements were taken through a sealable port in the cap with an optical DO probe (HACH
125 HQ40d Portable Meter, LDO101 Rugged Optical Dissolved Oxygen Probe). Water overlying
126 sediment in each core was stirred gently and continuously throughout the incubation by a
127 suspended magnetic bar, with a stirring rate below levels that would cause sediment disturbance.
128 12.5 mL of overlying water were removed through a port in the cap every few hours, placed into
129 12.5-mL Exetainers® (Labco Ltd., UK) with gas-tight septa, and preserved with HgCl_2 until
130 analysis for CO_2 , CH_4 , and N_2O concentrations with a gas chromatograph (GC) (Shimadzu
131 GC17A) equipped with a Methanizer (SRI Instruments, USA). Changes in GHG concentration
132 over time were used to calculate the flux of the three GHGs. Aliquots of water removed during
133 incubation were replaced with an equal volume of water collected from the same site, and care
134 was taken during all procedures to prevent the creation of headspace or entrapment of air bubbles

135 during incubation periods. The water volume and surface area of the sediment were taken into
136 consideration when calculating fluxes, along with correction for the addition or dilution of
137 constituents by the replacement of water (Roberts et al. 2012).

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

144 *Core processing* – Immediately following each incubation, overlying water was carefully
145 removed (with syringe) from above the sediment-water interface. To estimate sediment porosity,
146 a sub-sample ($\sim 3 \text{ cm}^3$) of surface sediments was pulled into a syringe, volume recorded, then
147 sediments were placed into a conical bottom tube, wet weight was recorded, sediment was dried
148 at 60°C for 4 days, and finally dry weight was recorded. Sediment porosity (mL pore volume per
149 mL sample volume) was calculated as water volume (sample wet weight – dry weight) divided
150 by sample volume. Dried sediments were later ground until they were capable of passing through
151 a 250- μm sieve and analyzed for elemental organic C (C_{org}), organic N (N_{org}), $\delta^{13}\text{C}$ (‰) and $\delta^{13}\text{N}$
152 (‰) using an ANCA GSL2 elemental analyzer interfaced to a Hydra 20-22 continuous-flow
153 isotope ratio mass-spectrometer (Sercon Ltd., UK) at the Water Studies Centre, Monash
154 University. This was repeated for sediments at depths greater than 10 cm within the core as well.
155 Remaining core material was rinsed over a 300- μm sieve. To evaluate trophic position and the
156 contribution of methanotrophic bacteria to invertebrate diets, midge larvae and oligochaetes were
157 analyzed for $\delta^{13}\text{C}$ (‰) and $\delta^{13}\text{N}$ (‰). A few specimens were removed from each core, allowed to

158 clear their guts for 24 hours, and dried at 60°C for 48 hours. These were weighed to the nearest
159 μg , and then analyzed for C_{org} and N_{org} content and stable isotope ratios ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) using the
160 methods described above. Data are provided in the supporting information (Appendix S1: Fig.
161 S1).

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

168 *Statistical analysis*

169 To determine the relative importance of invertebrate density among a set of factors best
170 explaining variability in benthic fluxes of CH_4 , N_2O , and CO_2 , we compared multiple linear
171 regression models using Akaike's Information Criterion (AIC) and an information theoretic
172 approach (Burnham and Anderson 2002). For all parameters, values from individual sediment
173 cores (four per site) were averaged together in a given season, and treated as a single replicate.
174 Parameters initially selected for possible inclusion in multiple regression models were
175 invertebrate density, which included density of oligochaetes and midge larvae (Tanypodinae
176 and/or non-Tanypodinae), in both large ($>1\text{mm}$) and small (0.3-1 mm) size classes. In addition,
177 temperature, water column chlorophyll, sediment %C, and dissolved oxygen saturation (DO%)
178 were included as environmental factors that may influence GHG flux. DO% was included both
179 for the inhibitory effects that oxygen availability has on denitrification and CH_4 production, and
180 also as an indicator of the presence of benthic microalgae, which may compete with N_2O -

181 producing bacteria for NO_3^- , and would not be detected with water column chlorophyll
182 measurements. For N_2O flux models, water column NO_x (nitrate + nitrite) concentration was
183 included as a model parameter, but water column nutrients were excluded from other models
184 after initial examination failed to show statistically significant correlations with response
185 variables.

186 Explanatory variables were tested for multicollinearity with Pearson's correlation
187 coefficient (r) matrices and variance inflation factors (VIF), and any two variables with $r \geq 0.60$
188 were not included in the same model. The predictive abilities of models were compared by using
189 leave-one-out cross-validation (LOOCV) (James et al. 2013). Predicted GHG flux values were
190 regressed against observed GHG fluxes, and root-mean-squared errors (RMSE) were calculated
191 for each model, with lower RMSE indicating improved predictive power. All analyses were
192 conducted in R software (R Development Core Team 2008).

193 Average CO_2 , CH_4 , and N_2O fluxes were predicted for increasing densities of benthic
194 invertebrates using parameter estimates from the model with lowest RMSE for each GHG. For
195 CO_2 and CH_4 , the average flux was reported for four different scenarios: (1) low temperature and
196 low sediment C concentration (C_{org}), (2) low temperature and high C_{org} , (3) high temperature and
197 low C_{org} , and (4) high temperature and high C_{org} . Low and high values for temp and C_{org} were set
198 at the 1st and 3rd quartile of field-collected data: 13 and 25°C, and 4 and 8% C_{org} in sediments.
199 For N_2O , average flux was predicted for high and low temperatures, for 1st and 3rd quartiles of
200 $\text{NO}_x\text{-N}$ availability (0.7 and 40 μM), and also for 10th and 90th percentiles (48 and 151%) of
201 dissolved oxygen saturation.

202 **Results**

203 *Benthic greenhouse gas flux*

204 Benthic fluxes of CO₂ and CH₄ were higher in summer than in winter (Fig. 2, F_{1,22} =
205 23.89 and 5.10, $p < 0.001$ and < 0.05 , respectively), and seasonal differences were strongest in
206 stormwater treatment wetlands (hereafter referred to as treatment wetlands), where benthic fluxes
207 were higher in inlets during the summer than in outlets for CH₄ (season×inlet F_{1,8} = 21.06, $p <$
208 0.005) and CO₂ (season×inlet F_{1,8} = 16.35, $p < 0.005$). While overall fluxes of GHG were not
209 significantly different between wetland types, inlet fluxes in treatment wetlands were higher than
210 fluxes in golf course ponds (hereafter referred to as gc ponds) during the summer for CH₄
211 (season×site F_{2,16} = 11.26, $p < 0.001$) and CO₂ (season×site F_{2,16} = 11.04, $p < 0.001$, Fig. 2).
212 There were no significant differences in benthic N₂O fluxes either seasonally, among site types,
213 or among locations within sites.

214 *Benthic invertebrates*

215 Invertebrate communities in all sites were dominated by oligochaetes and midge larvae.
216 Oligochaetes accounted for $\sim 82 \pm 10\%$ and $60 \pm 20\%$ (mean \pm 95% C.I.) of average total
217 invertebrate density in treatment wetlands and gc ponds, respectively (Fig. 3, Appendix S1:
218 Table S3). Tubificinae (family Naididae) was the most abundant subfamily of oligochaetes,
219 accounting for $78 \pm 9\%$ and $85 \pm 14\%$ of all oligochaetes encountered in treatment wetlands and
220 gc ponds (Appendix S1: Table S4). Oligochaete density did not differ significantly between
221 treatment wetlands and gc ponds (F_{1,22} = 2.15, $p = 0.16$), or between inlets and outlets within
222 treatment wetlands (F_{1,8} = 2.36, $p = 0.16$). However, oligochaete density was significantly higher
223 in inlets of treatment wetlands than in gc ponds (F_{1,12} = 5.73, $p < 0.05$), with densities as high as
224 508,886 m⁻² (Table S4). Although oligochaete density did not undergo statistically significant
225 seasonal changes, it was reduced by 97% between summer and winter in the inlet of the

226 Huntingdale Road treatment wetland, following the removal of upper layers of sediment during
227 wetland maintenance (reduced from $478,634 \pm 24,580$ to $11,472 \pm 4,231^{-2}$ [mean \pm 95% C.I.]).

228 Midge larvae accounted for $16 \pm 7\%$ and $34 \pm 19\%$ of average total invertebrate density
229 in treatment wetlands and gc ponds, respectively (Appendix S1: Tables S3, S5). Midge larval
230 density did not differ significantly between seasons or site types, and there was no significant
231 difference in midge larval density between the families Tanypodinae and Chironominae (paired
232 t-test, $t_{25} = 2.06$, $p = 0.50$, Table S5). Almost all Tanypodinae present in our sites were
233 *Procladius* spp., and stable isotope values suggest a non-predatory diet, as $\delta^{15}\text{N}$ of Tanypodinae
234 (7.68 ± 1.62 , range -0.52 to 10.50) were not significantly different from those of Chironominae
235 (8.37 ± 0.53 , range -0.18 to 13.56) or oligochaetes (7.25 ± 0.65 , range 4.16 to 18.43).

236 *Drivers of greenhouse gas flux and the importance of invertebrates*

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

243 The predictive abilities of CO_2 and CH_4 models were substantially improved with the
244 inclusion of invertebrates (Table 1, Figs. 4,5). Each doubling in density of large benthic
245 invertebrates or oligochaetes corresponded to a $\sim 15\%$ and $\sim 42\%$ average increase in CO_2 and
246 CH_4 flux, respectively. The RMSE of the best model excluding invertebrates was 17% higher
247 (poorer predictive power) than that of the top-performing CO_2 flux model, which included
248 temperature, density of all large oligochaetes and chironomids, and sediment C_{org} content (Table

249 1, Fig. 4). The improvement of CH₄ flux prediction with the inclusion of benthic invertebrate
250 density in models was even greater, with the RMSE of the best model excluding invertebrates
251 being 29% higher (poorer predictive ability) than that of the top-performing CH₄ flux model,
252 which included temperature, density of large oligochaetes, and sediment C_{org} content (Table 1,
253 Fig. 5).

254 In the case of N₂O flux, inclusion of invertebrate density did not substantially improve
255 the predictive ability of models. All of the highest-ranked models ($\Delta i < 2$) models included
256 temperature, nitrate + nitrite (NO_x), oxygen % saturation (DO) and an interaction term
257 (NO_x×DO), but the RMSE of models excluding or including invertebrates were similar (within
258 4.5%) in predictive ability (Table 1, Fig. 6).

259 Discussion

260 Animals may play critical roles in the function of urban ecosystems, and there is
261 mounting evidence that sediment-dwelling invertebrates influence GHG flux in aquatic
262 ecosystems (Stief et al. 2010, Bonaglia et al. 2014, Nogaro and Burgin 2014, Poulsen et al. 2014,
263 Welsh et al. 2015). The invertebrates dominating faunal communities in the current study
264 frequently reach high densities in degraded wetlands and lakes, due to their abilities to withstand
265 hypoxia, eutrophication, and heavy metal contamination (Wiederholm 1980, Datry et al. 2003,
266 Nogaro and Mermillod-Blondin 2009, Pignoret et al. 2016). Using a unique dataset, our analysis
267 shows that CO₂ and CH₄ flux in urban wetlands is positively correlated to the densities of these
268 ubiquitous, pollution-tolerant invertebrates, suggesting faunal enhancement of GHG flux. Each
269 doubling in density of large benthic invertebrates (Tubificid oligochaetes + midge larvae) or
270 oligochaetes alone corresponded to a ~15% and ~42% average increase in CO₂ and CH₄ flux,
271 respectively. Thus, urbanization, by causing shifts in benthic fauna communities, may alter GHG

272 budgets of cities. As conditions in urban aquatic ecosystems often favor the development of
273 faunal communities dominated by Tubificid oligochaetes (Palmer 1968, Paul and Meyer 2001),
274 stimulatory effects on both CO₂ and CH₄ emissions may occur as a result. This suggests a
275 functional link between faunal community structure and GHG flux enhancement that may be
276 globally widespread among urban aquatic ecosystems.

277 The substantial stimulation of CO₂ and CH₄ flux with increasing invertebrate density in
278 the present study (Figs. 4,5) is likely due to several factors, including: (1) enhanced upward gas
279 transport and diffusion through invertebrate burrows, (2) rapid cycling of organic matter between
280 oxic and anoxic layers of sediment, due to conveyor-belt feeding by oligochaetes (Fig. 1) and (3)
281 simultaneous enhancement of oxygenation and aerobic microbial respiration in upper sediment
282 layers due to increased porosity, and enhanced anoxia (and therefore methane production) in
283 lower layers of sediment due to microbial and oligochaete respiration. While it has been
284 suggested that invertebrates grazing on methanotrophic bacteria have the potential to enhance
285 CH₄ fluxes in aquatic ecosystems (Kankaala et al. 2007), this seems unlikely in the current study.
286 $\delta^{13}\text{C}$ values as low as -38.4 ‰ suggest the possibility that midge larvae may have consumed
287 methanotrophic bacteria at a few of our study sites (Appendix S1: Fig. S1) (Jones et al. 2008),
288 but this was rare and not observed for oligochaetes, which were the only taxa with densities
289 significantly correlated to CH₄ flux.

290 Oligochaete bioturbation enhances sediment porosity and diffusion (Wang and Matisoff
291 1997, Mermillod-Blondin et al. 2003), which could facilitate upward transport of CO₂ and CH₄
292 through sediments. Oligochaete and chironomid burrows also allow for increased penetration of
293 oxygen from overlying water into sediments, which enhances aerobic respiration and flux of CO₂
294 (Nogaro and Burgin 2014). However, while increased sediment porosity may enhance CH₄

295 *transport*, increased movement of sediments via conveyor-belt feeding (Matisoff et al. 1999)
296 may be the most plausible mechanism by which oligochaetes contribute to enhanced CH₄
297 *production*. Through feeding head-down in sediments, and defecating above the sediment
298 surface, oligochaetes rapidly transport reduced organic matter from deeper sediments upward to
299 the oxic sediment-water interface. As fecal pellets accumulate in successive layers at the
300 sediment surface while organic particles are consumed in deeper sediments by oligochaete
301 feeding, this causes an enhanced movement of sediments from surface to deeper anoxic sediment
302 layers. This downward transport of particles can be rapid (Fisher et al. 1980), with sediment
303 velocities as high as 3.66 cm d⁻¹ 100,000 individuals m⁻² measured for larger oligochaetes
304 (Matisoff et al. 1999). The rate of sediment turnover caused by oligochaetes often exceeds
305 sedimentation rates in their absence by more than an order of magnitude (Fisher et al. 1980,
306 Robbins et al. 1989). Presumably, during this process labile organic particles (such as sinking
307 phytoplankton, oligochaete fecal pellets, or guano) spend a great amount of time in deeper
308 anoxic layers of sediment, allowing for enhanced CH₄ production, while in the absence of
309 bioturbation, a greater proportion of labile organic particles are respired at the oxic sediment-
310 water interface, resulting in C being respired primarily as CO₂. Oligochaete density was not
311 correlated to the quantity of C in sediments, but may have varied among sites in response to
312 differences in sediment C *quality*. The disentangling of the relative importance of these drivers
313 has the potential to vastly improve our understanding of the drivers of CH₄ flux in wetlands, as
314 well as our ability to forecast changes in wetland GHG flux in response to urbanization and shifts
315 in faunal community structure.

316 Why was stronger invertebrate enhancement of N₂O emission not detected in the current
317 study, even though oligochaete and midge larval densities were high? This is likely due to the

318 combined effects of NO_3^- availability, temperature, and sediment organic matter content. Benthic
319 invertebrates can enhance the flux of N_2O by creating alternating oxic and anoxic conditions
320 within sediments during burrow ventilation, thereby enhancing coupled nitrification-
321 denitrification (Stief et al. 2009, Hölker et al. 2015). Furthermore, the ingestion of denitrifying
322 bacteria briefly exposes them to anoxic conditions within invertebrate guts, which enhances
323 production of N_2O via incomplete denitrification (Stief et al. 2009). However, the activity of
324 denitrifying microbes (and invertebrate enhancement of their activity) is enhanced by
325 temperature and the availability of NO_3^- (Stief and Schramm 2010). Compared with previous
326 laboratory studies, wetlands in the current study had low concentrations of NO_3^- in water
327 overlying sediments (median = $1.96 \mu\text{mol NO}_3^- \text{N L}^{-1}$, range: below detection to $77.96 \mu\text{mol N}$
328 L^{-1}). Several studies have found enhanced N_2O emission by invertebrates to be dependent upon
329 temperature and NO_3^- thresholds (Stief et al. 2010, Stief and Schramm 2010). For example, Stief
330 et al. (2010) showed that N_2O emissions from midge larvae were positively stimulated by
331 temperature at NO_3^- concentrations of 250 and $500 \mu\text{mol N L}^{-1}$, but not at concentrations of 10
332 and $50 \mu\text{mol N L}^{-1}$. Likewise, larval N_2O emissions were stimulated by NO_3^- availability, but
333 only above a temperature threshold somewhere around 4–10°C. In the current study, water
334 column temperature and NO_3^- availability were negatively correlated ($R^2 = 0.32$, $t_{1,24} = -3.37$, $p <$
335 0.005). During the winter, when NO_3^- concentrations were elevated (median = 36.27, range =
336 $0.57\text{-}77.96 \mu\text{mol N L}^{-1}$), temperatures were approaching the threshold below which the
337 stimulatory effects of NO_3^- on larval N_2O emission might be reduced (median = 11.9, range =
338 $8.6\text{-}14.8 \text{ }^\circ\text{C}$). During the summer, when temperatures were high (median = 23.5, range = 18.8-
339 $27.3 \text{ }^\circ\text{C}$), NO_3^- concentrations were low (median = 0.21, range = below detection- $61.97 \mu\text{mol N}$
340 L^{-1}).

341 While benthic invertebrates can enhance N₂O production within the walls of their
342 burrows or emit N₂O directly from their guts (Stief et al. 2009, Heisterkamp et al. 2010, Bonaglia
343 et al. 2014), the N₂O flux that ultimately escapes to the water column depends upon redox
344 conditions within the surrounding sediments. Sediment N₂O emissions are highest at
345 intermediate redox (de Bie et al. 2002, Seo and DeLaune 2010), and high organic matter content
346 in sediments will lead to more strongly reducing conditions, which consume N₂O and result in
347 higher fluxes of N₂. Other studies have observed invertebrate enhancement of N₂O flux at low
348 temperatures and low concentrations of NH₄⁺ and NO₃⁻, but these were either conducted in sites
349 with substantially lower (<0.3%) sediment organic C content (Bonaglia et al. 2014, Welsh et al.
350 2015) than those in the current study (2.1-12.6 %C, median = 5.5%C), or measured emission of
351 N₂O from invertebrates incubated without surrounding sediments (Heisterkamp et al. 2010).
352 Organic matter in surrounding sediments may enhance N₂ emissions relative to N₂O, as was
353 likely the case in studies by Stief and Schramm (2010) and Welsh et al. (2015), where N₂O
354 fluxes from animals incubated without sediments were substantially higher than fluxes measured
355 from invertebrates *in situ* (within sediments). Likewise, Nogaro and Burgin (2014) measured
356 significant stimulation of N₂ flux at higher densities of oligochaetes, but differences in N₂O flux
357 among worm density treatments were only moderately significant ($p = 0.064$). Although their
358 incubations were run with NO₃⁻ concentrations ranging from 100-470 μM N (~51-240× higher
359 than the median value in the current study, 1.96 μM N), the sediment utilized in their core
360 incubations contained substantially more C (~15% organic C) than in the current study (median
361 5.5% C), which likely resulted in the conversion of NO₃⁻ to N₂ rather than N₂O prior to escaping
362 the sediment. These results underscore the need to consider seasonality (i.e. variability in NO₃⁻

363 availability and temperature) and the context-dependent nature (e.g. incubation conditions,
364 sediment C content) of invertebrate effects on GHG fluxes.

365 The apparent multiplicative enhancement effects of temperature, sediment C content, and
366 benthic invertebrate abundance on CO₂ and CH₄ flux in the current study (Figs. 4, 5) suggest a
367 deleterious positive feedback with continuing climate change and urbanization. Shifts toward
368 oligochaete-dominated invertebrate communities are a common consequence of eutrophication,
369 pesticide, and heavy metal pollution in aquatic ecosystems (Palmer 1968, Wiederholm 1980,
370 Winner et al. 1980, Paul and Meyer 2001, Devine and Vanni 2002, Verdonschot 2006). As
371 regions of the world become increasingly urbanized, if the impacts of increased metal mobility,
372 hypoxia, and nutrient loading on wetlands are not kept in check, shifts in aquatic invertebrate
373 communities toward oligochaete dominance may become more widespread. As wetland CO₂ and
374 CH₄ fluxes increase due to higher temperatures and oligochaete densities, additional atmospheric
375 warming may result, causing greater enhancement of wetland GHG flux. This is one of the first
376 field studies of the influence of invertebrate communities on urban wetland GHG emissions.
377 Further research is needed in order to confirm whether the mechanisms suggested by our results
378 are broadly applicable, but our findings open a new line of inquiry in the investigation of
379 community structure effects on urban ecosystem function and global wetland GHG budgets.

380 **Acknowledgements**

381 We thank Keryn L. Roberts and Brandon K. Winfrey, who assisted with field sampling in
382 Melbourne, Australia. Vera Eate, Keralee Brown, and Tines Hines provided assistance with
383 laboratory analyses at Monash University, Benedikt Fest and Steve Livesley assisted with
384 greenhouse gas analysis at the University of Melbourne, and Jennifer Gonzalez and Guillermo
385 Mendoza assisted with sorting and identification of preserved invertebrates at Scripps Institution

386 of Oceanography. Meredith K. Meyers assisted with the creation of Figure 1. We also thank
387 Teresa Mackintosh, and William Steele from Melbourne Water, who fostered discussion and
388 facilitated access to constructed wetland sites in Australia; and Dave Mason, Michael Freeman,
389 Robert Sim, Chris Allen, and Stuart Cooper who generously provided access and resources to
390 facilitate sampling ponds at their golf clubs and courses. Finally, we thank two anonymous
391 reviewers who provided helpful critiques of the ms. Support was provided by an NSF PIRE
392 program sub award to LAL from UC Irvine OISE-1204866.

393 **Literature cited**

- 394 Allinson, G., P. Zhang, A. Bui, M. Allinson, G. Rose, S. Marshall, and V. Pettigrove. 2015.
395 Pesticide and trace metal occurrence and aquatic benchmark exceedances in surface
396 waters and sediments of urban wetlands and retention ponds in Melbourne, Australia.
397 *Environmental Science and Pollution Research* **22**:10214-10226.
- 398 APHA. 2012. Standard methods for the examination of water and wastewater. 22 edition.
399 American Public Health Association Publication, APHA, AWWA, WEF.
- 400 Bonaglia, S., F. J. A. Nascimento, M. Bartoli, I. Klawonn, and V. Brüchert. 2014. Meiofauna
401 increases bacterial denitrification in marine sediments. *Nature Communications* **5**:5133.
- 402 Burgin, A. J., J. G. Lazar, P. M. Groffman, A. J. Gold, and D. Q. Kellogg. 2013. Balancing
403 nitrogen retention ecosystem services and greenhouse gas disservices at the landscape
404 scale. *Ecological Engineering* **56**:26-35.
- 405 Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A
406 Practical Information-Theoretic Approach. Springer, New York.
- 407 Butman, D., S. Stackpoole, E. Stets, C. P. McDonald, D. W. Clow, and R. G. Striegl. 2016.
408 Aquatic carbon cycling in the conterminous United States and implications for terrestrial

- 409 carbon accounting. Proceedings of the National Academy of Sciences of the United
410 States of America **113**:58-63.
- 411 Cao, M., S. Marshall, and K. Gregson. 1996. Global carbon exchange and methane emissions
412 from natural wetlands: Application of a process-based model. Journal of Geophysical
413 Research **101**:14399-14414
- 414 Carpenter, S. R., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith.
415 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecological
416 Applications **8**:559-568.
- 417 Covich, A. P., M. A. Palmer, and T. A. Crowl. 1999. The Role of Benthic Invertebrate Species in
418 Freshwater Ecosystems: Zoobenthic species influence energy flows and nutrient cycling.
419 BioScience **49**:119-127.
- 420 Datry, T., F. Hervant, F. Malard, L. Vitry, and J. Gibert. 2003. Dynamics and adaptive responses
421 of invertebrates to suboxia in contaminated sediments of a stormwater infiltration basin.
422 Archiv für Hydrobiologie **156**:339-359.
- 423 Davidson, N. C. 2014. How much wetland has the world lost? Long-term and recent trends in
424 global wetland area. Marine and Freshwater Research **65**:934–941.
- 425 de Bie, M. J. M., J. J. Middelburg, M. Starink, and H. J. Laanbroek. 2002. Factors controlling
426 nitrous oxide at the microbial community and estuarine scale. Marine Ecology Progress
427 Series **240**:1-9.
- 428 Deemer, B. R., J. A. Harrison, S. Li, J. J. Beaulieu, T. DelSontro, N. Barros, J. F. Bezerra-Neto,
429 S. M. Powers, M. A. dos Santos, and J. A. Vonk. 2016. Greenhouse gas emissions from
430 reservoir water surfaces: A new global synthesis. BioScience.

- 431 Devine, J. A., and M. J. Vanni. 2002. Spatial and seasonal variation in nutrient excretion by
432 benthic invertebrates in a eutrophic reservoir. *Freshwater Biology* **47**:1107-1121.
- 433 Fisher, J. B., W. J. Lick, P. L. McCall, and J. A. Robbins. 1980. Vertical mixing of lake
434 sediments by tubificid oligochaetes. *Journal of Geophysical Research* **85**:3997-4006
- 435 Hamer, A. J., and K. M. Parris. 2013. Predation modifies larval amphibian communities in urban
436 wetlands. *Wetlands* **33**:641-652.
- 437 Hassall, C. 2014. The ecology and biodiversity of urban ponds. *WIREs Water* **1**:187-206.
- 438 Heisterkamp, I. M., A. Schramm, D. d. Beer, and P. Stief. 2010. Nitrous oxide production
439 associated with coastal marine invertebrates. *Marine Ecology Progress Series* **415**:1-9.
- 440 Hill, M. J., J. Biggs, I. Thornhill, R. A. Briers, D. G. Gledhill, J. C. White, P. J. Wood, and C.
441 Hassall. 2016. Urban ponds as an aquatic biodiversity resource in modified landscapes.
442 *Global Change Biology*.
- 443 Hölker, F., M. J. Vanni, J. J. Kuiper, C. Meile, H.-P. Grossart, P. Stief, R. Adrian, A. Lorke, O.
444 Dellwig, A. Brand, M. Hupfer, W. M. Mooij, G. Nützmann, and J. Lewandowski. 2015.
445 Tube-dwelling invertebrates: tiny ecosystem engineers have large effects in lake
446 ecosystems. *Ecological Monographs* **85**:333-351.
- 447 James, G., D. Witten, T. Hastie, and R. Tibshirani. 2013. *An Introduction to Statistical Learning:
448 with Applications in R*. Springer Science+Business Media, New York.
- 449 Jenny, J.-P., P. Francus, A. Normandeau, F. Lapointe, M.-E. Perga, A. Ojala, A. Schimmelmann,
450 and B. Zolitschka. 2016. Global spread of hypoxia in freshwater ecosystems during the
451 last three centuries is caused by rising local human pressure. *Global Change Biology*
452 **22**:1481-1489.

- 453 Jones, R. I., C. E. Carter, A. Kelly, S. Ward, D. J. Kelly, and J. Grey. 2008. Widespread
454 contribution of methane-cycle bacteria to the diets of lake profundal chironomid larvae.
455 Ecology **89**:857-864.
- 456 Kankaala, P., G. Eller, and R. I. Jones. 2007. Could bacterivorous zooplankton affect lake
457 pelagic methanotrophic activity? Fundamental and Applied Limnology **169**:203-209.
- 458 Kaushal, S. S., P. M. Groffman, L. E. Band, C. A. Shields, R. P. Morgan, M. A. Palmer, K. T.
459 Belt, C. M. Swan, S. E. G. Findlay, and G. T. Fisher. 2008. Interaction between
460 urbanization and climate variability amplifies watershed nitrate export in Maryland.
461 Environmental Science & Technology **42**:5872-5878.
- 462 Kaye, J. P., P. M. Groffman, N. B. Grimm, L. A. Baker, and R. V. Pouyat. 2006. A distinct urban
463 biogeochemistry? TRENDS in Ecology and Evolution **21**:192-199.
- 464 Lagauzère, S., P. Boyer, G. Stora, and J.-M. Bonzom. 2009. Effects of uranium-contaminated
465 sediments on the bioturbation activity of *Chironomus riparius* larvae (Insecta, Diptera)
466 and *Tubifex tubifex* worms (Annelida, Tubificidae). Chemosphere **76**:324-334.
- 467 Lorenzen, C. J. 1967. Determination of chlorophyll and phaeo-pigments: Spectrophotometric
468 equations. Limnology and Oceanography **12**:343-346
- 469 Mander, Ü., G. Dotro, Y. Ebie, S. Towprayoon, C. Chiemchaisri, S. F. Nogueira, B. Jamsranjav,
470 K. Kasak, J. Truu, J. Tournebize, and W. J. Mitsch. 2014. Greenhouse gas emission in
471 constructed wetlands for wastewater treatment: A review. Ecological Engineering **66**:19-
472 35.
- 473 Matisoff, G., X. Wang, and P. L. McCall. 1999. Biological redistribution of lake sediments by
474 tubificid oligochaetes: *Branchiura sowerbyi* and *Limnodrilus hoffmeisteri*/*Tubifex tubifex*.
475 Journal of Great Lakes Research **25**:205-219.

- 476 McPhearson, T., S. T. A. Pickett, N. B. Grimm, J. Niemelä, M. Alberti, T. Elmqvist, C. Weber,
477 D. Haase, J. Breuste, and S. Qureshi. 2016. Advancing urban ecology toward a science of
478 cities. *BioScience* **66**:198-212.
- 479 Mermillod-Blondin, F., J.-P. Gaudet, M. Gérino, G. Desrosiers, and M. Creuzé des Châtelliers.
480 2003. Influence of macroinvertebrates on physico-chemical and microbial processes in
481 hyporheic sediments. *Hydrological Processes* **17**:779-794
- 482 Mitsch, W. J., B. Bernal, A. M. Nahlik, Ü. Mander, L. Zhang, C. J. Anderson, S. E. Jørgensen,
483 and H. Brix. 2013. Wetlands, carbon, and climate change. *Landscape Ecology* **28**:583-
484 597.
- 485 Nisbet, E. G., E. J. Dlugokencky, and P. Bousquet. 2014. Methane on the Rise-Again. *Science*
486 **343**:493-495.
- 487 Nogaro, G., and A. J. Burgin. 2014. Influence of bioturbation on denitrification and dissimilatory
488 nitrate reduction to ammonium (DNRA) in freshwater sediments. *Biogeochemistry*
489 **120**:279-294.
- 490 Nogaro, G., and F. Mermillod-Blondin. 2009. Stormwater sediment and bioturbation influences
491 on hydraulic functioning, biogeochemical processes, and pollutant dynamics in
492 laboratory infiltration systems. *Environmental Science and Technology* **43**:3632–3638.
- 493 Palmer, M. F. 1968. Aspects of the respiratory physiology of *Tubifex tubifex* in relation to its
494 ecology. *Journal of Zoology* **154**:463-473
- 495 Paul, M. J., and J. L. Meyer. 2001. Streams in the Urban Landscape. *Annual Review of Ecology*
496 and Systematics **32**:333-365.
- 497 Pigneret, M., F. Mermillod-Blondin, L. Volatier, C. Romestaing, E. Maire, J. Adrien, L. Guillard,
498 D. Roussel, and F. Hervant. 2016. Urban pollution of sediments: Impact on the

- 499 physiology and burrowing activity of tubificid worms and consequences on
500 biogeochemical processes. *Science of The Total Environment* **568**:196-207.
- 501 Poulsen, M., M. V. W. Kofoed, L. H. Larsen, A. Schramm, and P. Stief. 2014. *Chironomus*
502 *plumosus* larvae increase fluxes of denitrification products and diversity of nitrate-
503 reducing bacteria in freshwater sediment. *Systematic and Applied Microbiology* **37**:51–
504 59.
- 505 R Development Core Team. 2008. A Language and Environment for Statistical Computing. . R
506 Foundation for Statistical Computing, Vienna, Austria.
- 507 Robbins, J. A., T. Keilty, D. S. White, and D. N. Edgington. 1989. Relationships among
508 Tubificid abundances, sediment composition, and accumulation rates in Lake Erie.
509 *Canadian Journal of Fisheries and Aquatic Sciences* **46**:223-231.
- 510 Roberts, K. L., V. M. Eate, B. D. Eyre, D. P. Holland, and P. L. M. Cook. 2012. Hypoxic events
511 stimulate nitrogen recycling in a shallow salt-wedge estuary: The Yarra River estuary,
512 Australia. *Limnology and Oceanography* **57**:1427-1442
- 513 Seo, D. C., and R. D. DeLaune. 2010. Fungal and bacterial mediated denitrification in wetlands:
514 Influence of sediment redox condition. *Water Research* **44**:2441-2450.
- 515 Stief, P., L. Polerecky, M. Poulsen, and A. Schramm. 2010. Control of nitrous oxide emission
516 from *Chironomus plumosus* larvae by nitrate and temperature. *Limnology and*
517 *Oceanography* **55**:872–884.
- 518 Stief, P., M. Poulsen, L. P. Nielsen, H. Brix, and A. Schramm. 2009. Nitrous oxide emission by
519 aquatic macrofauna. *Proceedings of the National Academy of Sciences of the United*
520 *States of America* **106**:4296-4300.

- 521 Stief, P., and A. Schramm. 2010. Regulation of nitrous oxide emission associated with benthic
522 invertebrates. *Freshwater Biology* **55**:1647–1657.
- 523 Syakila, A., and C. Kroeze. 2011. The global nitrous oxide budget revisited. *Greenhouse Gas*
524 *Measurement and Management* **1**:17-26.
- 525 van Asselen, S., P. H. Verburg, J. E. Vermaat, and J. H. Janse. 2013. Drivers of wetland
526 conversion: a global meta-analysis. *PLoS ONE* **8**:e81292.
- 527 Verdonschot, P. F. M. 2006. Beyond masses and blooms: the indicative value of oligochaetes.
528 *Hydrobiologia* **564**:127-142.
- 529 Verhoeven, J. T. A., B. Arheimer, C. Yin, and M. M. Hefting. 2006. Regional and global
530 concerns over wetlands and water quality. *TRENDS in Ecology and Evolution* **21**:96-
531 103.
- 532 Walsh, C. J., A. H. Roy, J. W. Feminella, P. D. Cottingham, P. M. Groffman, and R. P. Morgan
533 II. 2005. The urban stream syndrome: current knowledge and the search for a cure.
534 *Journal of the North American Benthological Society* **24**:706–723.
- 535 Wang, X., and G. Matisoff. 1997. Solute transport in sediments by a large freshwater
536 oligochaete, *Branchiura sowerbyi*. *Environmental Science and Technology* **31**:1926-
537 1933.
- 538 Welsh, D. T., D. Nizzoli, E. A. Fano, and P. Viaroli. 2015. Direct contribution of clams
539 (*Ruditapes philippinarum*) to benthic fluxes, nitrification, denitrification and nitrous
540 oxide emission in a farmed sediment. *Estuarine, Coastal and Shelf Science* **154**:84-93.
- 541 Wiederholm, T. 1980. Use of benthos in lake monitoring. *Journal (Water Pollution Control*
542 *Federation)* **52**:537-547.

- 543 Winner, R. W., M. W. Boesel, and M. P. Farrell. 1980. Insect community structure as an index of
544 heavy-metal pollution in lotic ecosystems. Canadian Journal of Fisheries and Aquatic
545 Sciences **37**:647-655.
546

In Press - do not distribute

547 **Table 1. Comparison of candidate multiple regression models explaining variation in benthic CO₂, CH₄, and N₂O flux.** For each

548 greenhouse gas (GHG), the top model as selected by Akaike's information criterion, the model with the best predictive power (lowest

549 RMSE, in bold) and the top model excluding invertebrates are provided. C_p is Mallows' C_p (a measurement of model error), AICc is

550 Akaike's Information Criterion corrected for small sample size, Δ_i is the difference between the candidate and best model's AICc, and

551 RMSE is the root-mean-square error (model predictive ability).

| GHG (response) | Environmental parameters | Invertebrate parameters | C _p | R ² | AICc | Δ _i | RMSE |
|------------------|---|-------------------------|----------------|----------------|------|----------------|---------------|
| CO ₂ | temp, %C | Invert (large) | 3.66 | 0.84 | 27.7 | | 0.3981 |
| | temp, %C | | 5.78 | 0.73 | 38.6 | 10.9 | 0.4653 |
| CH ₄ | temp, %C | Oligo (all) | 30.61 | 0.64 | 83.0 | | 1.0357 |
| | temp, %C | | 48.12 | 0.39 | 93.7 | 10.7 | 1.3354 |
| N ₂ O | temp, NO _x , DO, NO _x *DO | Oligo (small) | 1.70 | 0.62 | 10.9 | | 0.2877 |
| | temp, NO _x , DO, NO _x *DO | | 1.92 | 0.53 | 12.4 | 1.5 | 0.2994 |

552 *temp* = temperature, %C = sediment C content (upper 3 cm), *Invert* = Oligochaeta + Chironomidae density, *Oligo* = Oligochaeta density, *all* = >0.3mm, *large*

553 = >1mm, *small* = 0.3–1mm, *NO_x* = total initial NO₃ + NO₂ (mg) present in overlying water of sediment core, *DO* = field measurement of percent saturation of

554 dissolved O₂ in water column, *NO_x*DO*% = interaction between NO_x and DO%.

564 **Figure Legends**

565 **Figure 1.** Wetland sediment inhabited by a midge larva (Diptera: Chironomidae) in its U-shaped
566 burrow, and oligochaete worms (Oligochaeta: Tubificinae) feeding head-down in sediments.
567 Oligochaete consumption of organic particles from deeper sediment layers and subsequent
568 translocation and accumulation of particles at the sediment-water interface causes a downward
569 movement of sediment, the rate of which is determined by oligochaete size and density. Particles
570 move downward until they reach the zone of oligochaete feeding, where they are ingested and
571 transported rapidly above the sediment-water interface. This process, which may be repeated
572 several times until particles pass below the zone of oligochaete feeding, results in enhanced
573 transport and “conveyor belt” cycling of organic particles between oxic and anoxic layers of
574 sediments. Meanwhile, midge larvae ventilate their U-shaped burrows in shallower layers of
575 sediment, alternately drawing nitrate and oxygen as well as organic particles into burrows,
576 thereby enhancing oxygenation of sediments, nitrification and denitrification, and the production
577 of N_2 and N_2O in shallow sediments.

578 **Figure 2.** Benthic fluxes of CO_2 , CH_4 , and N_2O in golf course ponds ($n = 40$) and constructed
579 treatment wetlands ($n = 63$) sampled January-March (summer) and August-September
580 (winter/spring) in Melbourne, Australia. For treatment wetlands, filled diamonds overlaying box
581 plots indicate average flux measurements in wetland inlets, while hollow diamonds indicate
582 average flux measurements in wetland outlets. Each diamond is the average of four GHG flux
583 measurements per site. Note differences in y axis scales among panels.

584 **Figure 3.** Average density of oligochaete worms (Oligochaeta), chironomid midge larvae
585 (Chironomidae), and all other invertebrates (Other) in sediment cores collected from golf course

586 ponds ($n = 40$) and stormwater treatment wetlands ($n = 63$) in summer and winter. Error bars
587 indicate 95% confidence intervals.

588 **Figure 4.** Estimated effects of large benthic invertebrates (> 1 mm) on benthic CO_2 flux at
589 varying temperatures and sediment carbon (C) concentrations in urban wetlands. Low and high
590 values for temperature and C are set at the 1st and 3rd quartile of field-collected data: 13 and
591 25°C , and 4 and 8% C in sediments.

592 **Figure 5.** Estimated effects of oligochaetes on benthic CH_4 flux at varying temperatures and
593 sediment carbon (C) concentrations in urban wetlands. Low and high values for temperature and
594 C are set at the 1st and 3rd quartiles of field-collected data: 13 and 25°C , and 4 and 8% C in
595 sediments.

596 **Figure 6.** Estimated effects of small oligochaetes (0.3-1 mm) on benthic N_2O flux at (a) low and
597 (b) high dissolved oxygen % saturation, with varying temperature and water column nitrate +
598 nitrite (NO_x) concentrations in urban wetlands. Low and high values for temperature and NO_x
599 are set at the 1st and 3rd quartiles of field-collected data: 13°C and 25°C , and 0.7 and $40.0 \mu\text{M}$
600 $\text{NO}_x\text{-N}$. Dissolved oxygen is set at 10th and 90th percentiles (48 and 151% saturation).

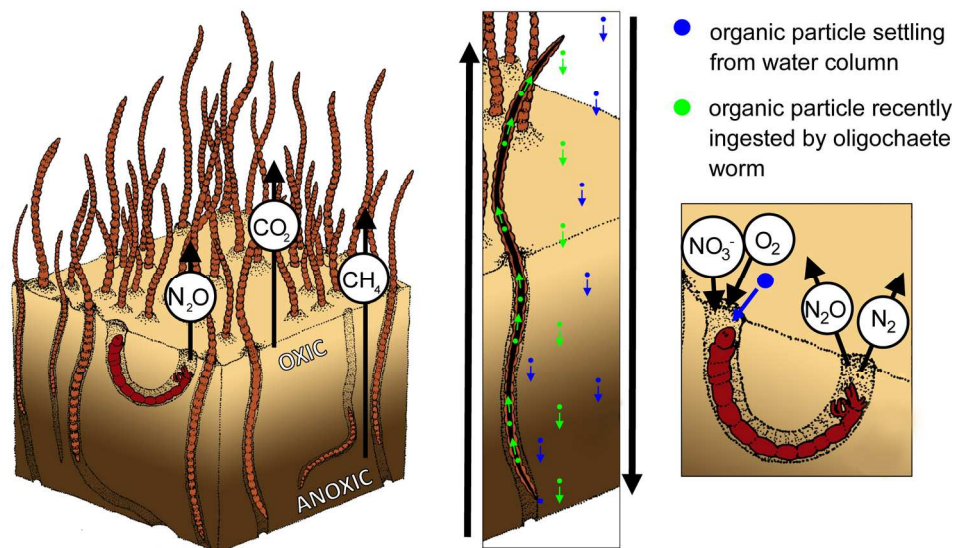


Figure 1. Wetland sediment inhabited by a midge larva (Diptera: Chironomidae) in its U-shaped burrow, and oligochaete worms (Oligochaeta: Tubificinae) feeding head-down in sediments. Oligochaete consumption of organic particles from deeper sediment layers and subsequent translocation and accumulation 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.

85x48mm (600 x 600 DPI)

In Press

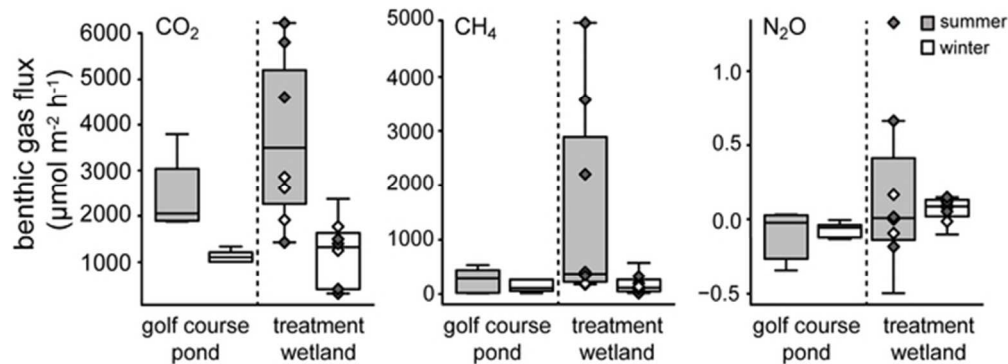


Figure 2. Benthic fluxes of CO₂, CH₄, and N₂O in golf course ponds (n = 40) and constructed treatment wetlands (n = 63) sampled January-March (summer) and August-September (winter/spring) in Melbourne, Australia. For treatment wetlands, filled diamonds overlaying box plots indicate average flux measurements in wetland inlets, while hollow diamonds indicate average flux measurements in wetland outlets. Each diamond is the average of four GHG flux measurements per site. Note differences in y axis scales among panels.

54x19mm (300 x 300 DPI)

In Press - do not

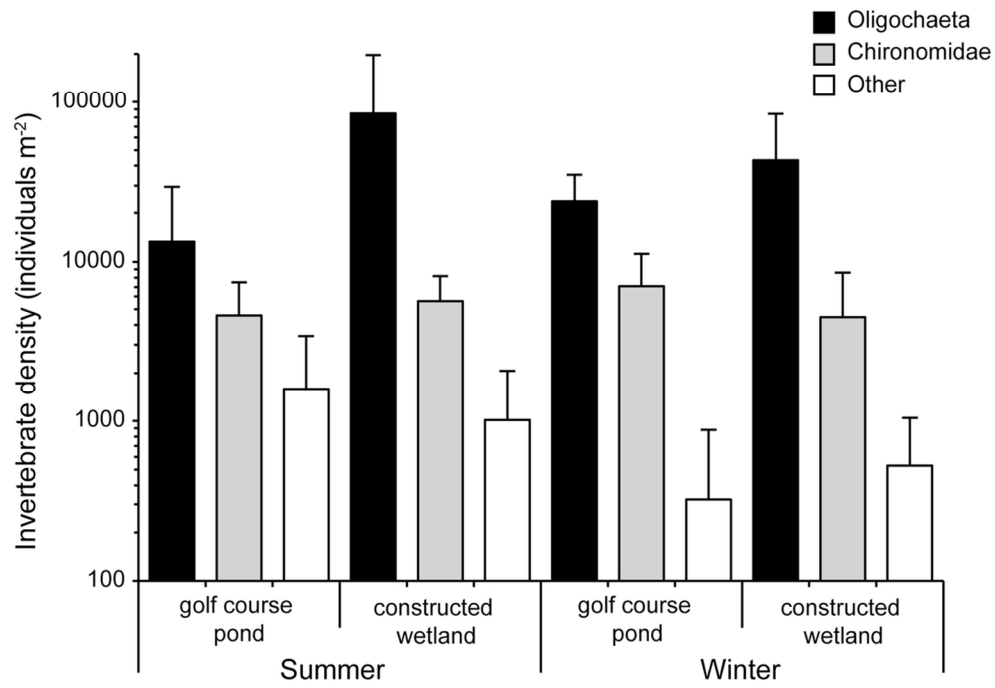


Figure 3. Average density of oligochaete worms (*Oligochaeta*), chironomid midge larvae (*Chironomidae*), and all other invertebrates (*Other*) in sediment cores collected from golf course ponds ($n = 40$) and stormwater treatment wetlands ($n = 63$) in summer and winter. Error bars indicate 95% confidence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

106x73mm (300 x 300 DPI)

In Press

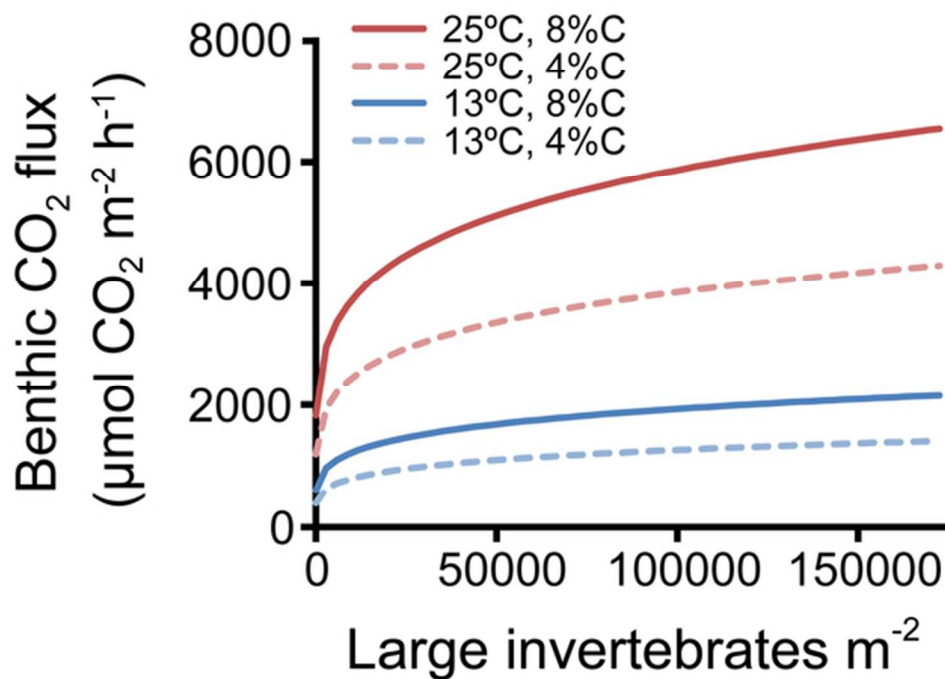


Figure 4. Estimated effects of large benthic invertebrates (> 1 mm) on benthic CO₂ flux at varying temperatures and sediment carbon (C) concentrations in urban wetlands. Low and high values for temperature and C are set at the 1st and 3rd quartile of field-collected data: 13 and 25°C, and 4 and 8% C in sediments.

56x41mm (300 x 300 DPI)

In Press

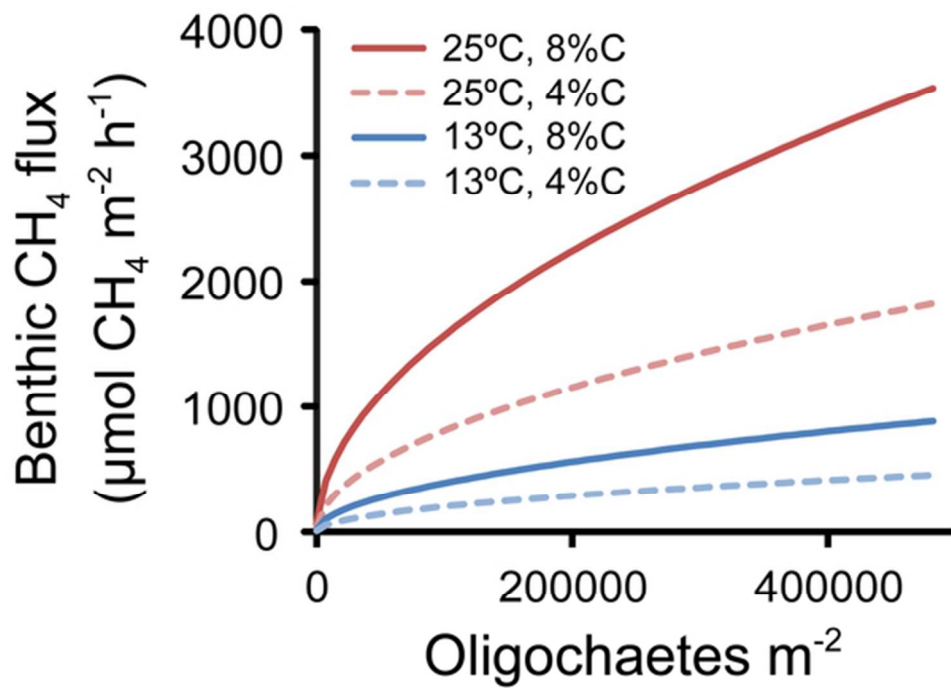


Figure 5. Estimated effects of oligochaetes on benthic CH₄ flux at varying temperatures and sediment carbon (C) concentrations in urban wetlands. Low and high values for temperature and C are set at the 1st and 3rd quartiles of field-collected data: 13 and 25°C, and 4 and 8% C in sediments.

53x37mm (300 x 300 DPI)

In Press -

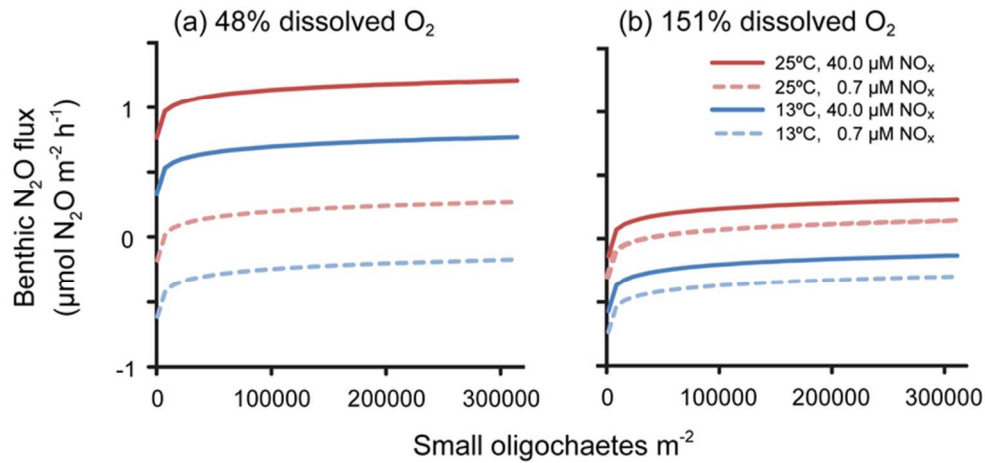


Figure 6. Estimated effects of small oligochaetes (0.3-1 mm) on benthic N_2O flux at (a) low and (b) high dissolved oxygen % saturation, with varying temperature and water column nitrate + nitrite (NO_x) concentrations in urban wetlands. Low and high values for temperature and NO_x are set at the 1st and 3rd quartiles of field-collected data: 13°C and 25°C, and 0.7 and 40.0 $\mu\text{M } NO_x\text{-N}$. Dissolved oxygen is set at 10th and 90th percentiles (48 and 151% saturation).

69x31mm (300 x 300 DPI)

In Press - do not