

## Sampling considerations and assessment of Exetainer usage for measuring dissolved and gaseous methane and nitrous oxide in aquatic systems

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### Abstract

Pre-evacuated Exetainers are commonly used as measurement vials for the determination of methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) concentrations in liquid and gaseous samples from aquatic environments. The impact of residual air in these Exetainers on measurement accuracy is assessed. Residual air pressure in commercially available, pre-evacuated Exetainers varied between  $0.071 \pm 0.008$  atm and  $0.180 \pm 0.031$  atm in examined batches. This background contamination can lead to large errors when determining dissolved and gaseous CH<sub>4</sub> and N<sub>2</sub>O concentrations particularly at low concentrations. A method for Exetainer pretreatment is suggested and verified, to reduce the residual CH<sub>4</sub> and N<sub>2</sub>O. Vials are flushed (needle 30 G × 0.5", 0.3 mm) with nitrogen gas (N<sub>2</sub>) for 5 min, which reduces the background CH<sub>4</sub> and N<sub>2</sub>O concentrations to  $0.092 \pm 0.008$  ppm and  $0.016 \pm 0.001$  ppm, respectively, approximately 3–4% of their respective concentrations in air. To avoid an alteration of sample concentration by variable residual gas levels left during a pre-evacuation step, liquid and gaseous samples are injected into the N<sub>2</sub> filled Exetainers. For gaseous samples where large volumes of gas are available, Exetainers can alternatively be flushed with 100 mL of sampling gas. For gaseous samples, measured CH<sub>4</sub> and N<sub>2</sub>O concentrations of standard gases were statistically identical to their known concentrations. For liquid samples, measured CH<sub>4</sub> and N<sub>2</sub>O concentrations of liquid standard dilution series showed strong linear correlations with theoretically calculated concentrations (slope CH<sub>4</sub>: 1.04, slope N<sub>2</sub>O: 1.12). Sample concentrations remained constant over a minimum storage period of 6 weeks.

### Introduction

Greenhouse gases (GHG) have long-term influence on the climate, with methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) among the most powerful GHG. Their global warming potentials are 21 and 310 times, respectively, that of carbon dioxide (CO<sub>2</sub>), on a 100-year time horizon (IPCC 2007). Thus, there is a growing interest in monitoring the emissions of CH<sub>4</sub> and N<sub>2</sub>O from natural and engineered systems, including aquatic systems such as lakes, oceans, bays, estuaries, and rivers (Kiese et al. 2003; Allen et al. 2007; Ferron et al. 2007; DelSontro et al. 2010).

Various methods are used to quantify CH<sub>4</sub> and N<sub>2</sub>O emissions and to get insight into the production and consumption pathways of these gases in aquatic environments. Direct gas fluxes in form of bubbles (ebullition) formed at the bottom of aquatic systems are commonly measured by using funnels

anchored below the water surface that trap these gases (Galy-Lacaux et al. 1999; Casper et al. 2000; Joyce and Jewell 2003). The emissions of CH<sub>4</sub> and N<sub>2</sub>O are often measured using floating static (closed) or active (dynamic) chambers that trap emitted gases at the water–air interface. Gas accumulation in the chambers over time is then used for rate calculations (St. Louis et al. 2000). While floating chambers measure ebullition and diffusive water–air fluxes simultaneously (Duchemin et al. 1999; Silvennoinen et al. 2008; Bastviken et al. 2010), diffusive water–air fluxes are usually estimated using the thin boundary layer model. This model requires the gas concentration difference across the water and air interface along with the gas-transfer velocity (Liss and Slater 1974; Upstill-Goddard 2006). To get insight in the production and consumption of GHG in the sediments, interfacial fluxes between sediment and water body can be determined using either sediment core incubations in the laboratory (Nishio et al. 1982; Dong et al. 2002; Qu et al. 2003; Gihring et al. 2010) or automated or manual chamber incubations *in situ* (Breuer et al. 2000; Butterbach-Bahl et al. 2004; Haese et al. 2007; Smith et al. 2012).

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All the above-described methods require the measurement of CH<sub>4</sub> and N<sub>2</sub>O concentrations in liquid and/or gaseous samples. Often, the CH<sub>4</sub> and N<sub>2</sub>O concentrations in gas samples are measured using gas chromatography (GC) as this is a widely available and cost effective analysis technique. For liquid samples, GC is typically used to measure CH<sub>4</sub> and N<sub>2</sub>O concentrations in the headspace of a vial containing the sample, after gas-liquid equilibrium is established. Alternatively, gas equilibrium is reached in a sampling syringe and only the gas phase is transferred to a measurement vial. The dissolved CH<sub>4</sub> and N<sub>2</sub>O concentrations can then be calculated through the use of Henry's Law.

Different sampling vials have been used to measure CH<sub>4</sub> and N<sub>2</sub>O in liquid and gaseous samples with GC. Samples can be stored in crimp sealed glass vials with volumes ranging between, e.g., 20 mL and 125 mL (Bastviken et al. 2010; DelSontro et al. 2011; Grossart et al. 2011). This method, which has to date only been described for the measurement of CH<sub>4</sub> (Bastviken et al. 2010; DelSontro et al. 2011; Grossart et al. 2011), requires a relatively large amount of sample. Also, vial preparation and sample handling in the field is time consuming. Samples can also be stored in gas-tight syringes (Kreuzwieser et al. 2003; Ferron et al. 2007) or gas bags (Wang et al. 2009). Drawbacks for all these methods are that they are labor intensive as they require manual injections of sample aliquots into the GC, and thus, they are usually suitable for small number of samples.

As an alternative, pre-evacuated Exetainers are commonly used for the measurements of liquid and gaseous samples (Silvennoinen et al. 2008; Beaulieu et al. 2010; Allen et al. 2011; Grover et al. 2012; Zheng et al. 2012; Hyvonen et al. 2013). Pre-evacuated Exetainers are inexpensive (current price approximately: £0.25/vial, order code 039W, www.labco.co.uk), use little storage place and can be used with a GC autosampler. These features allow a fast throughput of high numbers of samples. Further, these Exetainers are particularly suitable for applications, where the sample volume is limited as the commonly used total tube volume is 12 mL. Pre-evacuated Exetainers are also suitable for gases other than CH<sub>4</sub> and N<sub>2</sub>O such as dissolved oxygen or nitrogen gas (e.g., Hamilton and Ostrom 2007). Exetainer vials with a vacuum are commercially available (pre-evacuated) or the vacuum can be self-created, either manually or with a vacuum pump. The original purpose of these vials was the suction of liquid samples from a syringe in medical applications (Hamilton and Ostrom 2007). This was later extended to measuring various gases in environmental samples and several studies investigated drawbacks and challenges of Exetainer vial usage. Glatzel and Well (2008) reported air leakage into the vials after piercing the septa which could be reduced using needles with a small diameter (0.45 mm). N<sub>2</sub>O leakage through the septum and adsorption of N<sub>2</sub>O by the butyl rubber septum have been reported to cause a 30% decrease of N<sub>2</sub>O concentrations after 1 yr of storage in initially helium filled and then self-evacuated Exetainers (Laughlin and Stevens 2003). The authors suggest to store and analyse the

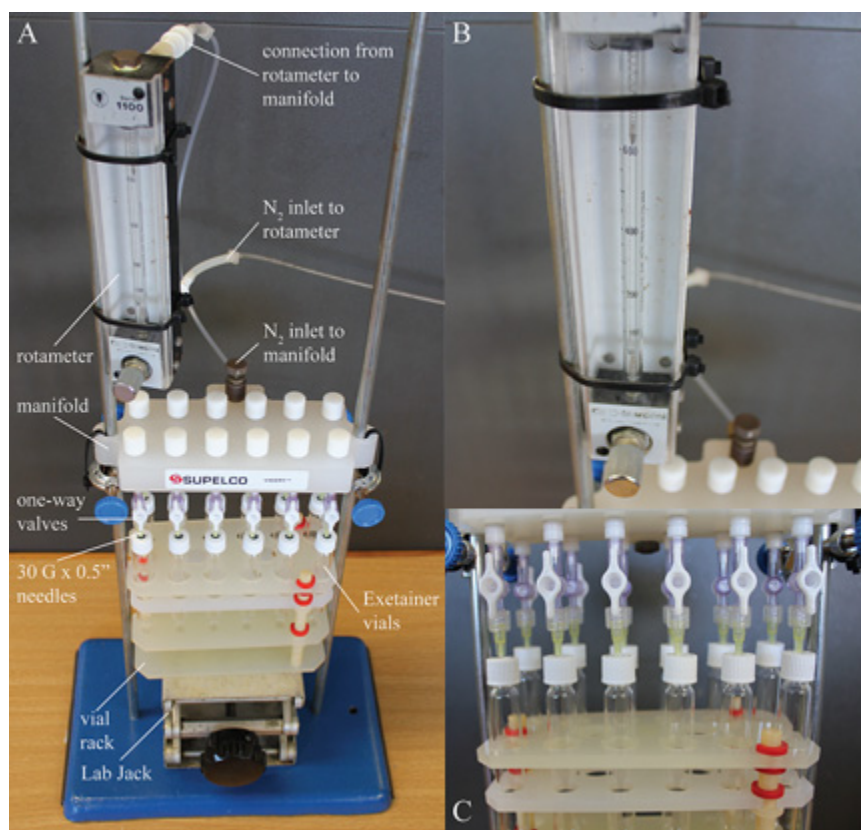
calibration gas along with the samples, to compensate for these errors (Laughlin and Stevens 2003). Hamilton and Ostrom (2007) found unacceptable levels of nitrogen gas (N<sub>2</sub>) in purchased, pre-evacuated Exetainers. They also reported that N<sub>2</sub> leakage through the lid (cap/septum) caused an increase of N<sub>2</sub> with sample storage time. To reduce N<sub>2</sub> contamination they self-evacuated Exetainers prior to sample injection and stored samples under water. The obvious drawbacks of this method are that the vial and sample preparation is time intensive and that the reduction of background has only been evaluated for N<sub>2</sub> measurements. For terrestrial environments, Hedley et al. (2006) proposed a procedure for the use of pre-evacuated Exetainers for the sampling and analysis of gaseous samples (analysed for CH<sub>4</sub>, N<sub>2</sub>O, and CO<sub>2</sub>) taken above the soil surface with static chambers. The method involves injecting a 25 mL portion of gaseous sample into 12 mL pre-evacuated Exetainers, creating overpressure before analysis with GC. However, the accuracy of the method was not fully analysed as, e.g., a background contamination by residual air was not investigated.

Although many studies were conducted to evaluate and to improve the use of pre-evacuated Exetainer vials for liquid and gaseous samples, the information is scattered. There has been to date no report on the comprehensive assessment of the suitability or accuracy of the vials for measuring gaseous or liquid samples with concentrations in various ranges. The lack of such information leaves a high-level of uncertainties with the results obtained with the use of these pre-evacuated vials. In this study, we systematically assess the usage of Exetainer vials for CH<sub>4</sub> and N<sub>2</sub>O concentration determination. We start by investigating the varying residual air pressure in commercially available, pre-evacuated Exetainer vials. To address the background contamination caused by the residual air, we propose two pretreatment methods, thereby significantly improving the accuracy of the Exetainer method for measuring gases in both liquid and gaseous samples, while maintaining the benefits of the Exetainer vial usage (cost efficiency, high sample throughput using autosampler with GC, and easy sample storage). In our proposed method for Exetainer usage, vials are flushed with N<sub>2</sub> to reduce residual concentration of gases of interests, which are CH<sub>4</sub> and N<sub>2</sub>O in our case. The N<sub>2</sub> flushed Exetainers are used for both liquid and gaseous samples. For cases where large volumes of sampling gas are available, we alternatively suggest flushing vials with the sample gas itself instead of N<sub>2</sub>. The liquid or gaseous samples are subsequently injected into the N<sub>2</sub> or sample gas filled Exetainers without pre-evacuation. We also investigate the effects of storage time (up to 6 weeks) on the measurement results.

## **Materials and procedures**

### **Residual air pressure in pre-evacuated Exetainers**

The sample vials used were Exetainers from Labco® Limited, Lampeter, UK (order code 039W) and were 12 mL pre-



**Fig. 1.** (A) Setup to flush 12 Exetainer vials with  $N_2$  gas. (B) Detailed view of the rotameter controlling the gas flow (C) and the connections between one-way valves and needles to the Exetainer vials.

evacuated borosilicate vials with round bottoms and white caps containing gray butyl rubber septa (13 mm diameter, 3 mm thickness).

To assess the residual air volume in the pre-evacuated Exetainer vials, we quantified the volume of water that is sucked into the vials by the existing vacuum. We used a 12 mL syringe filled with water fitted with a 23 G  $\times$  1.25", 0.64 mm needle (Livingstone International Pty Ltd, item no. DN23GX1.25LV) to pierce the rubber septum of the Exetainer lid. On piercing the rubber septum, the water sample was sucked into the Exetainer by the given vacuum. The syringe piston was removed to avoid friction between piston and syringe wall. The volume of water sucked into the vials (i.e., level of vacuum) was calculated by subtracting the weight of the empty Exetainers from the weight of the water filled Exetainers. The residual air volume was then calculated by subtracting the volume of the Exetainer that could be filled with water from the total Exetainer volume.

#### Proposed method for $CH_4$ and $N_2O$ measurement

As will be presented in the assessment section, residual air was found in all vials, either pre-evacuated or manually evacuated, with levels varying amongst vials and batches. Vials were manually evacuated (not previously pre-evacuated by

the manufacturer, thus, they had an intact new rubber septum) by withdrawing the air through the septum of the closed vial with a 50 mL syringe fitted with a 23 G  $\times$  1.25", 0.64 mm needle until no further suction was possible (at least two times the full volume of the syringe). To reduce the residual air contamination and eliminate the uncertainties associated with different levels of vacuum, we propose a method, in which the Exetainers are flushed and filled either with 99.999% pure  $N_2$  gas (BOC gases, Brisbane, Australia) or the gaseous sample itself at standard temperature and pressure. A special manifold (Fig. 1) is used to enable flushing and filling of the Exetainers with  $N_2$  gas with a high throughput (12 Exetainers per run). To ensure a stable flow of  $N_2$  into the Exetainer vials, the gas flow to the manifold is controlled with a rotameter at 600 cc  $min^{-1}$  (equals 50 mL  $min^{-1}$  of  $N_2$  flushing per Exetainer vial). The manifold used is a modified Visidry<sup>TM</sup> drying attachment (Sigma Aldrich, LLC., St. Louis, Missouri, U.S.A.), where the solid phase extraction tube adapters were replaced with one-way valves (John Morris Scientific Pty Ltd, item no. 30600-00) and disposable 30 G  $\times$  0.5", 0.3 mm needles (Terumo Medical Corporation, item no. NN-3013R) to pierce through the rubber septa of the Exetainer lids. While flushing, the Exetainer lids are kept slightly unscrewed to ensure that gas is able to

escape and no dangerous overpressure is created. After flushing for a certain time the Exetainers are removed from the manifold and ONLY then are the lids fully closed, thereby avoiding any build-up of overpressure in the vials. Only lids with new gray rubber septa (13 mm diameter, 3 mm height) not being previously pierced were used throughout whereas the Exetainer tubes can be washed and reused. The manifold mounted needles are regularly replaced, especially when bent.

An experiment to examine the efficiency of the N<sub>2</sub> flushing through the manifold mounted needles was conducted comparing two different cannula diameters (30 G × 0.5", 0.3 mm and 23 G × 1.25", 0.64 mm). Within this experiment, we also tested different N<sub>2</sub> flushing durations of 1 min, 2 min, 3 min, 4 min, 5 min, and 10 min to determine the shortest possible flushing time adequate to reduce gas concentrations for all gasses but the abundant gases N<sub>2</sub> or oxygen. MilliQ water (6 mL, deaerated by sparging with helium for 15 min) was injected into the N<sub>2</sub> flushed vials, mimicking the real sampling procedure for liquid samples, and the headspace gas was then analysed for remaining CH<sub>4</sub> and N<sub>2</sub>O concentrations using the GC as described below.

As an alternative approach for gaseous samples where large volumes of sampling gas are available, we propose flushing the Exetainers with the sampling gas. In this procedure, syringes fitted with a 23 G × 1.25", 0.64 mm needle and filled with the sampling gas itself are used for flushing the Exetainers and not the manifold described above. While flushing the vials, the lids of the Exetainers are slightly unscrewed and closed after the flushing process.

For measurements, 12 mL of a gas or 6 mL of a liquid sample is injected by syringes fitted with a 23 G × 1.25", 0.64 mm needle into an Exetainer filled with the sample gas itself or N<sub>2</sub> (without evacuating the vials), thus, creating an overpressure.

#### CH<sub>4</sub> and N<sub>2</sub>O concentration determination using GC

Both liquid and gaseous samples in the N<sub>2</sub> or sample gas flushed Exetainers, were analysed for CH<sub>4</sub> and N<sub>2</sub>O concentrations by injecting gas aliquots into a gas chromatograph (Agilent GC7890A, Agilent Technologies, Santa Clara, California, U.S.A.). Prior to the GC analysis vials containing liquid samples were stored upside down to prevent gas leakage from the headspace through the septum and refrigerated (4 °C) to decrease microbial processes as an additional measure next to sterile filtration (0.22 μm PES syringe filter). Liquid samples had a gaseous headspace of approximately 6 mL. They were taken out of the fridge 24 h prior to GC analysis to reach room temperature and to allow equilibration of the gases in headspace and water column.

The GC method was based on Agilent application SP1 7890-0468. For the pre-evacuated Exetainers, 250 μL of the gas sample were injected by a CTC CombiPAL autosampler headspace syringe (1 mL-syringe, filling speed: 100 μL s<sup>-1</sup>,

injection speed: 500 μL s<sup>-1</sup>) into a purged packed inlet (heated to 110 °C). With the proposed method, the overpressure created inside the vials during sample injection (see details above) allows the withdrawal of a bigger sample volume by the GC. Thus, for the proposed method, 3.5 mL of the gas sample were injected by a CTC CombiPAL autosampler headspace syringe (5 mL-syringe, filling speed: 100 μL s<sup>-1</sup>, injection speed: 500 μL s<sup>-1</sup>) to flush a 1 mL sample loop (heated to 100 °C). The injected samples of both sampling techniques were separated by two columns (column 1: Supelco 6 feet × 1/8-in stainless steel packed column (HayeSep Q 80/100), 21 mL min<sup>-1</sup> at 60 °C; column 2: Supelco 12 feet × 1/8-in stainless steel packed column (HayeSep Q 80/100), 21 mL min<sup>-1</sup> at 60 °C). A microelectron capture detector (ECD) at 250 °C was used for the analysis of N<sub>2</sub>O and a flame ionization detector (FID) at 350 °C for the analysis of CH<sub>4</sub>. The make-up gas for the ECD was 5% CH<sub>4</sub> in argon at 2 mL min<sup>-1</sup> and for the FID N<sub>2</sub> gas at 2 mL min<sup>-1</sup>. The temperature of the oven was maintained at 60 °C for 10 min, after which it was raised in 25 s to 110 °C and kept at this temperature for 2 min. The CTC CombiPAL autosampler was equipped with four VT32 racks accommodating a total of 128 Exetainer vials. The VT32 racks are originally made for headspace vials that have a bigger diameter (23 mm) than the Exetainer vials (15 mm). Therefore, 128 special spacers had to be made to overcome this problem. In addition Perspex covers were custom built for each rack to make sure that the vials would stay in the racks when the syringe withdrew the gas aliquot. The gas syringe was cleaned two times with air before the next sample injection. The CTC headspace syringes are open to atmosphere and, therefore, the gas samples were injected at room temperature and pressure. The GC was calibrated using standards with a range of 1.8 ± 0.02 ppm to 249.8 ± 1.3 ppm of CH<sub>4</sub> and 0.5 ± 0.01 ppm to 50.53 ± 0.51 ppm of N<sub>2</sub>O, which were prepared from certified gas standards (BOC gases, Brisbane, Australia).

Exetainer weights were measured before and after liquid samples were injected to determine the actual volume of the sample. Henry's law was used to calculate the concentrations in the liquid phase from the measured concentrations in the headspace. Henry's law states that the amount of dissolved gas at a certain temperature is directly proportional to the partial pressure of the same gas in the gas phase which is in equilibrium with the liquid phase (Henry 1803). The proposed approach of using N<sub>2</sub> flushed Exetainers for liquid sample measurements creates an overpressure inside the vials which needs to be accounted for when calculating the concentrations in the liquid phase.

#### Verification of the developed method for liquid sample measurements

To verify the accuracy of the proposed method for liquid sample measurements, we measured CH<sub>4</sub> and N<sub>2</sub>O concentrations in a series of liquid samples diluted from the same



dissolved CH<sub>4</sub> or N<sub>2</sub>O stock solutions with a known concentration. These results are then compared to the theoretically calculated CH<sub>4</sub> or N<sub>2</sub>O concentrations.

To obtain liquid samples of a known CH<sub>4</sub> or N<sub>2</sub>O concentration, a 1 L glass bottle with a magnetic stirrer bar inside was filled with milliQ water. The bottle was closed with a lid containing three ports. One port was connected to a gas cylinder to sparge the sample water with the test gas. The test gas contained 90 ± 1.8% mol CH<sub>4</sub>, 5 ± 0.1% mol CO<sub>2</sub>, and 5 ± 0.1% mol N<sub>2</sub> (Coregas Pty Ltd, Yennora, Australia) for the verification experiment for CH<sub>4</sub> and 0.094 ± 0.003% mol N<sub>2</sub>O which was in balance with N<sub>2</sub> for the verification experiment for N<sub>2</sub>O (Air Liquide Australia Ltd, Melbourne, Australia). A second port was used as a gas outlet to the fume hood. To the third port a 1 L multilayer FlexFoil® gas bag (SKC, Eighty Four, Pennsylvania, U.S.A.) was attached. The water was sparged with the gas for 30 min to ensure the water is saturated with CH<sub>4</sub> or N<sub>2</sub>O. The gas bag was filled with the same gas after sparging the water and remaining ports in the lid were closed with clamps. The glass bottle had a sampling port at the bottom, which was connected with tubing (John Morris Scientific Pty Ltd, Masterflex Norprene 06404-series) to a one-way valve (John Morris Scientific Pty Ltd, item no. 30600-00), to sample the CH<sub>4</sub> or N<sub>2</sub>O spiked samples. To avoid low pressure in the sample water bottle during the sampling process, gas from the gas bag filled up the increasing headspace. To obtain samples with different concentrations of the dissolved gases, different volumes of CH<sub>4</sub> or N<sub>2</sub>O spiked water and different volumes of deaerated milliQ water (deaerated by sparging with helium for 15 min) at ratios of 6: 0, 5: 1, 4: 2, 3: 3, 2: 4, 1: 5, 0.1: 5.9, 0.03: 5.97 (mL of spiked water: mL of deaerated water) were injected into Exetainers to achieve a total liquid volume of 6 mL. Prior to this, the Exetainers were flushed by the manifold for 5 min with N<sub>2</sub> (cannula size 30 G × 0.5", 0.3 mm). The concentration of CH<sub>4</sub> or N<sub>2</sub>O in the headspace was analysed using the GC as described above.

#### Verification of the developed method for gaseous sample measurements

To verify the accuracy of the two methods suggested for gaseous sample measurements, two standard gases (BOC gases, Brisbane, Australia) were taken as the gas samples. The measured results were compared to the expected standard gas concentrations. The higher standard gas contained 249.8 ± 1.3 ppm CH<sub>4</sub> and 50.53 ± 0.51 ppm N<sub>2</sub>O, and the lower standard gas contained 1.8 ± 0.02 ppm CH<sub>4</sub> and 0.5 ± 0.01 ppm N<sub>2</sub>O, respectively.

For the first approach, designed for the application of gas sample measurements with small volumes of sampling gas available, Exetainers were flushed for 5 min with N<sub>2</sub> (cannula size 30 G × 0.5", 0.3 mm) to replace the residual air with N<sub>2</sub>. Afterwards, 12 mL of the sample gas was injected with a 25 mL gas tight, luer lock valve glass syringe (SGE

Analytical Science/Trajan Scientific Australia Pty Ltd, Ringwood, Australia) fitted with a 23 G × 1.25", 0.64 mm needle into the N<sub>2</sub> gas filled vial thereby producing an overpressure. Test results achieved by this approach needed to be corrected for the dilution of the sample gas by the N<sub>2</sub> gas.

For the second approach, designed for applications where large volumes of gas sample are available, Exetainers were flushed with the sample gas itself and not with N<sub>2</sub>, thus, avoiding a potential dilution of the gas samples by N<sub>2</sub> gas. We tested three different sample flushing volumes of 50 mL, 100 mL, and 250 mL (inserted with a 100 mL gas tight, luer lock valve glass syringe fitted with a 23 G × 1.25", 0.64 mm needle) before closing the cap and adding the actual sample (12 mL).

#### Effect of liquid and gaseous sample storage in Exetainers

The effect of liquid and gaseous sample storage in Exetainers prepared using the proposed methods were tested over a period of 43 d.

For the storage test of liquid samples in pretreated Exetainers, the Exetainers were first flushed with N<sub>2</sub> for 5 min and then they were flushed with either 3 × 100 mL of a low gas standard (1.8 ± 0.02 ppm CH<sub>4</sub>, 0.5 ± 0.01 ppm N<sub>2</sub>O) or 3 × 100 mL of a high gas standard (249.8 ± 1.3 ppm CH<sub>4</sub>, 50.53 ± 0.51 ppm N<sub>2</sub>O) using a 100 mL gas tight, luer lock valve glass syringe fitted with a 23 G × 1.25", 0.64 mm needle. Immediately after flushing, 6 mL of deaerated milliQ water (deaerated by sparging with helium for 15 min) was injected into the vials. The liquid samples in these vials were then vortexed to achieve gas-liquid equilibrium. A second storage experiment was conducted in parallel with environmental river water samples (27°29'30" S, 153°0'47" E, Brisbane River, Brisbane, Australia). For this, Exetainers were flushed for 5 min with N<sub>2</sub> and a filtered (0.22 μm PES syringe filter) river water sample of 6 mL was injected.

Exetainers for gaseous samples using the pretreatment approach for small gaseous sample volumes were prepared as described above as the verification and storage experiments were conducted using the same set of samples. For the approach, designed for applications where large volumes of gas sample are available, Exetainers were flushed with 100 mL of the sample gas itself, before the cap was closed and the actual sample (12 mL) was added.

#### Field verification of the effect of the proposed pretreatment: dissolved gas measurement as an example

Surface water CH<sub>4</sub> and N<sub>2</sub>O concentrations are commonly measured in limnological and oceanographic research for emission calculations using the thin boundary layer models (Abril et al. 2005; Ferron et al. 2007; DelSontro et al. 2010). To verify the possible improvement to accuracy achievable with the proposed pretreatment method, we monitored dissolved CH<sub>4</sub> and N<sub>2</sub>O water concentrations at one local site (Brisbane River estuary, Australia, 27°52'42" S, 152°99'45" E) during the transition from low tide (time 09:30 h) to high

**Table 1.** Tested Exetainer batches, their manufacturing dates and timeframe between the manufacturing and first test dates.

	Batch number	Manufacturing date	Timeframe between manufacturing date and first test date (April 2012)
Batch 1	2343	September 2010	19 months
Batch 2	2692	March 2011	13 months
Batch 3	2784	April 2011	12 months
Batch 4	3308	November 2011	5 months
Batch 5	0023	February 2012	2 months

tide (time 15:30 h). In the study, we used both N<sub>2</sub> flushed Exetainers (flushed for 5 min, no evacuation) and commercially available, pre-evacuated Exetainers. For both Exetainer vial approaches, liquid samples were taken 5 times in 1 h intervals between low and high tide. The samples were taken from approximately 20 cm below the water surface, and 6 mL of samples were then inserted with a 12 mL plastic syringe fitted with a 23 G × 1.25", 0.64 mm needle into the respective vials.

To further assess the impact of potential measurement errors on emission estimation, we estimated water–air emissions using the surface water concentrations measured with both approaches. The emissions were estimated using the thin boundary layer model equation  $F = k \times \Delta C = k \times (C_w - C_{eq})$ , where  $F$  is the flux ( $\mu\text{mol m}^{-2} \text{d}^{-1}$ ),  $k$  is the gas-transfer coefficient ( $\text{m d}^{-1}$ ), and  $\Delta C$  is the gas concentration difference across the water ( $C_w$ ) and air-equilibrium ( $C_{eq}$ ) interface (Cole et al. 2010). The measured surface water samples were used for  $C_w$ , whereas  $C_{eq}$  was calculated from the atmospheric CH<sub>4</sub> (1774 ppb) and N<sub>2</sub>O (319 ppb) concentrations based on their solubility (Yamamoto et al. 1976; Weiss and Price 1980; Forster et al. 2007). The gas-transfer coefficient  $k$  was estimated using the equation  $k = a \times U_{10}^2 \times (Sc/600)^{-x}$  developed by Wanninkhof (1992) with parameter  $a$  depending on the wind type ( $a = 0.31$  for short-term winds or  $a = 0.39$  for steady winds),  $U_{10}$  as the frictionless wind speed ( $\text{m s}^{-1}$ ) normalized to a 10 m height (Crusius and Wanninkhof 2003),  $Sc$  as the Schmidt number for CH<sub>4</sub> and N<sub>2</sub>O (Wanninkhof 1992) and  $x$  as a constant depending on the wind speed ( $x = 0.66$  for wind speed  $< 3 \text{ m s}^{-1}$  or  $x = 0.5$  for wind speed  $> 3 \text{ m s}^{-1}$ ). Wind speed data was obtained from a nearby weather station (27°29'46" S, 153°0'53" E), which logged (VantagePro2plus, Davis Instruments Corp., Hayward, California, U.S.A.) wind speed every minute (average readings from 1 min). We averaged the wind speed for our calculations over each hour incubation interval of the surface floating chamber.

### Statistical analysis

Statistical analysis of all results was performed with the program Statistica version 12 (StatSoft, Tulsa, Oklahoma,

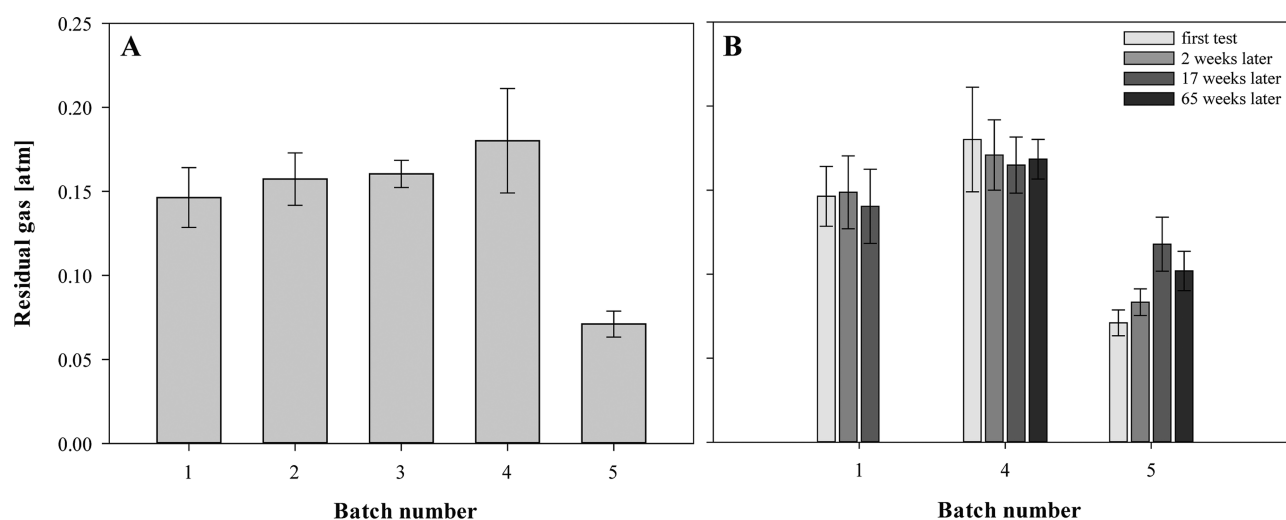
U.S.A.), using factorial or one-way analysis of variances (ANOVAs). To evaluate the importance of cannula diameter together with flushing time a factorial ANOVA was performed with cannula diameter and flushing time as the categorical predictors and CH<sub>4</sub> or N<sub>2</sub>O as the continuous variables. One-way ANOVAs were performed with residual air levels, flushing times, analysis days, or standard gases as the categorical predictor and CH<sub>4</sub> or N<sub>2</sub>O as the continuous variables. Data were log transformed where necessary to ensure normality of distribution and homogeneity of variance (Levene's test) (Zar 1984). Post hoc tests were performed using Fisher's LSD (least significant difference) Test (Zar 1984). The nonparametric Kruskal–Wallis (KW) test was used for data which failed to satisfy the assumptions of normality and homogeneity of data after being transformed.

### Assessment

#### Residual air pressure in pre-evacuated Exetainers

The residual air pressure that is left in the vials after pre-evacuation was assessed in five batches (Batches 1–5) manufactured on different dates. The tested Exetainer batches, their manufacturing date and the timeframe between the manufacturing and test dates are summarized in Table 1.

The purchased Labco® Exetainers were found to have an incomplete vacuum as a substantial residual air pressure varying between  $0.071 \pm 0.008 \text{ atm}$  and  $0.180 \pm 0.031 \text{ atm}$  remained inside the glass vials (Fig. 2A). The range of residual air pressure determined in our study is in agreement with De Brabandere et al. (2012) who reported an internal air pressure of 0.08 to 0.15 atm in evacuated Labco® Exetainers (flat bottomed and soda glass instead of round bottomed and borosilicate glass in our study, all other specifications are the same). Our results also showed a high variability of the residual air levels within Exetainers of a specific batch and also amongst the different Exetainer batches. The average residual air pressure in Batch 5 was significantly lower ( $F_{4,55}$ ,  $p < 0.001$ ) compared to all other batches ( $0.071 \pm 0.008 \text{ atm}$ ). In contrast to this, no significant difference ( $p > 0.05$ ) was found amongst the average residual air pressure of Batches 1–3 on the first measurement day, with residual air pressure varying between  $0.146 \pm 0.018 \text{ atm}$  and  $0.157 \pm 0.016 \text{ atm}$ . The residual air pressure of Batch 4 ( $0.180 \pm 0.031 \text{ atm}$ ) was slightly elevated as compared to the Batches 1–3. However, the residual air pressure of Batch 4 was only significantly different ( $F_{4,55}$ ,  $p < 0.05$ ) from residual air levels in Batch 1 but not significantly different from Batches 2 and 3. The low residual air level in Batch 5 might be due to the fact that the batch was manufactured at the latest date of all tested batches. As leaking often happens over time, batches with longer lifetime could have more loss of vacuum through leaking. However, Batch 4 which was only 3 months older than Batch 5 had a significantly lower vacuum level, thus, variations amongst batches



**Fig. 2.** (A) Residual air levels of five Exetainer batches (mean  $\pm$  standard deviation,  $n = 10$ ) tested at the same test date. (B) Residual air levels of three Exetainer batches (mean  $\pm$  standard deviation,  $n = 10$ ) tested on three test dates (Batch 1) and on four test dates (Batches 4 and 5).

could also reflect evacuation level variations by the manufacturer.

To determine if the residual air pressure in the vials is stable over time further tests were carried out. For this, three of the batches tested in the first sets of tests (Batches 1, 4, and 5) were tested again 2 weeks and 17 weeks after the initial testing and two of the batches (Batches 4 and 5) 65 weeks after the initial testing. An increasing trend of internal residual air between the first test date, 2 weeks and 17 weeks later was observed in Batch 5 (Fig. 2B), where significantly different results ( $F_{2,87}$ ,  $p < 0.05$ ) were detected amongst the testing dates. 17 weeks after the first test, residual air levels in Batch 5 were much closer to the ranges of Batches 1–4 than on the first test date. These results indicate that leakage of air into the vials may occur over time. However, results 65 weeks after the first test date showed no significant difference ( $p > 0.05$ ) from the results 17 weeks after the first test date. In contrast to Batch 5, the residual air levels of Batches 1 and 4 did not vary significantly amongst all the three or four test dates. The level of residual air pressure found in the pre-evacuated Exetainers and the extent of variation within a batch and between batches show that the usage of pre-evacuated Exetainers as storage and measurement vials can lead to a significant and not easily quantifiable contamination of samples. Manually evacuated Exetainers showed comparable levels of residual air to the pre-evacuated Exetainer (data not presented).

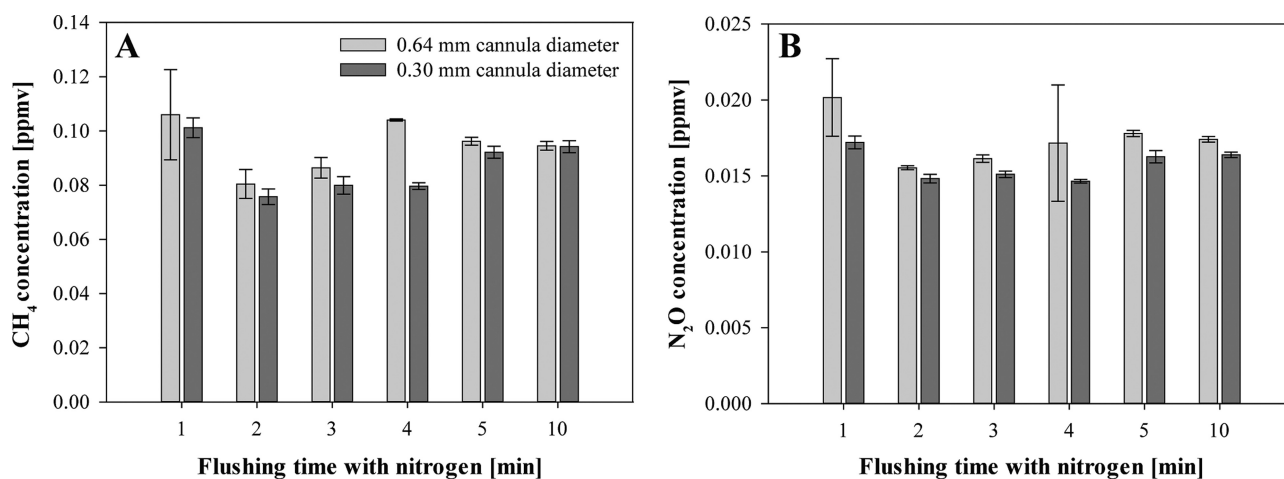
### Evaluation of proposed method

#### Choice of cannula diameters and optimization of $N_2$ flushing time

To reduce the previously described residual air contamination in Exetainers, we propose to flush the Exetainers with

$N_2$  gas for liquid and gas samples or, if a large volume of gas sample volumes is available, with the gaseous sample itself. The  $N_2$  flushing is conducted with a manifold where the tube adapters were replaced with disposable needles piercing the rubber septum of the Exetainer lids. The hole created by piercing should be as small as possible to avoid possible leaks and loss of pressure. Glatzel and Well (2008) advised to use a small cannula diameter of, e.g., 0.45 mm (26 G  $\times$  5/8") to minimize pressure loss and associated tightness. In this work, we used an even smaller cannula diameter of 0.3 mm (30 G  $\times$  0.5"). An experiment was conducted to verify if such a cannula is efficient in comparison to bigger cannula (23 G  $\times$  1.25", 0.64 mm) during the replacement of the air in the Exetainers with  $N_2$  thereby reducing the contamination of the samples. In addition, we also tested different  $N_2$  flushing durations (1 min, 2 min, 3 min, 4 min, 5 min, and 10 min) with both examined cannula diameters to determine the shortest possible flushing time. Six replicate Exetainers were tested per investigated cannula diameter and flushing duration.

Averaged  $CH_4$  and  $N_2O$  concentrations measured in the experiments shown in Fig. 3 indicate that comparable background concentrations were achieved with the smaller cannula size of 0.3 mm (30 G  $\times$  0.5") in comparison to the larger cannula. It is, therefore, advisable to choose the smaller cannula diameter to ensure that the subsequent leaking of gases through punctured holes is kept to a minimum. The concentrations of  $CH_4$  (Fig. 3A) and  $N_2O$  (Fig. 3B) in the tested vials showed no significant difference ( $p > 0.05$ ) with the small cannula size for the tested flushing durations of 2 min, 3 min, and 4 min and also no significant different ( $p > 0.05$ ) results for the flushing durations of 1 min, 5 min, and 10 min. Although a flushing time of 1 min did not give significant different results to 5 min, our personal preference



**Fig. 3.** Concentration of CH<sub>4</sub> (A) and N<sub>2</sub>O (B) in the Exetainers (mean  $\pm$  standard error,  $n = 6$ ) flushed with N<sub>2</sub> using two types of cannula diameter and using six different flushing times.

is a flushing time of 5 min to be on the safe side. A flushing time of 5 min reduces the background concentration of CH<sub>4</sub> to  $0.092 \pm 0.008$  ppm and of N<sub>2</sub>O to  $0.016 \pm 0.001$  ppm ( $n = 6$ ), slightly above their respective detection limit and was, thus, used in all following experiments.

The alternative sampling approach for gaseous samples which requires the flushing and filling of the Exetainers with the gaseous sample itself was carried out with the same needle (23 G  $\times$  1.25", 0.64 mm) used afterwards for sampling.

#### Verification of the developed method for liquid sample measurements and effect of liquid sample storage in Exetainers

To verify the accuracy of the proposed method for liquid sample measurements, we measured liquid samples with various "known" concentrations of dissolved CH<sub>4</sub> or N<sub>2</sub>O (three replicates per concentration) and compared these results to theoretically calculated CH<sub>4</sub> or N<sub>2</sub>O concentrations. We calculated the theoretical concentrations in the CH<sub>4</sub> or N<sub>2</sub>O spiked water and in the dilutions by using the solubility equations presented in Yamamoto et al. (1976) and Weiss and Price (1980).

Figure 4 compares the measured ( $y$ -axis) and theoretically calculated ( $x$ -axis) CH<sub>4</sub> (Fig. 4A) or N<sub>2</sub>O (Fig. 4B) concentrations. Results show a strong linearity between measured and calculated theoretical values for both dissolved gases (CH<sub>4</sub>:  $R^2 = 0.9998$ , N<sub>2</sub>O:  $R^2 = 0.9995$ ). The linear regression analysis shows that there is a consistent relative error between the measured and calculated theoretical results of 4.2% for CH<sub>4</sub> and 11.5% for N<sub>2</sub>O. The gas mixture used for spiking the samples had a gas concentration of  $90 \pm 1.8\%$  mol CH<sub>4</sub> and  $0.094 \pm 0.003\%$  mol N<sub>2</sub>O. Thus, in addition to measurement uncertainties, the concentration uncertainties of the gases may account for a deviation of 2.1–6.3% and 8.1–15.2% for CH<sub>4</sub> and N<sub>2</sub>O, respectively, between measured and calculated theoretical values. Another possible source of error

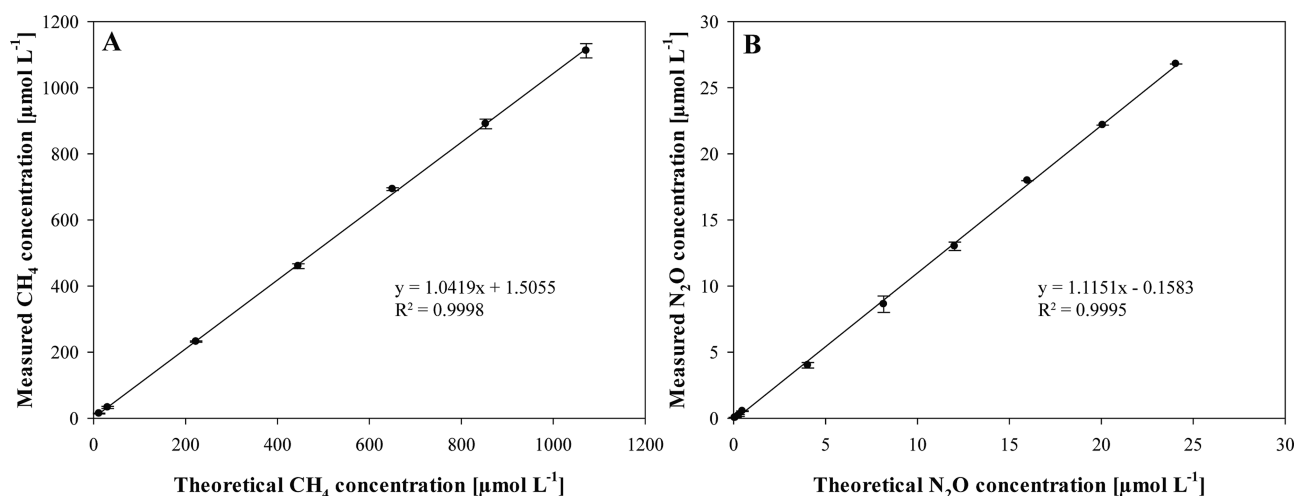
could be related to the solubility equations used in the calculations. Such errors could be minimized by recalibrating the parameters in these equations.

The effect of sample storage in Exetainers on liquid CH<sub>4</sub> and N<sub>2</sub>O concentrations was also conducted over a period of six week (measurements 1 d, 8 d, 15 d, 22 d, 29 d, 36 d, and 43 d after the sample injection) with five replicate Exetainers per test. This is important as taking samples in the field often means that analysis cannot be carried out immediately and storage is needed.

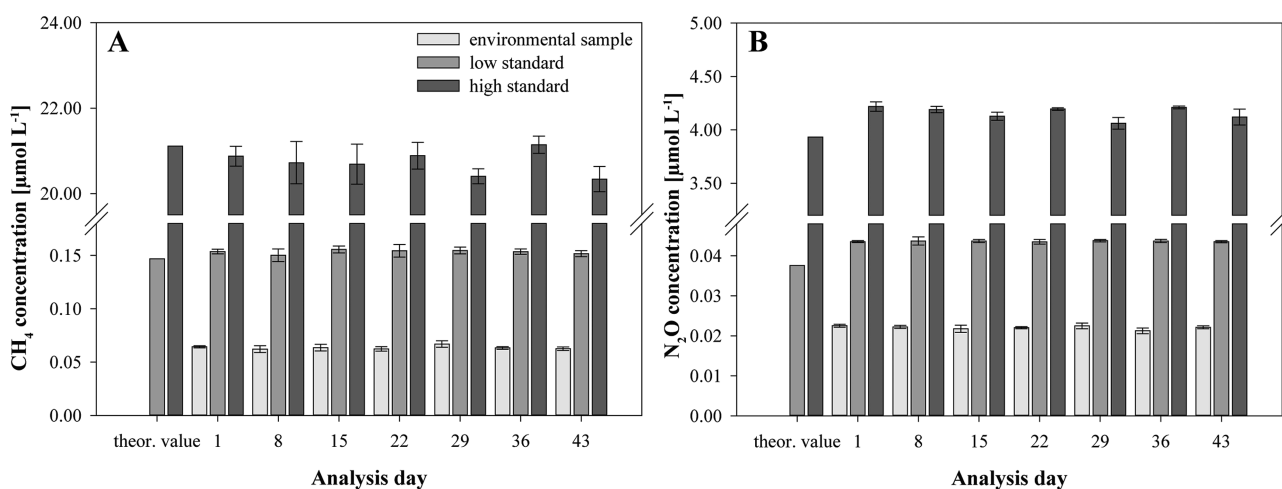
The CH<sub>4</sub> and N<sub>2</sub>O concentrations measured during the storage are presented in Fig. 5, together with the theoretically expected concentrations of the two used standards. The measured CH<sub>4</sub> (Fig. 5A) and N<sub>2</sub>O (Fig. 5B) concentrations in the Exetainers flushed with the low gas standard are not significantly different amongst all investigated analysis days ( $p > 0.05$ ). The measured concentrations are slightly higher than the theoretically expected value. This may be explained by errors during vial and sample handling, during GC operation, during the dilution of standards or by uncertainties related to the standard gas, as the higher values cannot be explained by leakage of the gases through the lid.

In the high standard case, the measured CH<sub>4</sub> concentrations on 8 d, 22 d, 36 d, and 43 d showed no significant differences ( $p > 0.05$ ) compared to the results on Day 1. However, the measured CH<sub>4</sub> concentrations on Day 15 and Day 29 were slightly lower than the values on Day 1 (Day 15: KW-H<sub>6,35</sub>,  $p < 0.05$ ; Day 29: KW-H<sub>6,35</sub>,  $p < 0.001$ ). Measured N<sub>2</sub>O concentrations for the high standard case showed no significant differences ( $p > 0.05$ ) compared to the results on Day 1, apart from Day 29 (KW-H<sub>6,35</sub>,  $p < 0.01$ ). Measured N<sub>2</sub>O concentrations were slightly higher than the theoretical values in all cases, again indicating that leakage of gases is not responsible for the differences observed.





**Fig. 4.** Method verification results for liquid samples showing the measured CH<sub>4</sub> (A) and N<sub>2</sub>O (B) concentration (mean ± standard error, *n* = 3) versus the theoretically calculated concentrations.



**Fig. 5.** CH<sub>4</sub> (A) and N<sub>2</sub>O (B) concentrations and their theoretical values in liquid samples filled in N<sub>2</sub> flushed Exetainer after a storage time of 1, 8, 15, 22, 29, 36, and 43 d (mean ± standard deviation, *n* = 5).

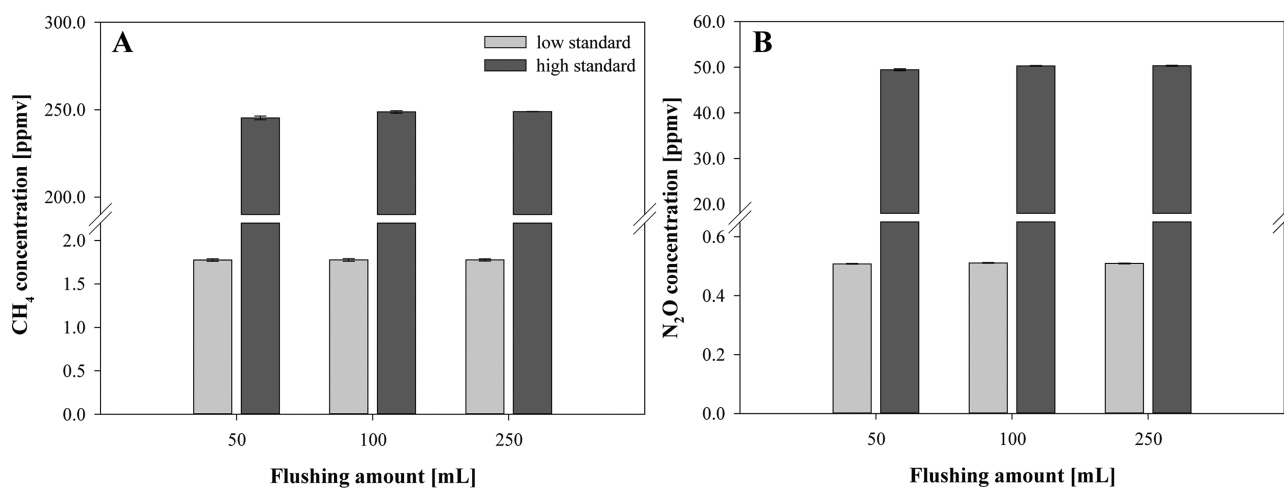
Exetainers with environmental samples also showed no significant difference (*p* > 0.05) for CH<sub>4</sub> results amongst all analysis days. The N<sub>2</sub>O results of the environmental samples were not significant different (*p* > 0.05) amongst analysis days apart from the measurements of Day 1 with Day 36 which were similar but statistically different (KW-H<sub>6,35</sub>, *p* < 0.05).

**Verification of the developed method for gaseous sample measurements and effect of gaseous sample storage in Exetainers**

Both methods proposed for gaseous sample measurements were tested to verify the method accuracy and effect of sample storage in Exetainer vials.

For the method designed for large available gas volumes, where sample gas is used for flushing, tests were done to

determine the gas flushing volume needed. Six replicate Exetainers were tested for flushing amounts of 50 mL, 100 mL, or 250 mL of a low gas standard or high gas standard before inserting the gaseous sample. CH<sub>4</sub> (Fig. 6A) results for the low standard gas showed no significant difference (*p* > 0.05) among flushing amounts of 50 mL, 100 mL, or 250 mL. Flushing with 100 mL or 250 mL of the high standard gas for CH<sub>4</sub> resulted in no significant difference (*p* > 0.05) of measured concentrations to the actual concentration of the sampled standard gas. However, flushing with 50 mL of the high standard gas resulted in significantly lower (KW-H<sub>2,18</sub>, *p* < 0.05) CH<sub>4</sub> concentrations than flushing with 100 mL or 250 mL. Best results for N<sub>2</sub>O (Fig. 6B) measurements were also achieved by flushing Exetainers with 100 mL or 250 mL (no significant different results, *p* > 0.05) of the sampled gas



**Fig. 6.** CH<sub>4</sub> (A) and N<sub>2</sub>O (B) concentrations of gaseous samples (samples: low and high gas standard, mean  $\pm$  standard deviation,  $n = 6$ ) after flushing with 50 mL, 100 mL, or 250 mL of a low gas standard or high gas standard before inserting the gaseous sample (approach designed for large volumes of sample gas).

for both standard gases. We conclude and recommend to flush each Exetainer with a minimum of 100 mL of the sampling gas before closing the lid and then injecting the sample.

Recovery rates of both of the proposed methods for gaseous sample measurements were tested for both gas standards to verify if measured concentration results were as expected from the standard gas concentrations (theoretical value). Five replicate Exetainers were used per suggested approach (approach for small volumes of sample gas and approach for large volumes of sample gas) and standard gas (low and high gas standard). The Exetainers for large gaseous sampling amounts were flushed with 100 mL of sampling gas, as determined above.

Results of the first approach designed for small volumes of sample gas (Fig. 7A,B) showed that the method is accurate for gas samples injected into N<sub>2</sub> flushed Exetainers. Results showed CH<sub>4</sub> and N<sub>2</sub>O recovery rates of 93% to 100% for both gas standards, as measured concentration results were close to expected from the standard gas concentrations (theoretical value). The approach designed for large sampling gas volumes (Fig. 7C,D) showed that the method achieves high quality and accuracy of results with CH<sub>4</sub> and N<sub>2</sub>O recovery rates of 98% to 100% for both gas standards. The measured concentration results were as expected from the standard gas concentrations (theoretical value).

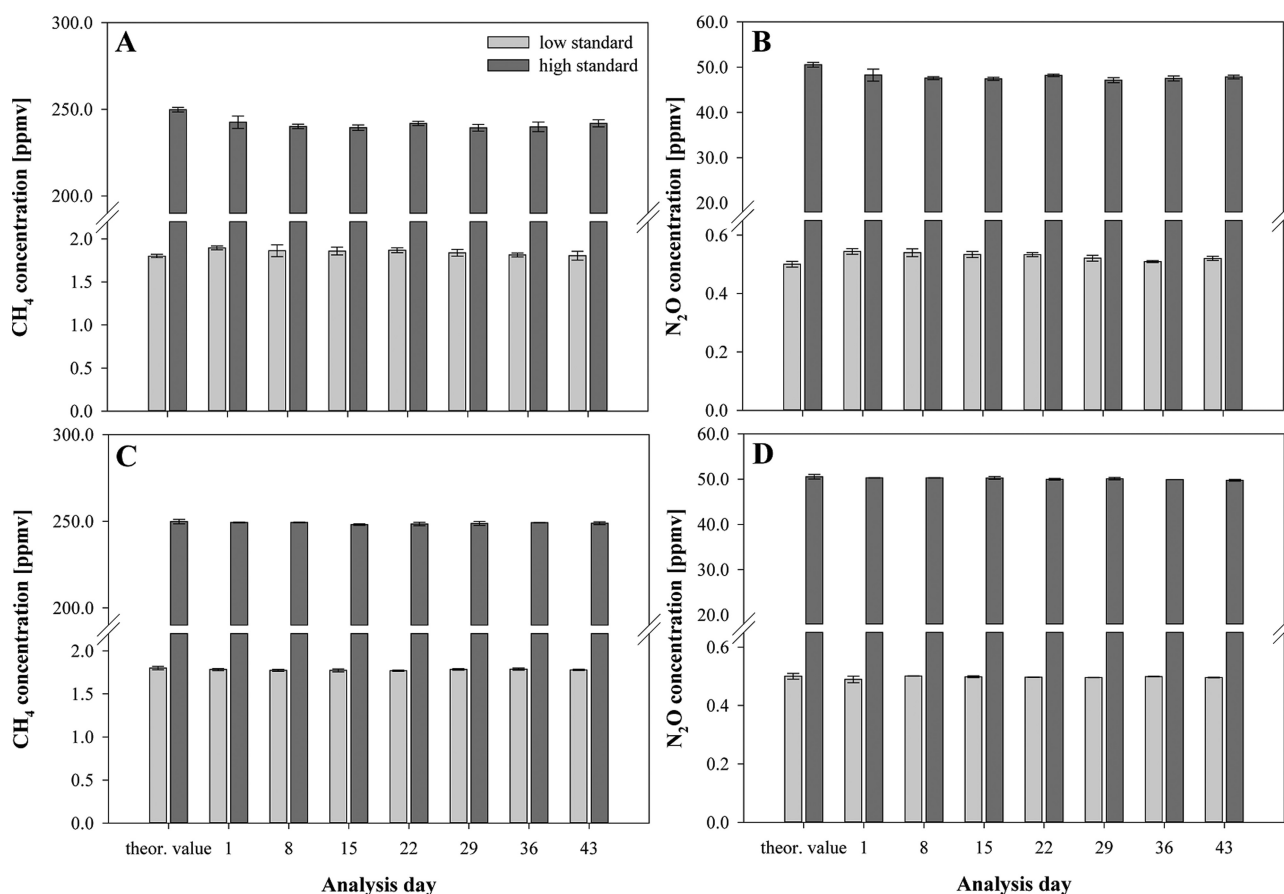
Both approaches for gaseous sample measurements achieved accurate results. Tests confirmed that for gaseous samples, the measured CH<sub>4</sub> and N<sub>2</sub>O concentrations of standard gases were statistically identical ( $p > 0.05$ ) to their known concentrations. For applications with low volumes of available sampling gas, we recommend to use Exetainers which were already filled with N<sub>2</sub> prior to injection of the small gas sample. If at least 112 mL (100 mL for flushing and

12 mL sample) of gaseous sample can be used it is advisable to flush the Exetainer first with 100 mL of the sample gas and insert then 12 mL of sample. This method avoids the dilution of the sample gas by N<sub>2</sub>. Also, the step of preflushing with N<sub>2</sub> is not necessary, saving costs for the N<sub>2</sub> gas and labor.

The experiment was also conducted to investigate if the gaseous CH<sub>4</sub> and N<sub>2</sub>O concentrations in stored samples are stable in the Exetainers over a period of 6 weeks (measurements were conducted on Day 1 (24 h after sample injection into Exetainers) and Day 8, 15, 22, 29, 36, 43 after the sample injection). The samples as previously described for testing the gaseous recovery rates of both approaches were used for the storage test. The CH<sub>4</sub> and N<sub>2</sub>O concentrations after a storage time of 1 d, 8 d, 15 d, 22 d, 29 d, 36 d, and 43 d are presented for gaseous samples in Fig. 7, together with the theoretically expected concentrations of the two used standard gases.

For both gas measurement approaches and both standard gases no significant concentration differences ( $p > 0.05$ ) were observed for CH<sub>4</sub> (Fig. 7A,C) amongst all investigated analysis days with a storage time of up to 43 d. Also, we observed no significant concentration differences ( $p > 0.05$ ) for N<sub>2</sub>O (Fig. 7B) using the high standard gas as a sample in the approach for smaller gas samples volumes amongst all analysis days up to 43 d. However, measured N<sub>2</sub>O concentrations for the same approach using the low standard gas as a sample were similar but statistically different between Day 1 and Day 36 (KW-H<sub>6,35</sub>,  $p < 0.01$ ); all other analysis days showed no significant differences ( $p > 0.05$ ) to Day 1.

Overall, our results demonstrate that liquid as well as gaseous CH<sub>4</sub> or N<sub>2</sub>O samples can be stored up to 6 weeks before analysis.



**Fig. 7.** Gaseous sample storage in Exetainers after a storage time of 1, 8, 15, 22, 29, 36, and 43 d (mean  $\pm$  standard deviation,  $n = 5$ ). CH<sub>4</sub> (A) and N<sub>2</sub>O (B) concentrations of gaseous samples (low and high gas standard) inserted in N<sub>2</sub> flushed Exetainer (approach designed for small volumes of sample gas). CH<sub>4</sub> (C) and N<sub>2</sub>O (D) concentrations of gaseous samples (low and high gas standard) after flushing with 100 mL of a low gas standard or high gas standard before inserting the gaseous sample (approach designed for large volumes of sample gas). The theoretical values give the used standard gas concentrations.

#### Comparison of two Exetainer vial approaches for liquid samples measurement

Surface water samples were taken in ten replicates with each type of the Exetainers, namely the N<sub>2</sub> flushed Exetainers and the purchased, pre-evacuated Exetainers.

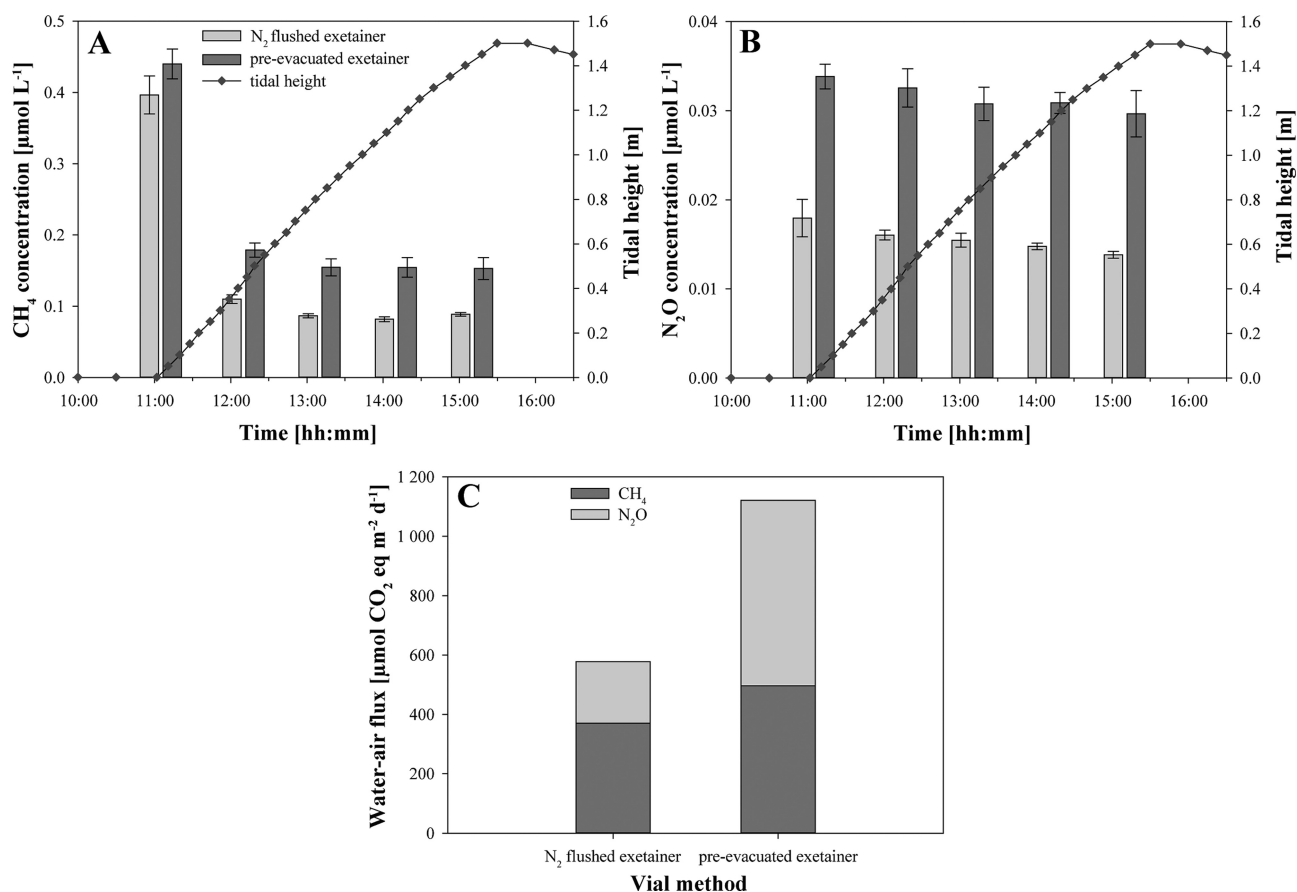
The CH<sub>4</sub> (Fig. 8A) and N<sub>2</sub>O (Fig. 8B) surface water concentration measured with both methods showed a similar trend. The highest CH<sub>4</sub> concentrations were measured at low tide and the concentrations were relatively stable for the remaining sampling points. In comparison, the N<sub>2</sub>O concentrations were relatively stable during the entire 5 h. However, the concentrations of both CH<sub>4</sub> and N<sub>2</sub>O measured with the purchased, pre-evacuated Exetainer vials were always higher, at 10–47% and 47–53%, respectively, in comparison to the N<sub>2</sub>-flushed vials.

Figure 8C shows estimated CH<sub>4</sub> and N<sub>2</sub>O emissions (expressed as CO<sub>2</sub> equivalents) using the thin boundary model, in conjunction with the surface water concentrations analysed using the two methods. The N<sub>2</sub>O emissions account

for 36% of the total emissions when expressed as CO<sub>2</sub> equivalents using the N<sub>2</sub> flushed Exetainers, in comparison with 56% when using pre-evacuated Exetainers. Due to a higher warming potential of N<sub>2</sub>O, any overestimation of N<sub>2</sub>O would result in a substantial increase in its contribution to the overall GHG emissions. Thus, the difference between two methods could provide different guidance in GHG mitigations, highlighting the importance of having accurate determination of N<sub>2</sub>O and CH<sub>4</sub> concentrations.

#### Detection limits using GC

All of the proposed approaches for CH<sub>4</sub> and N<sub>2</sub>O measurements in liquid and gaseous samples create an overpressure inside the vials. The created overpressure has the advantage to allow for a withdrawal of a larger sample volume, thus lowering the detection limit in the sample. A detection limit of 0.025 ppm and 0.006 ppm for CH<sub>4</sub> and N<sub>2</sub>O, respectively, could be achieved for samples analysed using the proposed method. These detection limits are considerably lower than



**Fig. 8.** CH<sub>4</sub> (A) and N<sub>2</sub>O (B) concentrations of liquid samples (mean  $\pm$  standard deviation,  $n = 10$ ) which were stored and analysed in N<sub>2</sub> flushed Exetainer vials and in purchased, pre-evacuated Exetainer vials. Sampling for all measurements was conducted at one site during the transition from low tide (time 09:30 h) to high tide (time 15:30 h). Water–air fluxes expressed as CO<sub>2</sub> equivalents, estimated using the thin boundary layer model for both methods (C). Values are averaged water–air fluxes ( $n = 50$ ) of all measurements.

the detection limits of the method with pre-evacuated Exetainers, where are 0.5 ppm for CH<sub>4</sub> and 0.1 ppm for N<sub>2</sub>O. Caution, though, should be taken when transport of the vials exposes the vials to large changes in pressure and temperature.

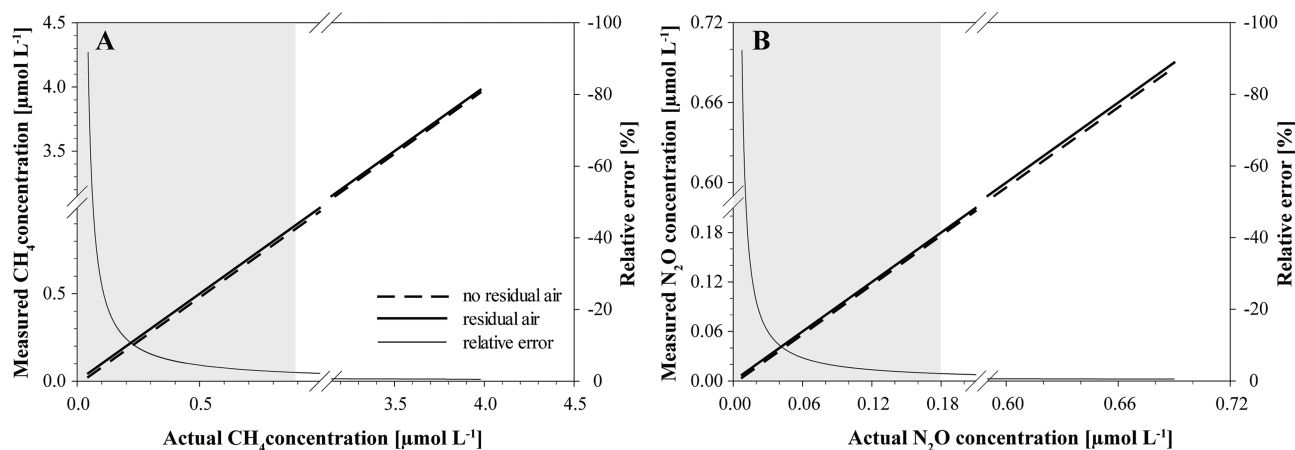
## Discussion

### Contamination of pre-evacuated Exetainers

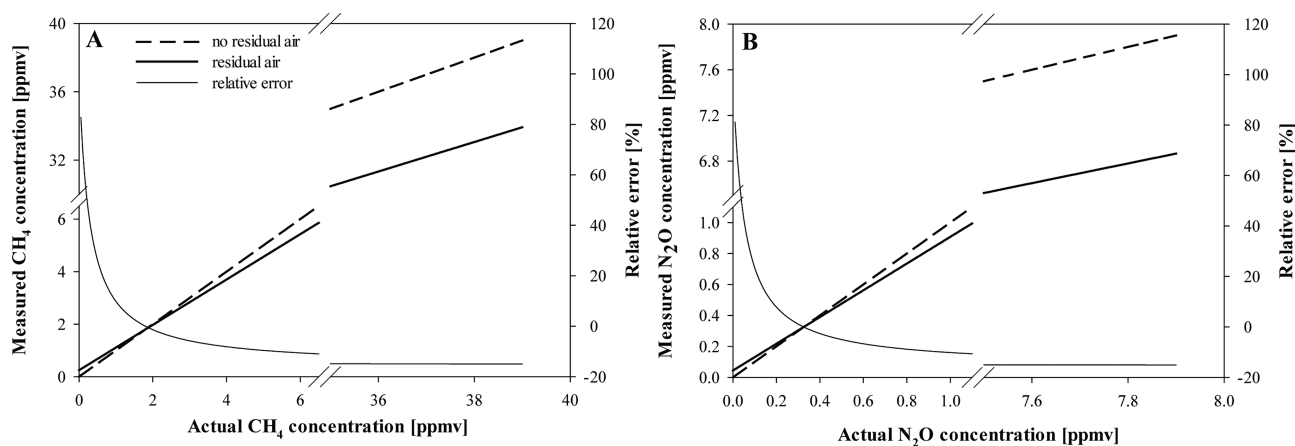
In the work presented, we found varying residual air pressure in the pre-evacuated Exetainer vials, which will lead to errors when determining CH<sub>4</sub> and N<sub>2</sub>O concentrations in liquid and gaseous samples. A theoretical assessment of the contamination effect of the residual air on CH<sub>4</sub>/N<sub>2</sub>O concentrations (calculated based on our results, assuming an average residual air pressure of 0.14 atm) in liquid samples against expected, noncontaminated CH<sub>4</sub>/N<sub>2</sub>O concentrations is shown in Fig. 9A,B. The contamination effect for liquid samples shows a constant absolute error at all CH<sub>4</sub> or N<sub>2</sub>O concentration levels and a decreasing relative error with increasing CH<sub>4</sub> or N<sub>2</sub>O concentrations. Low CH<sub>4</sub> or N<sub>2</sub>O

concentrations measurements of liquid samples are particularly sensitive to residual air. For example, measured concentrations of approximately 0.035  $\mu\text{mol L}^{-1}$  CH<sub>4</sub> or approximately 0.006  $\mu\text{mol L}^{-1}$  N<sub>2</sub>O in contaminated liquid samples would overestimate the actual concentration by approximately 60%. This is in general agreement with the observation made in the verification field study. The gray areas in Fig. 9A,B highlight the concentration ranges (CH<sub>4</sub>: 0.002  $\mu\text{mol L}^{-1}$  to 0.89  $\mu\text{mol L}^{-1}$ , N<sub>2</sub>O: 0.006  $\mu\text{mol L}^{-1}$  to 0.18  $\mu\text{mol L}^{-1}$ ) of aquatic environments such as estuaries, coastal waters, upwelling zones, or the open ocean [based on Bange et al. (1994, 1996)]. Our theoretical assessment shows that the relative error introduced by the residual air contamination in pre-evacuated Exetainers can be substantial (up to 90%). This stresses the importance of efforts to minimize any contamination effects within Exetainers when studying these systems. In comparison, the measurement of CH<sub>4</sub> and N<sub>2</sub>O in wastewater systems [CH<sub>4</sub>: 31  $\mu\text{mol L}^{-1}$  to 1563  $\mu\text{mol L}^{-1}$ , N<sub>2</sub>O: 1.14  $\mu\text{mol L}^{-1}$  to 39  $\mu\text{mol L}^{-1}$ ; based on Foley et al. (2009, 2010), Guisasaola et al. (2008), and Ren et al. (2013)] may not require pretreatment of the vials.





**Fig. 9.** Theoretical assessment of the contamination effect of the residual air on the measured  $\text{CH}_4$  (A) and  $\text{N}_2\text{O}$  (B) concentrations against the actual values in liquid samples. The gray areas highlight the concentration ranges of aquatic environments.



**Fig. 10.** Theoretical assessment of the contamination effect of the residual air on the measured  $\text{CH}_4$  (A) and  $\text{N}_2\text{O}$  (B) concentrations against the actual values in gaseous samples.

Figure 10A,B shows the theoretical assessment of the contamination effect of the residual air on  $\text{CH}_4$  or  $\text{N}_2\text{O}$  concentrations (calculated based on our results, assuming an average residual air pressure of 0.14 atm) in gaseous samples against true, noncontaminated  $\text{CH}_4$  or  $\text{N}_2\text{O}$  concentrations. At  $\text{CH}_4$  and  $\text{N}_2\text{O}$  concentrations below their respective residual air concentrations, the contamination leads to overestimates of the actual concentrations, with the relative errors increasing with decreasing concentrations. In contrast, at concentrations higher than the respective residual air concentrations, measurements would lead to underestimates of the actual concentration, with the relative errors increasing with increasing concentrations, approaching 14% (the residual air pressure used in the calculation) when the  $\text{CH}_4$  and  $\text{N}_2\text{O}$  concentrations are far higher than their concentrations in air. As an example, a measured concentration of, e.g., approximately 0.6 ppm  $\text{CH}_4$  or approximately 0.1 ppm  $\text{N}_2\text{O}$  in residual air contaminated gaseous samples would overesti-

mate the actual gaseous concentration by approximately 32%, whereas, e.g., approximately 4 ppm  $\text{CH}_4$  or approximately 0.8 ppm  $\text{N}_2\text{O}$  measured in contaminated gaseous samples would lead to an underestimation of the actual gaseous concentration by approximately 9%.

It has to be noted that the actual effect of the contamination on measured and published  $\text{CH}_4/\text{N}_2\text{O}$  gas concentrations may deviate from the data presented in these theoretical calculations and in the examples, as the actual error would depend on the type of calibration used for the GC. If Exetainers with a residual air pressure were used for the calibration standards the calibration results may partly compensate for the contamination effect.

#### Proposed method for $\text{CH}_4$ and $\text{N}_2\text{O}$ measurements

The growing interest in monitoring and quantifying  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions and getting insight into the production and consumption pathways of these gases in natural and

engineered aquatic systems highlights the need to accurately measure CH<sub>4</sub> and N<sub>2</sub>O concentrations in liquid and/or gaseous samples. We found that the commonly used pre-evacuated Exetainers as storage and measurement vials contaminate samples by varying residual air pressure. Our study, thus, also aimed to propose a method that minimizes any contamination effects within the vials and enables more accurate measurements of CH<sub>4</sub> and N<sub>2</sub>O in both liquid and gaseous samples.

The proposed method meets our aim by reducing the background CH<sub>4</sub> and N<sub>2</sub>O concentrations to  $0.092 \pm 0.008$  ppm and  $0.016 \pm 0.001$  ppm, respectively, approximately 3–4% of their respective concentrations in air. This is achieved by a pretreatment method, which involves flushing vials with N<sub>2</sub> gas (for small available volumes of liquid samples or gaseous samples) or the sample gas itself instead of N<sub>2</sub> (for large available volumes of gaseous samples). Tests confirmed the suitability of the method for liquid as well as gaseous samples. Flushing vials with N<sub>2</sub> gas entails costs for the N<sub>2</sub> gas and labor. The costs for the N<sub>2</sub> gas are rather low and the flushing of vials for high sample numbers can be quickly conducted using the manifold. The manifold used for flushing is easy to obtain and to set up. In our alternatively method for large available gaseous samples volumes, N<sub>2</sub> gas is not used and costs for the gas is negligible.

### Comments and recommendations

The presence of residual air and the variability of the residual air volume in pre-evacuated Exetainer vials present a problem for the accurate determination of CH<sub>4</sub> and N<sub>2</sub>O concentrations in both liquid and gaseous samples, particularly for samples containing relatively low levels of these gases. We propose a method in this work, in which the Exetainers are flushed and filled with N<sub>2</sub> gas. Thus, a large portion of residual air is removed and the contamination by background air for liquid or gaseous samples is reduced. Additionally, a second proposed method for gaseous samples that uses the sample gas itself to flush the Exetainer first has a further benefit of avoiding dilution of samples.

The injection of liquid and gaseous samples into Exetainers previously filled with N<sub>2</sub> or sample gas creates overpressure in the vials. This overpressure has the advantage that a higher volume of gas can be withdrawn from the sample headspace, which allows the lowering of the detection limit when the analysis is conducted by gas chromatograph.

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