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NOTE

Bridging between litterbags and whole-ecosystem experiments: a new approach for studying lake sediments

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ABSTRACT

Nearshore sediments have a major influence over the functioning of aquatic ecosystems, but predicting their response to future environmental change has proven difficult. Previous manipulative experiments have faced challenges controlling environmental conditions, replicating sediment mixing dynamics, and extrapolating across spatial scales. Here we describe a new approach to manipulate lake sediments that overcomes previous concerns about reproducibility and environment controls, whilst also bridging the gap between smaller microcosm or litterbag experiments and whole-ecosystem manipulations. Our approach involves submerging moderate-sized (~15 L) artificial substrates that have been standardised to mimic natural sediments within the littoral zones of lakes. We show that this approach can accurately mirror the absolute dissolved organic carbon concentrations and pH of pore water, and to a lesser degree inorganic carbon concentrations, from natural lake sediments with similar organic matter profiles. On a relative basis, all measured variables had similar temporal dynamics between artificial and adjacent natural sediments. Late-summer zooplankton biomass also did not differ between natural and artificial sediments. By offering a more realistic way to manipulate freshwater sediments than previously possible, our approach can improve predictions of lake ecosystems in a changing world.

Key words: Carbon cycling; food webs; lake sediments; terrestrial-aquatic linkages; scaling.

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INTRODUCTION

Freshwater sediments, particularly the top few centimetres in nearshore environments, are of key importance to global biogeochemical cycles and aquatic food webs because they are sites where large amounts of organic matter (OM) are transformed and mineralised (Wetzel, 2001). Annually, lake sediments are a sink for up to 0.6 Pg carbon (C) year^{-1} (Tranvik *et al.,* 2009), and emit around 0.05 and 0.2-1.2 Pg C (CO_2-equivalents) of CO_2 and CH_4, respectively (Bastviken *et al.,* 2004; Pace and Prairie, 2005). The high levels of OM processing consequently allow lake sediments to host phototrophic algal, bacterial, and fungal communities that can be many times more productive than in open waters (Fischer and Pusch, 2001; Vadeboncoeur *et al.,* 2002; Ask *et al.,* 2009; Wurzbacher *et al.,* 2010). Additionally, nutrient exchange between decomposing surface sediments and overlying waters promotes harmful algal blooms and contaminant release in human-impacted waters that can further influence food webs (Eggleton and Thomas, 2004; Carpenter, 2005).

Experiments provide a controlled way of predicting how sediment functioning might respond to future changes in OM inputs. Here we introduce a new experimental approach to bridge the gap between smaller microcosm or litterbag experiments and whole-ecosystem manipulations. The approach involves submerging containers with moderate-sized (~15 L) artificial sediments, informed by geochemical surveys of natural ecosystems, within real lakes. Our work builds upon a recent technique described by Orihel and Rooney (2012) by offering the potential to link fine-scale geochemical studies with large-scale ecological studies of entire communities. Ecological studies have rarely manipulated sediments that have been standardised to mimic natural conditions, despite this strategy being fundamental to fields such as environmental toxicology (Suedel and Rodgers, 1994; but see Feuchtmayr *et al.,* 2009). Traditionally, studies have added OM either to field-collected sediments, which have been relocated to laboratories or outdoor mesocosms (Wood and Richardson, 2009; Liboriussen *et al.,* 2011; Song *et al.,* 2013), or *in situ* by burying decomposing leaves into sediment surfaces (Herbst, 1980; Jackson *et al.,* 1995; Longhi *et al*., 2008; Costantini *et al.,* 2009; Marmonier *et al.,* 2010).

Our new experimental platform has at least three major advantages over previous methods for manipulating freshwater sediments. First, our approach permits a level of reproducibility that is unachievable in experiments that simply manipulate field-collected samples and are limited by pre-existing OM, *i.e.* OM can be added but never sub-

tracted from natural sediment. *In situ* experiments also cannot control the many parameters that vary horizontally across surface sediments at fine spatial scales (*i.e.* <10 m), such as geochemical composition (Korsman *et al.,* 1999; Yu *et al.,* 2015). By contrast, relocating field-collected sediments to new settings, such as the laboratory, may fail to replicate *in situ* conditions (Orihel and Rooney, 2012). Second, our approach offers a more realistic simulation of long-term mineralisation and decomposition processes than, for example, leaf litterbags, which have been widely used to study biogeochemistry and ecosystem metabolism (Herbst, 1980; Jackson *et al.,* 1995; Longhi *et al*., 2008; Costantini *et al.,* 2009). As an OM amendment, litterbags often sit flush on the sediment surface and are not directly incorporated into sediment profiles. Mesocosms similarly isolate experimental units on shore and away from natural waters, *e.g.* in cattle tanks. Isolation in each method is problematic because it prevents mixing with sediments and overlying surface waters, respectively, which may be important for processes such as oxidation and nutrient recycling. Finally, our approach is sufficiently large and designed in such a way that it allows continuous monitoring of geochemistry and biotic communities that would not be possible with other methods. Lab and litterbag experiments are also often ≤0.04 m^2 in size, so may have limited value for broader ecosystem-level generalisations. The only studies performed at a whole-ecosystem level have focused on removing entire sediment horizons, such as for lake restoration or fisheries management, without manipulating OM directly (Peterson, 1982). New approaches are therefore needed to manipulate natural sediments over large spatial scales.

Sediment boxes: creating a new world within lake benthic zones

We originally developed our sediment incubation approach to study linkages between terrestrial and aquatic ecosystems. Our interest was in manipulating the quantity and quality of terrestrial organic matter (tOM) that accumulated in littoral sites under different water qualities. Previously, we discovered that the productivity of bacteria, zooplankton, and young-of-the-year fish in a single lake environment was greater beneath catchments that received larger inputs of tOM (Tanentzap *et al.,* 2014). We were subsequently interested in testing how tOM was processed and mobilized into aquatic food webs at much greater resolution. The incubation approach therefore offered numerous advantages over our observational studies, not the least of which was the ability to control for variation in the delivery and processing of OM by catchments with different geomorphologies.

The premise of our sediment boxes involved mixing inorganic and organic substrates within containers that were submerged in the bottom of a common lake environment. We used 17.5-L (surface area: 0.19 m^2, depth: 0.13 m) open-top high-density polyethylene (HDPE) containers, as these could be replicated at high number and easily manoeuvred when waterlogged. In principle, the HDPE containers could be much larger. For example, we tried preparing 365 L containers, but were constrained by the availability of organic materials and weight of inorganic substances. Here, we outline the setup of our sediment boxes with integrated biogeochemical samplers, describe how we prepared a sediment material that mimicked the natural environment, and present some potential data that can be generated using this approach.

Box preparation

We made three important modifications to each HDPE container (Fig. 1a). First, we wanted to ensure mixing of the sediment bottom with the external environment, partly to avoid artificially promoting anoxic conditions. We therefore drilled nine 8-mm holes in the bottom of each container to ensure that the mesocosms were freely draining. Second, we wanted to sample sediment pore water at high resolution, so we permanently secured a 3-mL polypropylene syringe immediately beneath the sediment surface along one side of the HDPE container. The syringe was placed horizontally in the upper 1 cm of sediment, with the wall facing the sediment partially removed and covered in ca. 250 µm nylon mesh to avoid clogging (Fig. 1b). This sampler is effectively analogous with other methods for measuring pore water, such as dialysis (Carignan, 1985) or suction (Bertolin *et al.,* 1995) samplers, and studies interested in vertical profiling can of course layer syringes at different depths in the sediment. To each syringe, we connected nylon tubing (inside diameter: 3.2 mm, outside diameter: 4.8 mm), which met a float on the water surface and allowed us to sample without disturbing the lake bottom. Finally, we attached light and temperature loggers and customised redox probes (Swerhone *et al.,* 1999) to as many boxes as possible.

Sediment mixture

Here we outline how we created a sediment with 5% tOM on a dry weight basis to mimic the natural conditions in Lake Laurentian, Ontario, Canada (46°27'30" N, 80°56'0" W). Lake Laurentian is a small (157 ha area, 5.2 m maximum depth) circumneutral lake with relatively high dissolved organic carbon (DOC) concentrations around 7 mg L^{-1}. The lake is surrounded by boreal forest and located in a 970-ha conservation area with little contemporary human disturbance aside from hiking and skiing trails.

Our artificial sediment was prepared by mixing organic and inorganic particles to mimic size fractions and vertical structuring observed in a survey of nearshore lake

sediments with varying tOM inputs from adjacent streams (Supplementary Text). First, we mixed 0.25 kg of each of deciduous (primarily *Acer rubrum*, *Betula papyrifera*, *Populus tremuloides*, *Quercus* spp.) and coniferous (*Pinus* spp.) litterfall representative of the surrounding forest, where these two tree types occurred in relatively equal proportions. Litterfall had been air-dried at ca. 25°C over at least 7 days to a constant mass. Air-drying ensured no chemical and physical changes occurred from exposing material to higher temperatures (*e.g.* >50°C), thereby better reflecting natural processes. We sorted dried litterfall into <1 and 1-10 mm diameter fractions at a 3:7 ratio to reproduce average fragment sizes observed in the natural sediments (Supplementary Fig. S1).

Next, the tOM was homogenously mixed with 9.5 kg of minerogenic material consisting of clay/silt, fine sand, and sand/gravel (<0.063 mm, 0.063-1 mm, >1mm diameter particles, respectively), which was sourced from a local quarry. As our interest was in testing the effects of different tOM on sediment dynamics, we pre-mixed the clay/silt, fine sand, and sand/gravel fractions into a constant 2:5:3 ratio by dry-weight, respectively, to reproduce conditions seen in one of our sampling sites (site J; Supplementary Fig. S1). We chose site J, despite variability in composition among sites (Supplementary Fig. S1), because its relatively large size (area of stream-lake confluence zone=359 m^2; catchment area=39 ha) and gentle slope (mean catchment slope±standard deviation [SD] from a 10-m digital elevation model: 5.6±4.2°) were typical of geomorphology in the wider region (Rasmussen *et al.,* 1989). Moreover, we wanted to avoid high concentrations of clay/silt and fine sand that were less indicative of fluvial transport of tOM (Håkanson and Jansson, 1983). The final sediment mixture was then laid upon 7 kg of crushed granitic rock (ca. 2 mm diameter) and filled to a height of 0.08 m in each mesocosm (Fig. 1c). There was also no clear evidence of vertical structuring of organic matter or particle size in the top 10 cm of our natural sediment survey (Supplementary Fig. S1), so we did not incorporate such variation into our design. Lake sediments

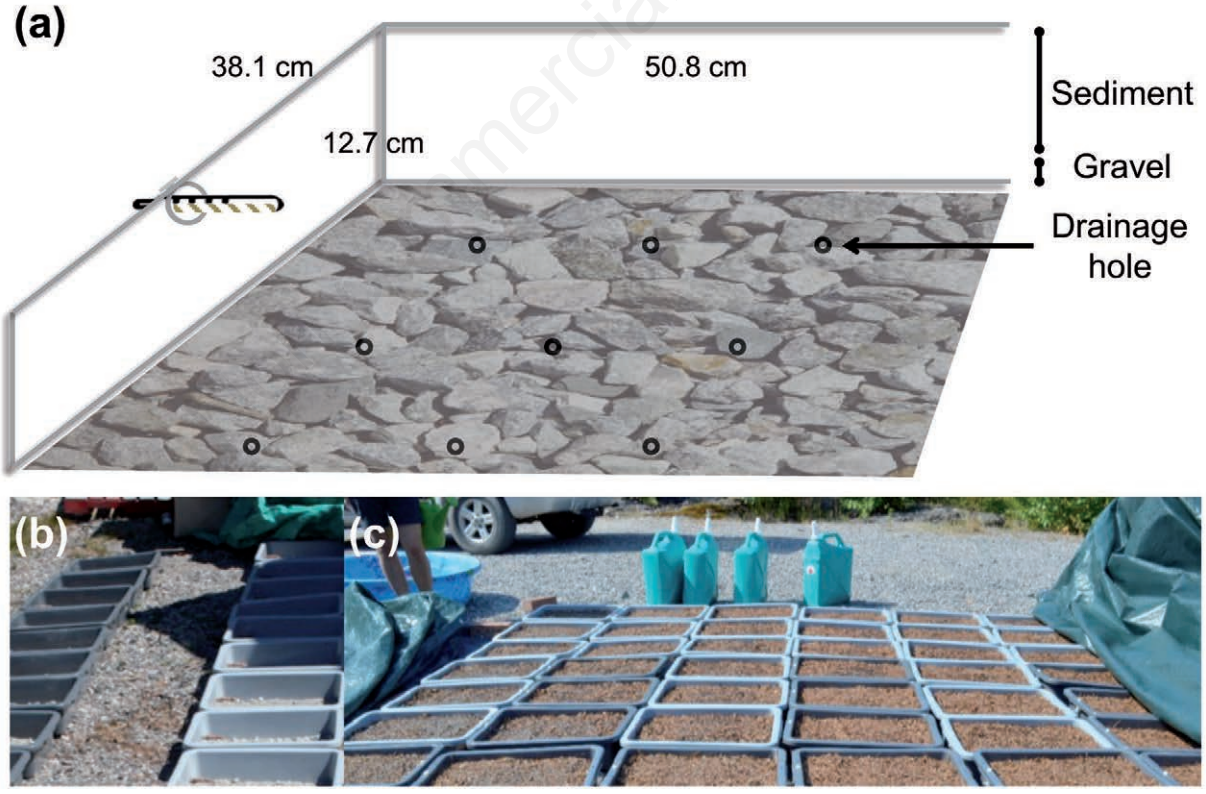

Fig. 1. Photo of experimental sediment boxes. a) Schematic drawn to scale (dimensions shown) of a sediment box, drainage holes, and sampling syringe with bottom wall exposed and wrapped in nylon mesh (denoted by hatched bars); the box is underlain by larger granitic bedrock and filled with artificial sediment consisting of a homogenous mixture of inorganic and organic material; the syringe is fastened with nylon cable ties onto a 64-mm nylon bolt drilled through the box lip; temperature-light loggers are also attached to the bolt with nylon cable ties. b) Boxes with granitic bedrock and drainage holes. c) Boxes filled with sediment containing progressively more tOM from left to right.

typically vary in their vertical structure over much greater depths than studied here because surface mixing dissipates (Håkanson and Jansson, 1983).

Finally, a 1 mm×1 mm nylon mesh was affixed to the top of each mesocosm to limit physical disturbance and provide standardised shading. Each box was then soaked for 5 to 7 days with Lake Laurentian water to ensure that the tOM became waterlogged and settled (Hoover *et al.,* 2010). We further minimised physical disturbance during initial lake installation by temporarily attaching HDPE lids to each container for 24-48 hours. After 1 month in the lake, we made an 80-mm wide slit in the centre of each mesh to promote colonisation by organisms larger than 1 mm in size.

Anchoring sediment boxes to the real world

Although our sediment boxes were constructed based upon detailed surveys of natural sediments, a key question is whether they functioned as in nature. To test this, we first compared pore water from natural surface sediments in Lake Laurentian to sediment boxes that were installed in the littoral zone between 22nd and 23rd July 2015 at a depth of ca. 0.5 m. Pore water samples were taken from the upper 1 cm of the natural sediments (hereafter 'lake bottom samples') by permanently installing one of our modified syringe samplers adjacent to each of three sediment boxes. The sediment boxes were built as described in the previous section (5% tOM) so as to mirror the mean OM content±SD associated with the lake bottom samples of 3.9±0.8%. After 14 months, the sediment boxes still had a mean OM content±SD of 5.9±2.2%.

Pore water samples were collected approximately every 3 days for two weeks from the lake bottom and sediment boxes for our comparison. The samples were collected 14 months after the initial installation. Each sampling syringe was connected to 122 cm of nylon tubing that was purged of water prior to extracting 45 mL of pore water into duplicate 60 mL syringes at the lake surface. To measure DOC, we filtered one of the samples through a 0.5 µm glass fibre filter (Macherey-Nagel MN 85/90) and into a 20-mL scintillation vial pre-acidified with 125 µl of 4N HCl. Samples were then run in NPOC mode on a Shimadzu TOC5000A analyser. We used the other pore water sample to measure total inorganic carbon (TIC) and CO_2 concentrations. We acidified the second 45 mL water sample to a pH of ca. 2.0 with 2 mL of 0.5N HCl *in situ*, introducing 13 mL of headspace to the syringe. We then shook the syringe for 2 min to equilibrate gasses in the acidified sample with the 13 mL of ambient-air headspace. After extracting 10 mL of the headspace into a separate airtight syringe, we detected both CO_2 and TIC as CO_2 within 48 h on a SRI 8610C gas chromatograph (0.5 mL sample loop, 105°C column temperature). Partial pressures of CO_2 and TIC in pore water were calculated from headspace concentrations after Åberg and Wallin (2014) by subtracting ambient air additions, applying the Bunsen solubility coefficient and ideal gas law, and accounting for pH and water temperature concurrently measured in the field.

We tested whether sediment boxes differed from lake bottom samples in pH and log-transformed concentrations of each of DOC, TIC, and CO_2 using linear mixed effects models fitted with restricted maximum likelihood. The models accounted for random variation from repeated measurements of individual syringes and sampling dates with sample type, either natural or artificial sediment, as a fixed explanatory effect. We then calculated 95% confidence intervals (CIs) from the fitted likelihood profiles and considered sediment boxes to differ from lake bottoms for a given response if the 95% CI for their effect excluded zero. All analyses were carried out with the *lme4* package in R v3.2.

We found that sediment boxes reproduced DOC and pH trends observed in pore waters of natural lake sediments with similar OM composition one year after installation, but had slightly more TIC and CO_2 (Fig. 2). Over a 14-day period, DOC and pH in sediment boxes were indistinguishable from lake bottoms (95% CI for difference: -0.73 to 1.21 mg L^{-1} and -0.05 to 0.22, respectively; black points overlapping grey polygons in Fig. 2 a,b). By contrast, TIC, which was mostly comprised of CO_2 (compare Fig. 2c and 2d), and CO_2 itself were 0.63 to 5.88 mg L^{-1} and 0.33 to 3.97 mg L^{-1} higher in the sediment boxes, respectively. Nonetheless, temporal trends in box TIC and CO_2 generally mirrored those in lake bottoms (Fig. 2 c,d).

We also compared zooplankton biomass between the natural surface sediments and sediment boxes studied for pore water in Lake Laurentian. Vertically-migrating animals were collected on two nights toward the end of our pore water sampling using a 500-mL funnel trap deployed at a height of 5 cm above the centre of each sediment sample as in Tanentzap *et al.,* (2014). Traps were collected the morning after deployment, immediately filtered through an 80-µm mesh, and live-sorted into pure zooplankton. To have sufficient mass for analyses, we pooled freeze-dried biomass between nights from the same trap. We then compared biomass between natural and artificial sediment using a linear model (*lm* function in R) with sample type as a fixed effect, while also accounting for sampling block, *i.e.* northerly or southerly exposure around the central dock (Fig. 3). Consistent with the pore water results, the mean (95% CI) zooplankton biomass of 0.31 (0.23 to 0.43) g m^{-2} sediment in the artificial boxes did not differ from the 0.31 (0.22 to 0.42) g m^{-2} sediment in the natural lake bottoms with similar OM content (95% CI for difference: -0.13 to 0.09 g m^{-2} sediment).

DISCUSSION

We found a relatively close correspondence between natural and artificial sediments. While CO_2 concentrations were slightly higher in our sediment boxes than adjacent lake bottoms, this likely reflects the more labile nature of the fresher tOM amendments. Labile tOM can be more quickly turned over through microbial biomass, elevating heterotrophic respiration and DOC production, the latter of which primes additional microbial activity, without necessarily increasing DOC concentrations (Bengtson and Bengtsson, 2007). We also found that littoral zooplankton occurred at similar standing biomass in natural and artificial sediments, although the colonisation of organisms larger than 1000 µm could have been limited by the mesh above each sediment box. Future work will now need to test whether these mesocosms similarly reflect other geochemical and biological variables in neighbouring lake bottoms (Dzialowski *et al.,* 2014), such as nitrogen fluxes and bacterial composition. Previous work with artificial sediments suggests that contained microbial communities may be less diverse (Goedkoop *et al.,* 2005), but has not attempted to replicate natural conditions as we have done here.

Field experiments aim to apply a perturbation to natural assemblages of organisms and generalise the findings, often across larger spatial and temporal scales (Pace, 2001). Our method aids this process by allowing us to consider biogeochemical and ecological dynamics in sediments and benthic habitats with greater realism than previously possible. For example, we found that we could recreate natural DOC concentrations in sediment pore water. This finding is important because whole-ecosystem experiments have differed from smaller lab-based incubations in estimates of DOC dynamics (Zwart *et al.,* 2016). Our method may therefore bridge contrasting approaches to studying lake sediments, *i.e*. lab *vs* whole ecosystem. Of course, our sediment boxes have caveats just like any other approach. First, there is an issue of scale and whether C dynamics across a 0.19 m² box can mirror those across the whole >50,000 m² area of littoral sediment in lakes such as Lake Laurentian. Second, container walls have been shown to promote periphyton

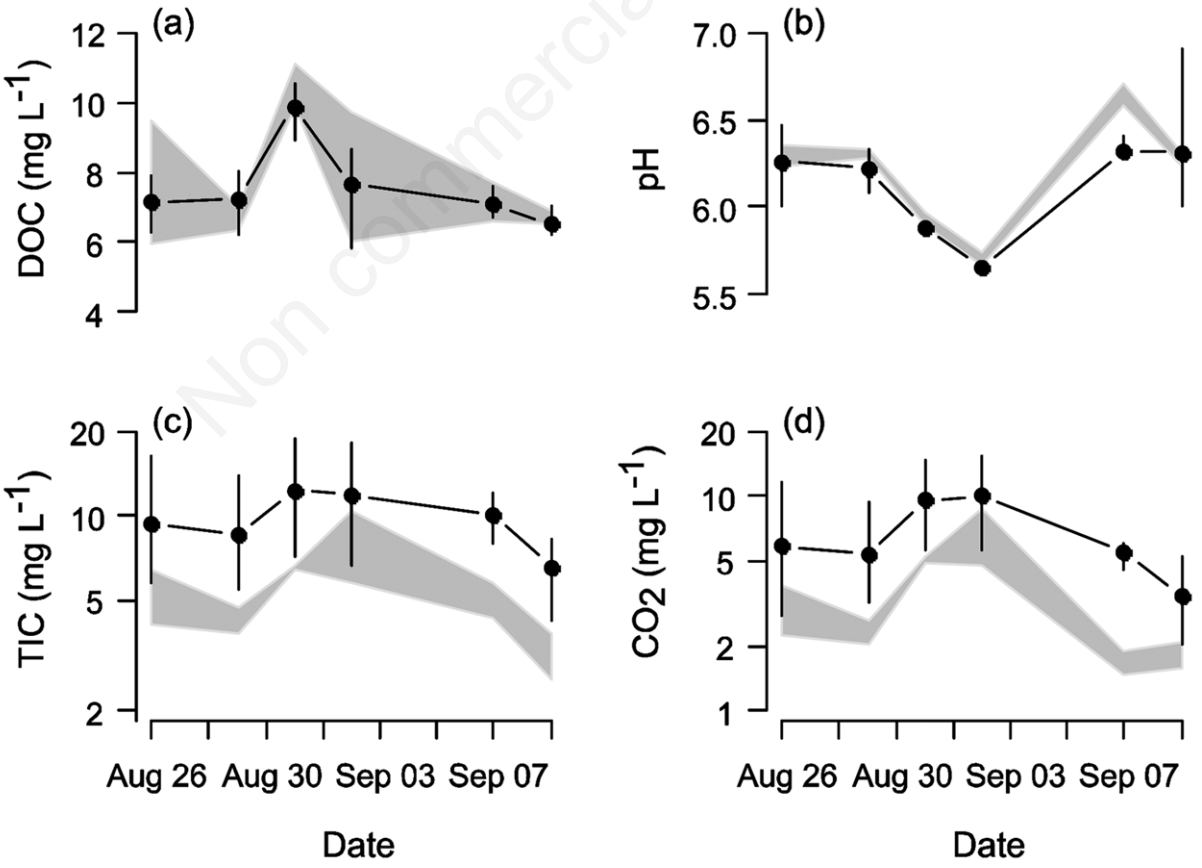

Fig. 2. Pore water chemistry in sediment boxes and their surrounding lake sediment during late summer 2016. a) DOC concentrations. b) pH. c) TIC concentrations. d) dissolved CO_2 concentrations. Black points are means, with vertical bars denoting the range of observations, in n=3 sediment boxes. Grey polygon is range of values in n=3 samplers installed in surrounding lake bottoms.

Fig. 3. Lake Laurentian study site with sediment boxes randomly deployed in a block design around three sampling docks. Left: view into lake from one sampling bay. Right: view into shore from other sampling bay.

growth and shift community composition (Chen and Kemp, 2004). We routinely cleaned container walls to minimise such effects, but have not quantified them as such. Container depth and surface area could also be reduced to minimise this wall effect, but at the cost of biological realism. Finally, all of our tOM amendments had a similar age. This differs from natural sediments where the same concentration of OM as in our boxes has accumulated over many years (Heathcote *et al.,* 2015). Amending sediments with tOM also bypasses the priming and inoculation of material with microorganisms as it is exported from land into receiving waters. We tried to account for some of this priming by pre-soaking sediments with water from the study lake, which should have bacterial communities that reflect broader flow networks (Ruiz-González *et al.,* 2015).

CONCLUSIONS

Here we have described a new experimental approach for studying the biogeochemistry and ecology of freshwater sediments at high spatial and temporal resolution. We have also shown that this method successfully mirrors both the absolute concentrations and temporal dynamics of biogeochemical parameters, especially those involved in carbon cycling, of natural sediments with similar organic matter inputs. Encouraged by our comparisons with natural lake bottoms, we believe that our method offers a way for better predicting how the important functions performed by lake sediments might respond to a changing world.

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