A novel injection technique: using a field-based quantum cascade laser for the analysis of gas samples derived from static chambers

Anne R. Wecking[1*], Vanessa M. Cave[2], Lìyǐn L. Liáng[3], Aaron M. Wall[1], Jiafa Luo[2], David I. Campbell[1], Louis A. Schipper[1*]

[1] School of Science and Environmental Research Institute, The University of Waikato, Private Bag 3105, Hamilton 3240, Aotearoa New Zealand
[2] AgResearch Ruakura, Private Bag 3123, Hamilton 3240, Aotearoa New Zealand
[3] Manaaki Whenua – Landcare Research, Palmerston North 4442, Aotearoa New Zealand

**Correspondence to*: Anne R. Wecking (arw35@students.waikato.ac.nz), Louis A. Schipper (louis.schipper@waikato.ac.nz)

Abstract. The development of fast-response analysers for the measurement of nitrous oxide (N_2O) has resulted in exciting opportunities for new experimental techniques beyond commonly used static chambers and gas chromatography (GC) analysis. For example, quantum cascade laser absorption spectrometers (QCL) are now being used with eddy covariance (EC) or automated chambers. However, using a field-based QCL EC system to also quantify N_2O concentrations in gas samples taken from static chambers has not yet been explored. Gas samples from static chambers are commonly analysed by GC that often requires labour and time consuming procedures off-site. Here, we developed a novel, field-based injection technique that allowed the use of a single QCL for: 1) micrometeorological EC; and 2) immediate manual injection of headspace samples taken from static chambers. To test this approach across a range of low to high N_2O fluxes, we applied ammonium nitrate (AN) at 0, 300, 600 and 900 kg N ha^{-1} (AN_0, AN_{300}, AN_{600}, AN_{900}) to plots on a pasture soil. After analysis, calculated N_2O fluxes from QCL (F_{N2O_QCL}) were compared with fluxes determined by a standard method, i.e. here laboratory-based GC (F_{N2O_GC}). Subsequent comparison of QCL and GC derived data was tested using orthogonal regression, Bland Altman and bioequivalence statistics. For the AN treated plots, the mean cumulative N_2O emissions across the seven day campaign were 0.97 (AN_{300}), 1.26 (AN_{600}) and 2.00 (AN_{900}) kg N_2O-N ha^{-1} for F_{N2O_QCL} and 0.99 (AN_{300}), 1.31 (AN_{600}) and 2.03 (AN_{900}) kg N_2O-N ha^{-1} for F_{N2O_GC}. These F_{N2O_QCL} and F_{N2O_GC} were highly correlated ($r = 0.996$, $n = 81$) based on orthogonal regression, in agreement following the Bland Altman approach (i.e. within ± 1.96 standard deviations of the mean difference) and shown to be for all intents and purposes the same (i.e. equivalent). The F_{N2O_QCL} and F_{N2O_GC} derived under near-zero flux conditions (AN_0) were weakly correlated ($r = 0.306$, $n = 27$) and not found to agree or to be equivalent. This was likely caused by the calculation of small but apparent positive and negative F_{N2O} when in fact the actual flux was zero, i.e. below the detection limit of static chambers. Our study demonstrated 1) that the capability of using one QCL to measure N_2O at different scales, including manual injections, offers a great potential to advance field measurements of N_2O (and other greenhouse gases) in future; and 2) that suitable statistics have to be adopted when formally assessing the agreement and difference (not only the correlation) between two methods of measurement.

1 Introduction

Accurate measurements of nitrous oxide (N_2O) emissions from agricultural land are crucial to quantify the contribution of the gas's radiative forcing to climate warming (Thompson et al., 2019). Nitrous oxide is a long-lived greenhouse gas with a global warming potential 265-times higher than that of carbon dioxide (CO_2) over 100 years, and is the largest contributor to the depletion of stratospheric ozone (IPCC, 2013; Ravishankara et al., 2009). Agricultural activities on intensively managed soils that receive high inputs of reactive nitrogen (N_r), mostly in the form of animal excreta and nitrogen fertiliser, are the main source of anthropogenic N_2O emissions (Reay et al., 2012). Reactive nitrogen facilitates microbial nitrification and denitrification in the soil with N_2O being an intermediate of these processes (Butterbach-Bahl et al., 2013; Firestone and Davidson, 1989). The production of N_2O in soils is controlled by a multitude of environmental and anthropogenic factors, e.g. soil moisture, nitrogen input and overall farm management, which often result in highly variable N_2O emissions (Erisman et al., 2013; Flechard et al., 2007; Rees et al., 2013). Adequate and precise flux measurements have, therefore, remained challenging (Cowan et al., 2020; Rapson and Dacres, 2014).

To date, the common method for measuring fluxes of N_2O (F_{N2O}) are closed, non-steady-state 'static chambers' (Hutchinson and Mosier, 1981; Lundegard, 1927); a method used for more than 95 % of all field studies (Lammirato et al., 2018; Rochette and Eriksen-Hamel, 2008; Rochette, 2011). Static chambers are relatively cost-efficient and easy to deploy in the field (de Klein et al., 2015; Velthof et al., 1996). Gas samples are extracted from the chamber headspace during an up to 60-minute enclosure and injected into pre-evacuated glass vials (Luo et al., 2007; Rochette and Bertrand, 2003; van der Weerden et al., 2011). Subsequent analysis of the gas samples is commonly conducted off-site, using gas chromatography (GC) (Luo et al., 2008a; Parkin and Venterea, 2010). However, measurements using static chambers are discontinuous and labour-intensive with uncertainties in F_{N2O} caused by alterations made to the soil environment after installation, pressure differences in the chamber headspace during sampling and the assumption of a linear increase/decrease in gas concentration with time (Chadwick et al., 2014; Christiansen et al., 2011; Denmead, 2008). Through time, different guidelines have been proposed to advance the standardisation of static chamber techniques (de Klein et al., 2015; Pavelka et al., 2018; Rochette, 2011) but essentially the basic method has remained unchanged for decades (Chadwick et al., 2014; Hutchinson and Mosier, 1981).

Alternative approaches to the static chamber method include the use of (semi-) automated chambers and micrometeorological techniques that allow F_{N2O} measurements at higher temporal frequency and resolution (Baldocchi, 2014; Pavelka et al., 2018; Rapson and Dacres, 2014). Recent developments in the technology of fast-response analysers have enabled e.g. tunable diode laser absorption spectrometers, Fourier transform infrared spectrometers and, in particular, continuous-wave quantum cascade laser absorption spectrometers (QCL) to be coupled to automated chambers (Brümmer et al., 2017; Cowan et al., 2014; Savage et al., 2014) or eddy covariance (EC) systems (Nemitz et al., 2018; Nicolini et al., 2013). Despite these recent advances in analyser technology, our understanding of the micro- and macro-scale processes that lead to the emission of N_2O has remained limited. While chamber measurements help to examine the interaction between soil processes and F_{N2O} at point scales (Luo et al., 2017), EC promotes the understanding of diurnal, seasonal and annual F_{N2O} dynamics at field to ecosystem scale (Cowan

et al., 2020; Liáng et al., 2018). Some studies have aligned chamber and EC measurements to determine the full range of processes that drive F_{N2O} dynamics across these different scales but still relied on the use of more than one analyser for measuring F_{N2O} (Jones et al., 2011; Tallec et al., 2019; Wecking et al., 2020a).

In this study, we tested whether a single field-deployed QCL could be used for manual injections of gas samples taken from static chambers to allow nearly concurrent measurements of chamber N_2O samples alongside continuous EC. Field measurements using a QCL for both these purposes have, to our knowledge, not yet been conducted. Our objective was to examine whether chamber F_{N2O} determined by field-based QCL (F_{N2O_QCL}) were equivalent to F_{N2O} determined by laboratory GC (F_{N2O_GC}). An important component of this comparison was to demonstrate that manual injections into the QCL offer a robust method for the use in field environments. Our analysis, therefore, reached beyond the sole comparison of two analytic devices (QCL and GC) but as well discussed the method's real-world application. An evidence of concept, i.e. assessing if the injection method would result in for practical purposes equivalent F_{N2O_GC} and F_{N2O_QCL}, was provided by statistical tests: 1) orthogonal regression; 2) Bland Altman and 3) bioequivalence analyses.

2 Methods

2.1 Study site

This study was conducted at Troughton Farm, a commercially operating 199 ha dairy farm in the Waikato region, 3 km east of Waharoa (37.78°S, 175.80°E, 54 m a.s.l.), North Island, New Zealand. The farm had been under long-term grazing for at least 80 years with micrometeorological measurements using a QCL EC system made since November 2016 (Liáng et al., 2018; Wecking et al., 2020a). Mean annual temperature and precipitation, recorded at a climate station 13 km to the south-west of the farm (1981–2010), were 13.3 °C and 1249 mm, respectively (NIWA, 2018). The experimental site comprised three paddocks (P51, P53, P54) in the north of the farm with each sized about 2.8 ha. Soils were formed in rhyolitic and andesitic volcanic ash and rhyolitic alluvium. The dominant soil type based on the New Zealand soil taxonomy was a Mottled Orthic Allophanic soil (Te Puninga silt loam) (Hewitt, 2010). Plots used for the static chamber measurement of this study were located on P53 around 50 m to the south-west of the EC system. The physical distance between chamber plots and EC tower ensured that the EC footprint did not experience cross-contamination from chamber N_2O fluxes (Wall et al., 2020).

2.2 Experiment design

One intensive field campaign was conducted between 10 and 16 September 2019. The campaign's primary purposes were to 1) manually collect gas samples from static chambers comprising potentially low to high N_2O concentrations (C_{N2O}); 2) analyse these samples on-site using QCL and off-site using GC; 3) to quantify and compare resulting C_{N2O} and F_{N2O}. A thorough description of the QCL operating in EC mode has been provided by Liáng et al. (2018) and Wecking et al. (2020a).

2.2.1 Static chamber measurements

The static chamber trial comprised a randomised block design of circular treatment and control plots each of which included three replicates per treatment/control. Ammonium nitrate (AN) fertiliser was used as a treatment and applied at different rates to ensure production of a wide range of low to high C_{N2O} in the chamber headspace for subsequent flux measurements. The three application rates were 300 (AN_{300}), 600 (AN_{600}) and 900 kg N ha^{-1} (AN_{900}), while the control plots (AN_0) did not receive any AN. The rates of AN applied were to match nitrogen loading commonly found in cattle excreta patches, which are the main sources of N_2O in grazed pastures (Selbie et al., 2015). Separate areas adjacent to the twelve chamber plots were established to collect soil samples for laboratory analyses of soil moisture and soil mineral nitrogen (N_{min}). Soil moisture and water-filled pore space (WFPS) were analysed and calculated using the methods described in Wecking et al. (2020a). Soil N_{min} was derived from field-moist soil samples extracted in 2M KCl (Mulvaney, 1996) and measured colorimetrically using a Skalar SAN++ flow analyser (Skalar Analytical B. V., Breda, Netherlands). Both, NH_4^+ and NO_3^-, were expressed in units kg ha^{-1} using a site-specific soil dry bulk density of 0.73 g cm^{-3} (Wecking et al., 2020a).

Flux measurements were made on the day of treatment application and throughout the following six days with chamber gas samples collected on nine occasions (Table S1). The sampling followed a standardised chamber technique (de Klein et al., 2003; de Klein et al., 2015; Luo et al., 2008b) and was carried out daily at 10 AM (NZDT) (van der Weerden et al., 2013). Additional sampling was conducted at noon on 12 and 15 September. Before sampling, PVC lids were fitted to water-filled base channels that provided a gas-tight seal over the 10 L headspace of the chambers. Gas samples were taken from this headspace during a 45 min enclosure period at four times – t_0, t_{15}, t_{30} and t_{45} – per chamber (Pavelka et al., 2018). A sampling port served to extract air from the chamber headspace by using a 60 mL plastic syringe (Terumo Corp., Tokyo, Japan). After flushing the syringe three times with air from the chamber headspace, the following procedure was applied to ensure that GC and QCL analysis received identical headspace samples: 1) after flushing, 60 mL of sample air was extracted from the chamber headspace; 2) 10 mL of the sample was discarded to flush the syringe needle; 3) 15 mL was transferred into a pre-evacuated, septum-sealed, screw-capped 5.6 mL glass vial (Exetainer, Labco Ltd., High Wycombe, UK); 4) the syringe needle was flushed again by discarding a further 10 mL; and 5) a second pre-evacuated glass vial was over-pressurised with 15 mL, and the remainder discarded. The procedure was repeated for each sample resulting in a total of 2 × 432 samples, i.e. two replicated sample batches for subsequent GC (1 × 432 samples) and QCL (1 × 432 samples) analyses. All samples remained in the septum-sealed Exetainers until analysis.

2.2.2 Laboratory gas chromatography

Gas chromatography was conducted on the first sample batch at the New Zealand National Centre for Nitrous Oxide Measurements (NZ-NCNM) at Lincoln University, New Zealand. Automated analysis (GX-271 Liquid Handler, Gilson Inc., Middleton, WI) was performed using a SRI 8610 GC (SRI Instruments, Torrance, CA, USA) and a Shimadzu GC-17a (Shimadzu Corp., Kyoto, Japan) equipped with a ^{63}Ni-electron capture detector. The analysis followed standard procedures

described in detail by de Klein et al. (2015). Oxygen-free, ultra high purity nitrogen (N_2) was used as the carrier gas (mobile phase) at a flow rate of 0.4 L min^{-1}. The measurement frequency was set to 1 Hz. Sample Exetainers experienced a storage time of up to two weeks prior to their analysis which was due to transportation from the field site to the laboratory. The run time during GC analysis was about eight minutes per sample.

2.2.3 Field quantum cascade laser absorption spectrometry

The second batch of N_2O samples was analysed collectively on the day after the last chamber sampling, 17 September, by manual injection into a continuous-wave quantum cascade laser absorption spectrometer (QCL, Aerodyne Research Inc., Billerica, MA, USA). Briefly, QCL uses infrared (IR) light energy which is passed through a 0.5 L multiple pass absorption cell with a pathlength of 76 m. Inside the cell, N_2O absorbs IR light energy which then is quantified as equivalent to the compositional N_2O concentration of the gas sample measured (Nelson et al., 2004).

For the purpose of our analysis, we switched the QCL from its continuous measurement (EC) mode to an 'injection mode'. The injection mode conversion took less than 30 minutes: a stainless steel three-way valve (Swagelok, Solon, OH, USA) mounted to the air inlet of the QCL allowed re-direction of the air flow from the primary inlet tube of the EC system into a second, 1 m long Bev-A-line tube (4 mm internal diameter). At its end, the tube was connected to a pressure regulator and a bottle of oxygen free, industrial grade N_2 carrier gas (BOC Ltd., NZ). Two stainless steel, T-junction connectors (Swagelok, Solon, OH, USA) were fitted to the sample tube allowing overflow of excess carrier gas through a 0.45 µm PTFE membrane filter (ThermoFisher, Scientific, NZ) and sample injection through a septum-sealed port (Fig. 1). A dry scroll vacuum pump (XDS35i, Edwards, West Sussex, UK) was used for both EC measurements and manual injections to continuously draw either air or carrier gas through the QCL sample cell.

[FIGURE 1 ABOUT HERE]

Once the injection line had been established, the flow rate was reduced from an initial 15 L min^{-1} used for EC to 1 L min^{-1} for manual injections, based on Lebegue et al. (2016), Savage et al. (2014) and Brümmer et al. (2017). The reduction in flow was monitored using a RMA-SSV flow meter (Dwyer Instruments, PTY. Ltd., Michigan City, IN, USA) while setting the inlet control valve of the QCL to 2 V (using the TDLWintel software command) before manually adjusting inlet and outlet control valves of the QCL device further until the desired flow rate was achieved. Prior to sample injection, a minimum lag time of ten minutes was applied to let temperature and pressure of the QCL and its temperature-controlled enclosure box return to steady-state, i.e. 35 ± 0.5 Torr, 33.5 °C laser temperature and QCL enclosure box temperature of 30 ± 0.1 °C.

Standards of certified N_2O concentration (range 0.2 to 100 ppm) were injected before, during and after each sample run and complemented QCL analysis (Table S2). Ten out of the twelve N_2O standards were provided by the NZ-NCNM (except 0.321 and 0.401 ppm) and, therefore, were identical to those used for GC (Sect. 2.2.2). The QCL measurements were made at 10 Hz frequency with 1 mL of sample air extracted from each sample Exetainer and manually injected into the flow of N_2 carrier gas by using a 1 mL glass syringe (SGE International PTY Ltd., VIC, Australia). The glass syringe was flushed with N_2 gas after each injection to avoid cross-contamination of samples and N_2O standards. The selection of syringe type, flow rate and the

usage of N_2O standards were based on preliminary tests conducted in advance of the actual field campaign. Finally, it was important to keep a record of the injected sample sequence to allow for later re-identification of samples in the raw output data of the QCL.

2.3 Data processing

GC and QCL analyses resulted in the output of peak area data from the injected N_2O standards and chamber derived N_2O samples (Fig. S1). Data processing, therefore, first had to determine the relationship between peak area and (known) N_2O concentration (C_{N2O}) of the injected standards. To compute the final but initially unknown C_{N2O} of chamber N_2O samples, peak area data from N_2O standards were fitted to linear and quadratic (second-order-polynomial) models (de Klein et al., 2015; van der Laan et al., 2009). Whereas de Klein et al. (2015) recommended the use of quadratic curves models as the standard curve for C_{N2O} standards measured by GC analysis, we found that both linear and quadratic models adequately fitted C_{N2O} standards derived from QCL. Using a linear fit ultimately resulted in on average 3 % smaller F_{N2O_QCL} (range -0.5 % to -4.3 %) than using a quadratic model. Nonetheless, since the quadratic fit suited lower C_{N2O} better than a linear fit, quadratic models were used to build the standard curves from injected standards of known C_{N2O} (Fig. S2). The actual quadratic model used to calculate final C_{N2O} of the gas samples was based on a selection of standards fitted to the expected minimum and maximum range of real sample C_{N2O}; which in our study was between 0.3–10 ppm. Output data from GC were processed in PeakSimple software (SRI Instruments, Torrance, CA, USA) and Excel (Microsoft Corp. Redmond, WA, USA). MATLAB R2017a scripting (MathWorks Inc., Natick, MA, USA) was used for data derived from the QCL.

2.4 Flux calculation

The N_2O flux in mg N_2O-N m^{-2} hr^{-1} was calculated for both data streams, GC (F_{N2O_GC}, n = 108) and QCL (F_{N2O_QCL}, n = 108), by applying a linear regression function to the increase in chamber headspace C_{N2O} between time t_0 and t_{45} following Eq. (1) (van der Weerden et al., 2011):

$$F_{N2O\_GC} \text{ and } F_{N2O\_QCL} = \frac{\Delta N_2O}{\Delta T} \times \frac{M}{Vm} \times \frac{V}{A} \quad (1)$$

where ΔN_2O is the increase in headspace C_{N2O} (µL N_2O L^{-1} (ppmv)) over time; ΔT is the enclosure period (in hours); M is the molar weight of nitrogen in N_2O (44 g mol^{-1}); Vm is the molar volume of gas (L mol^{-1}) at the mean air temperature recorded at each sampling occasion; V is the chamber headspace volume (m^3); and A is the area covered by the chamber base, here 0.0415 m^2. All F_{N2O} were converted to units of nmol N_2O m^{-2} s^{-1}. The integration of F_{N2O_GC} (n = 84) and F_{N2O_QCL} (n = 84) determined at 10 AM sampling was used to quantify the proportion of applied nitrogen emitted as N_2O (E_{N2O}) across the seven day trial in units kg N_2O-N ha^{-1} based on Luo et al. (2007) and Wecking et al. (2020a).

2.5 Statistical analyses

The statistical analysis for C_{N2O} data (C_{N2O_GC} and C_{N2O_QCL}, each n = 432) and resulting F_{N2O} (F_{N2O_GC} and F_{N2O_QCL}, each n = 108) was conducted in Genstat® (Version 19, VSN International, Hemel Hempstead, UK). After testing for normality using a Shapiro-Wilk test and homogeneity of variance by examining residual and fitted values, we applied three different statistical approaches to compare GC with QCL data: 1) orthogonal regression, 2) Bland Altman and 3) bioequivalence statistics.

The orthogonal regression analysis used standardised C_{N2O} and F_{N2O} data following Eq. (2):

$$standardised\ C_{N2O}\ and\ F_{N2O} = \frac{(x - mean)}{standard\ deviation} \quad (2)$$

The core of this orthogonal regression was a principal component analysis which, in contrast to ordinary least square regression, allowed for measurements errors in both the response and the predictor variable by minimising the squared residuals in vertical and horizontal direction. While orthogonal regression returned a Pearson correlation coefficient r that provided information about the strength of the linear relationship between GC and QCL data, we found that r did not include any predication about the level of agreement between the two methods (Bland and Altman, 1986; Giavarina, 2015). The degree to which GC and QCL data would agree was, for that reason, determined by using Bland Altman statistics that quantified the bias (i.e. the mean difference) and the limits of agreement between the two methods. The limits of agreement were calculated from the mean and the standard deviation (SD) of the difference between GC and QCL data. We defined that 95 % of all data points had to be within ± 1.96 SD of the mean difference (Giavarina, 2015). The Bland Altman analysis was conducted for individual F_{N2O} as well as for mean F_{N2O} across replicates of the same treatment.

Still, testing for correlation and agreement did not determine whether GC and QCL data would effectively and for practical purposes be the same (termed 'equivalent'). We, therefore, used bioequivalence statistics to assess the biological and analytical relevance of the difference between the two methods. The first part of this analysis comprised an one-way analysis of variance (ANOVA) for F_{N2O} which was subset by treatment (AN_0, AN_{300}, AN_{600}, AN_{900}) and analytical device (GC, QCL). Results from this ANOVA determined the 90 % confidence intervals (CI) of the mean difference between F_{N2O_QCL} and F_{N2O_GC}. In bioequivalence statistics, the 90 % CI (at a standard power level of 80 %) is generally preferred instead of using a 95 % CI that often serves to establish a statistical difference between two methods or treatments rather than proving no difference. An important component of the analysis was to also define the equivalence range, i.e. the maximum acceptable difference, between the new (QCL) and the standard method (GC). Bioequivalence statistics acknowledge that two methods will never be exactly the same. Defining an acceptable equivalence range is, thus, an important precondition and might in some cases be even provided by a regulatory authority. While commonly used in pharmaceutical research (Bland and Altman, 1986; Giavarina, 2015; Patterson and Jones, 2006; Rani and Pargal, 2004), the concept of bioequivalence has not broadly been applied in environmental sciences. Therefore, an acceptable equivalence range for N_2O data based on the use of different analysers and methods has yet to be defined. We determined that the maximum acceptable difference of F_{N2O_QCL} in our study had to be as small as possible and within ± 5 % of the mean difference of the standard method (F_{N2O_GC}). The null hypothesis (F_{N2O_QCL} is

different from F_{N2O_GC}) was rejected when the 90 % CI of the difference (F_{N2O_QCL}-F_{N2O_GC}) was entirely within the predefined equivalence range at a significance level of 5 %. Following the same principles, we conducted a bioequivalence analysis for C_{N2O_QCL} and C_{N2O_GC}.

3 Results and discussion

3.1 Environmental conditions and soil variables

Daily mean air temperatures during the seven-day chamber campaign ranged from 8.3 to 12.8 °C. The WFPS of the soil within the chambers and associated plots did not fall below 73.9 % with a mean of 79.5 %. Cumulative rainfall in September 2019 was 119 mm compared to only 2 mm occurring during the seven days of the campaign. As expected, soil NH_4^+ and NO_3^- levels increased with increasing application of AN fertiliser. The highest values of N_{min} measured at AN_{900} plots were 265 kg NH_4^+ ha^{-1} and 268 kg NO_3^- ha^{-1}. The mean background levels of soil NH_4^+ and NO_3^- were around 2 kg ha^{-1}. At the end of the campaign, soil NH_4^+ levels for all treatments had decreased by less than half while the amount of soil NO_3^- remained similar to the initial level measured on the day of treatment application (Table S3).

3.2 Comparing GC and QCL derived data

3.2.1 Magnitude and general variability

Measurements resulted in a wide range of F_{N2O} but followed the same temporal and treatment-dependent patterns for both F_{N2O_GC} and F_{N2O_QCL}. The magnitude of individual fluxes was between -0.10 and 22.24 nmol N_2O m^{-2} s^{-1} for F_{N2O_GC} and -0.07 and 22.81 nmol N_2O m^{-2} s^{-1} for F_{N2O_QCL}. The mean F_{N2O} (n = 27) from chamber plots that received the highest application rate of AN fertiliser (AN_{900}) was 13.22 nmol N_2O m^{-2} s^{-1} ± 1.47 (± standard error of the mean, SEM) for F_{N2O_GC} and 13.27 nmol N_2O m^{-2} s^{-1} ± 1.43 for F_{N2O_QCL}. Similarly, the AN_{600} treatment had a mean F_{N2O} of 8.51 nmol N_2O m^{-2} s^{-1} ± 0.98 (F_{N2O_GC}) and 8.33 nmol N_2O m^{-2} s^{-1} ± 0.9 (F_{N2O_QCL}). The mean F_{N2O} for AN_{300} was 6.61 nmol N_2O m^{-2} s^{-1} ± 0.78 (F_{N2O_GC}) and 6.48 nmol N_2O m^{-2} s^{-1} ± 0.69 (F_{N2O_QCL}). At control plots, F_{N2O} were close to zero (Fig 2; Table S3). We found that treatment F_{N2O} increased from a near zero background flux to ≥ 8.5 nmol N_2O m^{-2} s^{-1} on the second day of the campaign. From then, AN_{300} fluxes gradually decreased with time whereas F_{N2O} for AN_{600} and AN_{900} remained relatively elevated until the last day of the trial (Fig. 2). These temporal trends align with Cowan et al. (2020) who observed N_2O emissions to peak within seven days after urea and AN fertiliser application; and found that F_{N2O} returned to background levels after two or three weeks. Similarly, short-term responses of F_{N2O} to AN application were also determined by others, e.g. Bouwman et al. (2002); Jones et al. (2007) and Cardenas et al. (2019). However, for our study AN treatment effects on F_{N2O} were of secondary interest. Different rates of AN fertiliser were only applied to result in a wide range of F_{N2O} (low to high) and thereby to allow for a methodological comparison of GC and QCL data.

[FIGURE 2 ABOUT HERE]

3.2.2 AN treatment flux and concentration data

The correlation between calculated F_{N2O_GC} and F_{N2O_QCL} and between C_{N2O_GC} and C_{N2O_QCL} across all treatments was high with an r value of 0.996 resulting from orthogonal regression (Fig. 3a, 3b). For both cases, major axis, ordinary and inverse least squares were nearly identical to a 1:1 line. All three regression models could therefore be used similarly well to predict the strength of the linear relationship between F_{N2O_GC} and F_{N2O_QCL} and C_{N2O_GC} and C_{N2O_QCL}, respectively (Table S4). The results of the orthogonal regression analysis suggested that QCL delivered equivalent data to the GC method. The Bland Altman statistic quantified a percentage difference between the two methods for F_{N2O} (i.e. F_{N2O_GC} and F_{N2O_QCL} treatment means) of not smaller than -11.2 % and not greater than +9.2 % (Table S5). The percentage difference between individual F_{N2O_GC} and F_{N2O_QCL} (not treatment means) was slightly greater but in only less than 3 % of all cases exceeded +10 % and -15 %, which was likely due to the higher variability of F_{N2O} between individual replicates of the same treatment. For both cases, ≥ 95 % of all data points were well within the pre-defined limits of agreement ± 1.96 SD (Fig. 4b). The overall mean difference (bias) between F_{N2O_GC} and F_{N2O_QCL} was 0.1 nmol N_2O m^{-2} s^{-1} (Fig. 4b). However, this small bias might be practically irrelevant when compared with the overall detection limit of static chambers and other general uncertainties. Neftel et al. (2007), for instance, quantified a chamber detection limit of 0.23 nmol N_2O m^{-2} s^{-1} whereas Parkin et al. (2012) reported 0.03 nmol N_2O m^{-2} s^{-1}. At the annual scale, Flechard et al. (2007) and others (e.g. Jones et al., 2011; Rochette and Eriksen-Hamel, 2008) showed that the uncertainty of integrated fluxes can be as high as 50 % when using the static chamber method.

[FIGURE 3 and 4 ABOUT HERE]

3.2.3 Control flux and concentration data

In contrast to the strong comparability of GC and QCL data at AN treatment sites, F_{N2O_GC} and F_{N2O_QCL} measured at control plots (AN_0) were only poorly correlated (r = 0.3064) (Fig. 3c). The model-fit of major axis, ordinary and inverse least squares indicated that the regression of F_{N2O_GC} on F_{N2O_QCL} (and vice versa) was not identical, i.e. differed in the minimisation of squared residuals in vertical and horizontal direction. Likewise, this also applied to C_{N2O_GC} and C_{N2O_QCL} (Fig. 3d). Mean F_{N2O} ranged from a minimum of -0.05 to a maximum of only 0.21 nmol N_2O m^{-2} s^{-1} (Table S3). Consequently, Bland Altman statistics determined only small quantitative differences between F_{N2O_GC} and F_{N2O_QCL}. When computing the percentage difference between these F_{N2O_GC} and F_{N2O_QCL}, we found near-zero F_{N2O} from AN_0 plots were less consistent in relative terms than treatment F_{N2O} (Fig. 4, Table S5). However, these inconsistencies were generally small and did not appear of great biological interest.

More generally, QCL analysis resulted in slightly higher C_{N2O} than GC, which explains why the calculated F_{N2O_QCL} at AN_0 plots were higher than F_{N2O_GC} (Table S5). However, whether this finding was related to the potentially higher sensitivity of the QCL device or due to other possible variations in sampling procedures was not resolved. Instead, we found that the disagreement between the GC and QCL method was likely related to ambient N_2O concentrations in the chamber headspace that remained between 300-400 ppb and showed a non-linear response with time, regardless of which analytic device was

used. This might have resulted in the calculation of very small but apparent positive and negative F_{N2O}, when in fact the actual flux was zero (*Type I error* as defined by Parkin et al. (2012)). The integration of C_{N2O} with time to calculate F_{N2O}, therefore, likely included this error; rather than being caused by uncertainties associated with measurement procedures or analytic device (Kroon et al., 2008). Hence, the deviation of F_{N2O} determined at control sites (AN_0) from treatment F_{N2O} (AN_{300}, AN_{600}, AN_{900}) has to be taken into account when evaluating the above results and mathematical principles (Sect. 3.2.2). Since static chamber measurements often include near-ambient C_{N2O} and F_{N2O} equal or near-zero, F_{N2O} from control plots were kept in the manuscript for the purpose of completeness.

3.2.4 Cumulative N_2O emissions

Cumulative N_2O emissions across the seven-day campaign were quantified slightly greater for the GC (E_{N2O_GC}) than the QCL (E_{N2O_QCL}) method. The mean difference between E_{N2O_GC} and E_{N2O_QCL} for the control (AN_0) and each treatment, AN_{300}, AN_{600} and AN_{900}, was -0.011, +0.0023, +0.050 and +0.028 kg N ha^{-1}, respectively. This was a difference of less than 4 % in total N_2O emissions during deployment (Fig. 5).

[FIGURE 5 ABOUT HERE]

3.3 Measurement performance of QCL analysis

The measurement precision of QCL, and particularly GC, have been generally well-reviewed (de Klein et al., 2015; Lebegue et al., 2016; Rapson and Dacres, 2014). The precision of common GC analysers is < 0.5 ppb (Rapson and Dacres, 2014; van der Laan et al., 2009) while the precision of QCL was found to be about 0.3 ppb for measurements made at 10 Hz and 0.05 ppb for 1 Hz; but in some cases might be even higher (~1 ppt) (Curl et al., 2010; Rapson and Dacres, 2014; Savage et al., 2014). Zellweger et al. (2019), for instance, used laboratory QCL for the calibration of N_2O reference standards to inform the internationally accepted calibration scale of the Global Atmosphere Watch Programme of the World Meteorological Organisation. Similarly, Rosenstock et al. (2013) preferred lab-based QCL to verify the accuracy and precision of different photoacoustic spectrometers.

However, the analytic precision was also found to depend on factors other than the technical performance of the analytic device. Rannik et al. (2015) indicated that the performance (and thus the precision of F_{N2O}) of an analyser to measure static chamber derived gas samples is likely more limited by the precision of the chamber system than by errors related to analysis or post-processing of the data itself. Imprecisions might be caused by several factors, e.g. chamber type and dimension, experimental set-up, deployment time and preferred sampling method, all of which would lead to differences in the flux detection limit (Sect. 3.2.2). In contrast, the sources of uncertainty in our study were most likely related to: 1) insufficient evacuation of Exetainers leading to the sporadic dilution of gas samples and N_2O standards; and 2) variation of sample volume when injected into the QCL, which might not have been equal to 1 mL in practice and, thus, could have resulted in slight variations of output peak area. In agreement with these observations, de Klein et al. (2015) found that half the measurement

uncertainty could be explained by the variability of gas sample volume in the sample Exetainers. The inclusion of a fixed volume sample loop when injecting gas samples into the QCL might help to reduce this source of error.

As the N_2O analysis using QCL was conducted in a temperature and pressure controlled environment, variations in these parameters were unlikely. The temperature dependency of N_2O analysis by QCL was described as being linear by Lebegue et al. (2016) with variations less than 0.02 ppb $°C^{-1}$. To reduce the uncertainty of output peak area, we recommend a constant baseline flow of N_2 carrier gas at constant pressure (slightly higher than ambient) and temperature for manual injections made into the QCL device. Depending on the EC QCL system, an initial lag time of 10 to 30 min before injections might be required in order to assemble the operational set-up (Section 2.2.3) and ensure sufficient stabilisation of pressure and temperature in the QCL sample cell. Given a flow rate of 1 L min^{-1}, rapid injections into the QCL become possible shortly afterwards with a delay between single injections of 1 mL sample volumes of only 5 to 8 sec. Sample concentrations at the same volume but > 20 ppm N_2O required a longer delay time between individual injections (> 20 sec) to enable sufficient flushing of the QCL sample cell and to avoid cross-contamination (Fig. S1). The identification of suitable delay times was straight forward and could be accessed easily in real-time by visually examining the peak progression in TDLWintel. When observing the peak progression, for instance, it became noticeable that the injection of blanks (N_2 carrier gas) did not result in any changes of baseline flow. However, we did not determine the extend to which spontaneous but small variations in the flow rate of N_2 carrier gas would have affected the resulting output peak areas. Further uncertainties of true output peak areas might have also been associated with processing and curve fitting procedures applied to the raw dataset in MATLAB that likely led to small underestimations.

3.4 QCL injections

3.4.1 The concept of bioequivalence

Using the Pearson correlation coefficient and the coefficient of determination for comparing two or more quantitative methods is a generally preferred approach in the field of N_2O research. Comparisons of different methods for N_2O analysis made in the literature most commonly used orthogonal (Jones et al., 2011) and linear regression (Brümmer et al., 2017; Cowan et al., 2014; Tallec et al., 2019), Students t-tests (Christiansen et al., 2015) or were based on raw data (Savage et al., 2014). However, correlation studies as such have limitations when assessing the comparability between two methods since a correlation analysis only identifies the relationship between two variables, not the difference (Giavarina, 2015). Bland Altman and bioequivalence statistics overcome this limitation by assessing the degree of agreement between methods.

An important aspect of statistical hypothesis testing is that the null hypothesis is never accepted. But failure to reject the null hypothesis is not the same as proving no difference. A bioequivalence assessment allows the statistical assessment of whether two methods (e.g. measurement devices, drug treatment) are effectively the same. Central to a bioequivalence analysis is the "equivalence range" that defines the size of the acceptable difference for which the values are similar enough to be considered equivalent. This becomes important when considering that even with the most precise analytical design and the most tightly

controlled experimental conditions, e.g. F_{N2O_GC} and F_{N2O_QCL} will never be exactly the same (Rani and Pargal, 2004). However, if the difference is sufficiently small for 'practical purposes', F_{N2O_GC} and F_{N2O_QCL} can be considered effectively the same. Here, an accepted evidence of bioequivalence for F_{N2O_QCL} was that the 90 % confidence interval of the difference F_{N2O_QCL}-F_{N2O_GC} (corresponding to a test with size 0.05) was within a ± 5 % difference of F_{N2O_GC}.

The equivalence range will vary depending on the objective of the research or guidelines provided by a regulatory authority but commonly does not exceed ± 20 % (Rani and Pargal, 2004; Ring et al., 2019; Westlake, 1988). In our study, a small equivalence range of ± 5 % was preferred to test the difference between F_{N2O_QCL} and F_{N2O_GC} since such recommendations did not exist.

Overall, our results showed that F_{N2O_GC} and F_{N2O_QCL} from AN_{300}, AN_{600} and AN_{900} plots provided evidence of bioequivalence. The 90 % confidence intervals of the difference (F_{N2O_GC}-F_{N2O_QCL}) were quantified 0.127 (AN_{300}), 0.185 (AN_{600}) and -0.043 (AN_{900}) nmol N_2O m^{-2} s^{-1} and well within the pre-defined equivalence range of ± 5 % (Fig. 6e, Table S 6). At control sites (AN_0), F_{N2O_GC} and F_{N2O_QCL} did not provide evidence for bioequivalence. However, the failure to establish equivalence for AN_0 sites was due to the overall limitation of the static chamber method to provide 'real' F_{N2O}; rather than based on failure of the statistical principle (Sect. 3.2.3). On the contrary, when tested for C_{N2O} instead of F_{N2O}, equivalence was confirmed for t_0 and t_{15} but did not apply for t_{30} and t_{45} (Fig. 6a). Again, failure to establish equivalence was likely related to limitations of the static chamber method that, in this case, was indicated by the lower boundary of the 90 % CI remaining outside the predefined equivalence ranges. Another possible reason for not accepting equivalence for GC and QCL derived data at AN_0 sites could have been the maximum acceptable difference between the two methods itself. We defined (Sect. 2.5) that this difference had to be within ± 5 % of the mean difference of the standard method (i.e. GC). However, it has to be taken into consideration that the accepted evidence of bioequivalence would have led to different results if the percentage mean difference had been set to, for instance, ± 10 %. Consequently, accepting a greater mean difference between the two methods would have resulted in determining bioequivalence for C_{N2O_GC} and C_{N2O_QCL} even at ambient concentration. More generally, we found that positive values of the 90 % CI of the difference indicated that the difference between the two methods (GC-QCL) resulted in higher C_{N2O_GC} and F_{N2O_GC}. Negative values, instead, showed that the difference GC-QCL led C_{N2O_QCL} and F_{N2O_QCL} to be higher than C_{N2O_GC} and F_{N2O_GC}. The overall difference between the two methods did not exceed ± 0.1 ppm for C_{N2O} and ± 0.38 nmol N_2O m^{-2} s^{-1} for F_{N2O} (Fig. 6e).

[FIGURE 6 ABOUT HERE]

To the best of our knowledge, bioequivalence has not broadly been applied in the greenhouse gas literature to identify and to discuss the range at which a difference in F_{N2O_GC} and F_{N2O_QCL} could be considered relevant when using different analytical methods. Defining the magnitude of F_{N2O} (e.g. in nmol N_2O m^{-2} s^{-1}) at which a unit difference would actually become relevant, however, is important when using different methods to quantify, compare and ultimately upscale N_2O emissions. We, therefore, recommend bioequivalence or other statistical approaches (e.g. Bland Altman) for more formally assessing the agreement between two methods in the future.

3.4.2 Strengths and weaknesses

The employment of a QCL analyser offers an alternative approach for the injection of N_2O samples taken from static chambers, particularly as F_{N2O_QCL} were generally equivalent to F_{N2O_GC}. Using a QCL for the purpose of manual injections can be conducted without much disruption to other measurements (e.g. EC or automated chambers) and, therefore, helps justify the initially higher capital and general running costs involved with operating a QCL device. Additional labour effort and time associated with sample storage and transport necessary for laboratory GC do not necessarily apply for field-based injections into a QCL. Once established, a QCL system has relatively low maintenance and offers a straightforward application for manual injections in addition to EC or other measurements. In our study, the assembly of the injection set-up required little equipment and was installed within 30 min. This allowed for a rapid analysis after chamber sampling without greatly interfering with other measurements, such as EC, that were offline during the time of manual injection into the QCL. Nonetheless, we recommend to collectively inject a great number of N_2O samples in order to minimise the downtime of EC measurements and other interferences made to the QCL. For instance, we were able to inject a total of around 700, 1 mL samples (432 samples, 268 standards) within four hours into the QCL (Table 1). Prior to QCL analysis samples had been kept in septum-sealed Exetainers that can store gas samples for up to 28 days at any temperature between -10 and 25°C (Faust and Liebig, 2018). We acknowledge that sporadic dilution of N_2O samples might still have occurred for both GC and QCL analyses due to sample storage in and insufficient evacuation of sample Exetainers (de Klein et al., 2015). Despite this potential source of uncertainty, storing N_2O samples in Exetainers also enabled repeated injections from the same sample for multiple times and allowed sample injections at suitable times, i.e. postponing analysis if EC measurements were of higher importance or if weather conditions (e.g. precipitation) did not support manual injections into the QCL. Similar to GC, QCL injections required consumables (N_2 carrier gas, N_2O standards) but, in contrast, time and costs associated with laboratory work were substantially less (Table 1).

[TABLE 1 ABOUT HERE]

4 Conclusion

Previously, QCL had been used either in conjunction with EC or coupled to automated chambers. Here, we showed that one QCL device could be used as a practical tool for the analysis of static chamber derived N_2O samples without major disruption to these other measurement tasks. We found treatment N_2O concentrations (C_{N2O_QCL}) and fluxes (F_{N2O_QCL}) from QCL agreed with results based on laboratory GC (C_{N2O_GC}, F_{N2O_GC}). The percentage difference between treatment F_{N2O_GC} and F_{N2O_QCL} was not smaller than -11.2 % and not greater than +9.2 % with a mean difference between the two of only 0.1 nmol N_2O m^{-2} s^{-1}. Deviation between the GC and QCL methods was determined only for close to zero F_{N2O} at control plots where F_{N2O_GC} and F_{N2O_QCL} values were found outside the predefined equivalence range. However, this was likely due to the calculation of very small but apparent positive and negative F_{N2O} when in fact the actual flux was zero; rather than being caused by uncertainties related to GC or QCL analysis itself. Equivalence was evidenced for all other F_{N2O_GC} and F_{N2O_QCL}, i.e. it was

confirmed that GC and QCL data were for practical purposes the same. We found that using Bland Altman and bioequivalence statistics in addition to regression analysis served the comparison of GC and QCL particularly well. Yet, these two statistical approaches have not broadly been used in the field of greenhouse gas research to compare different analytical methods or to discuss the magnitude at which a difference in F_{N2O}, or other greenhouse gas fluxes, would become relevant. Since correlation studies identify the relationship between two methods but not the difference, we recommend that bioequivalence or other suitable statistical approaches are used for more formally assessing the agreement between two methods. Finally, QCL offers a great potential to interlink different methods of gas measurements across different temporal and spatial scales. In the future, this capability might not only be important for rapid field analysis of N_2O samples but equally also applies to the measurement of other gas species (e.g. CO_2, CH_4) and gas isotopomers of interest.

Data availability

Data were deposited at the University of Waikato Research Commons, see (Wecking et al., 2020b) https://researchcommons.waikato.ac.nz/handle/10289/13539

Supplements to this manuscript exist.

Author contributions

ARW, VC, JL and LS designed the experiment. ARW performed the field work. ARW conducted the post-processing of GC and QCL data using MATLAB scripts provided by AW and DC. ARW performed the statistical analysis with inputs and contributions from VC. VC and LS commented on the results of the initial data analysis. ARW wrote and revised the manuscript with contributions from VC, AW, LL, JL, DC and LS.

Competing interests

The authors declare that they have no conflict of interest.

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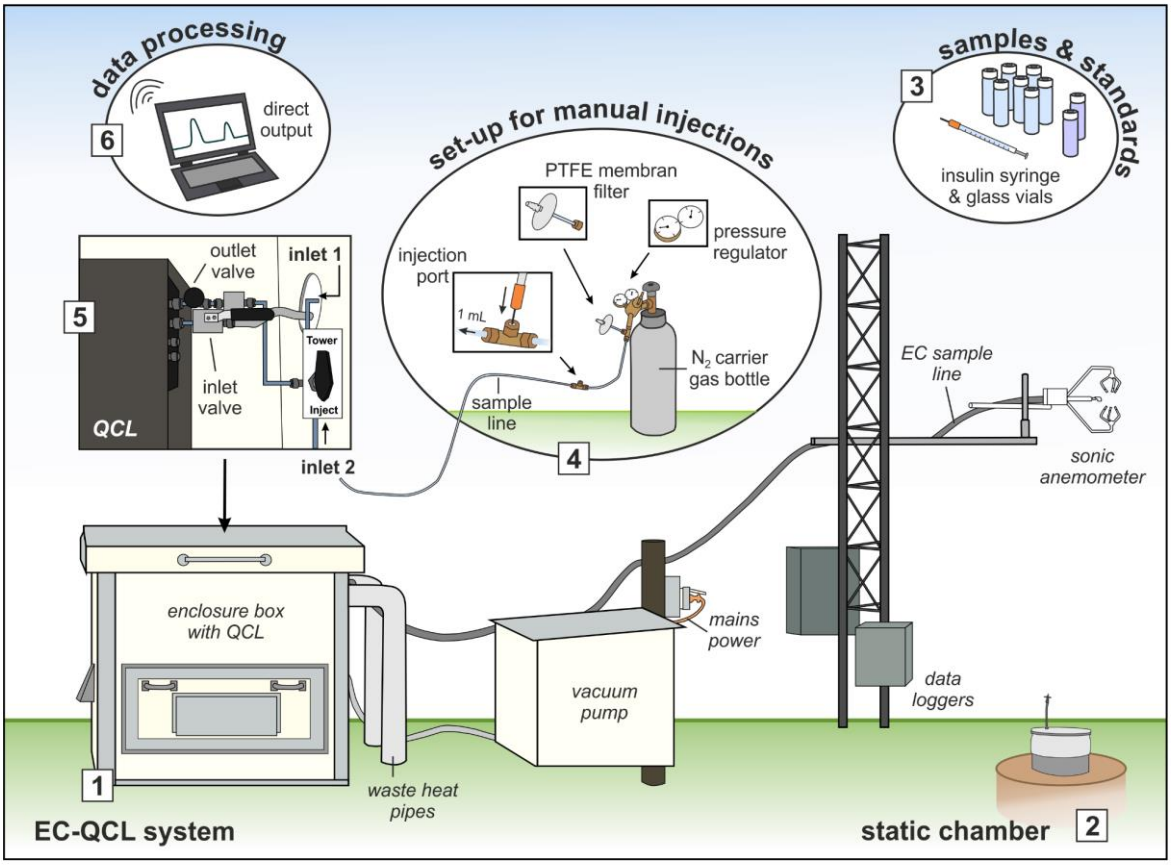

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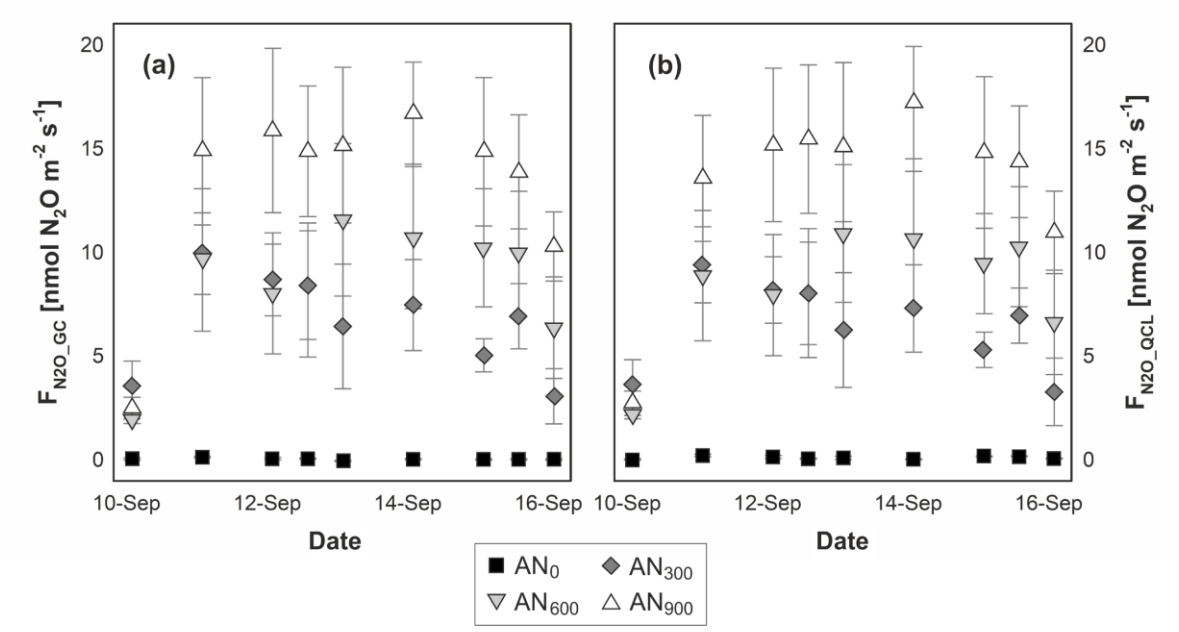

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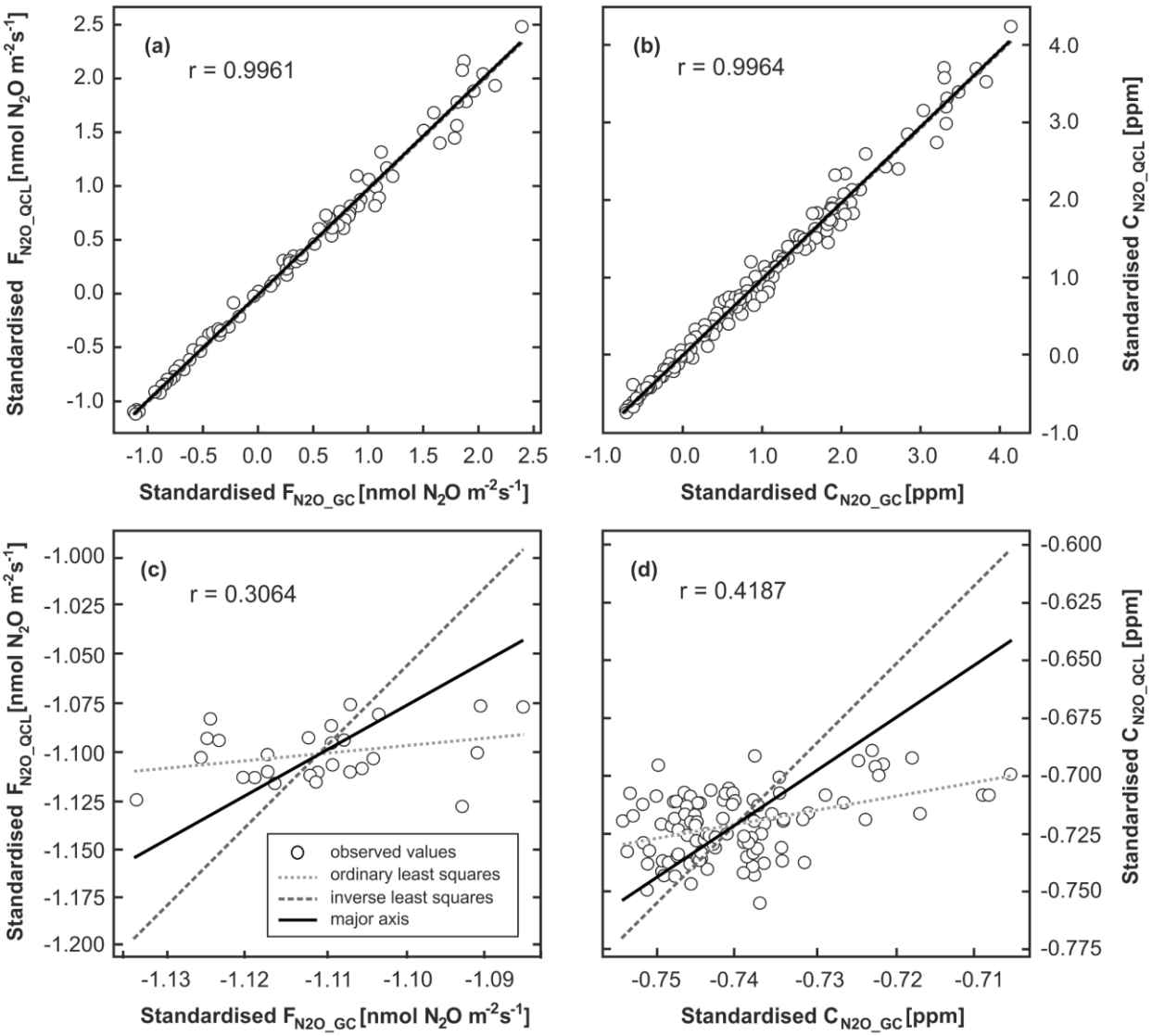

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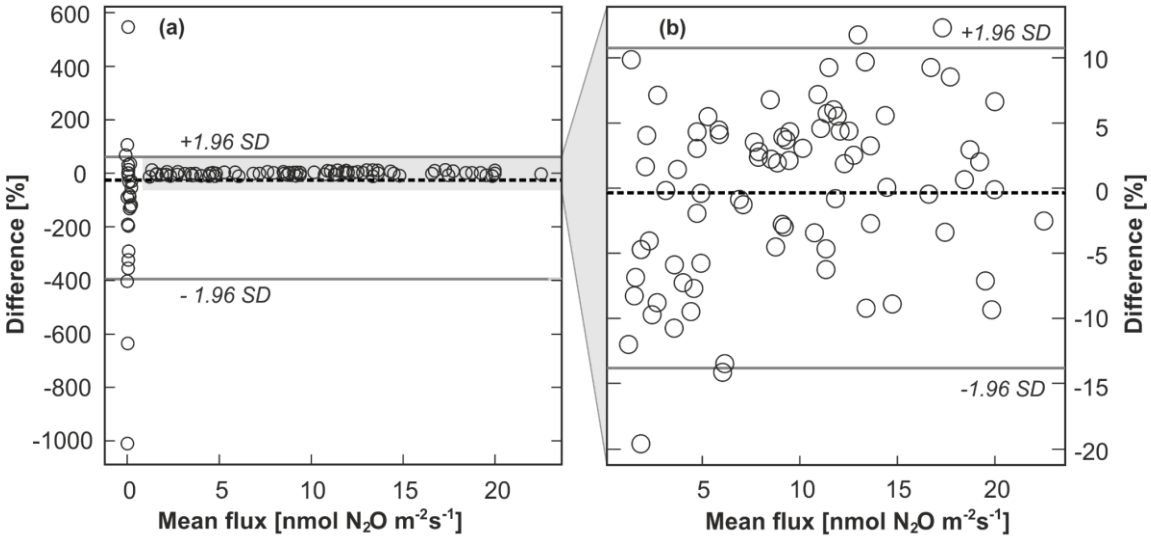

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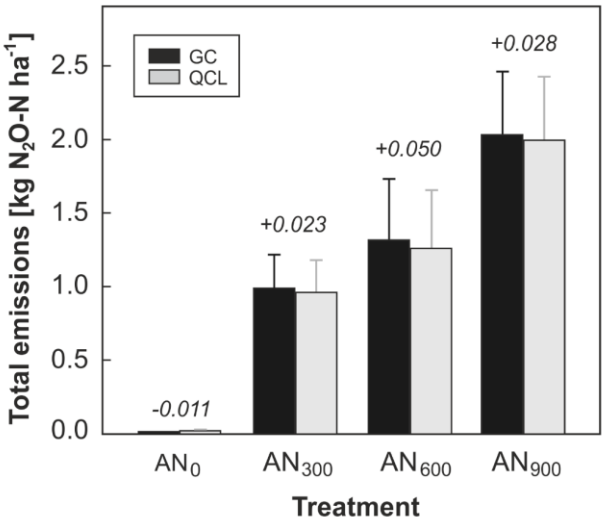

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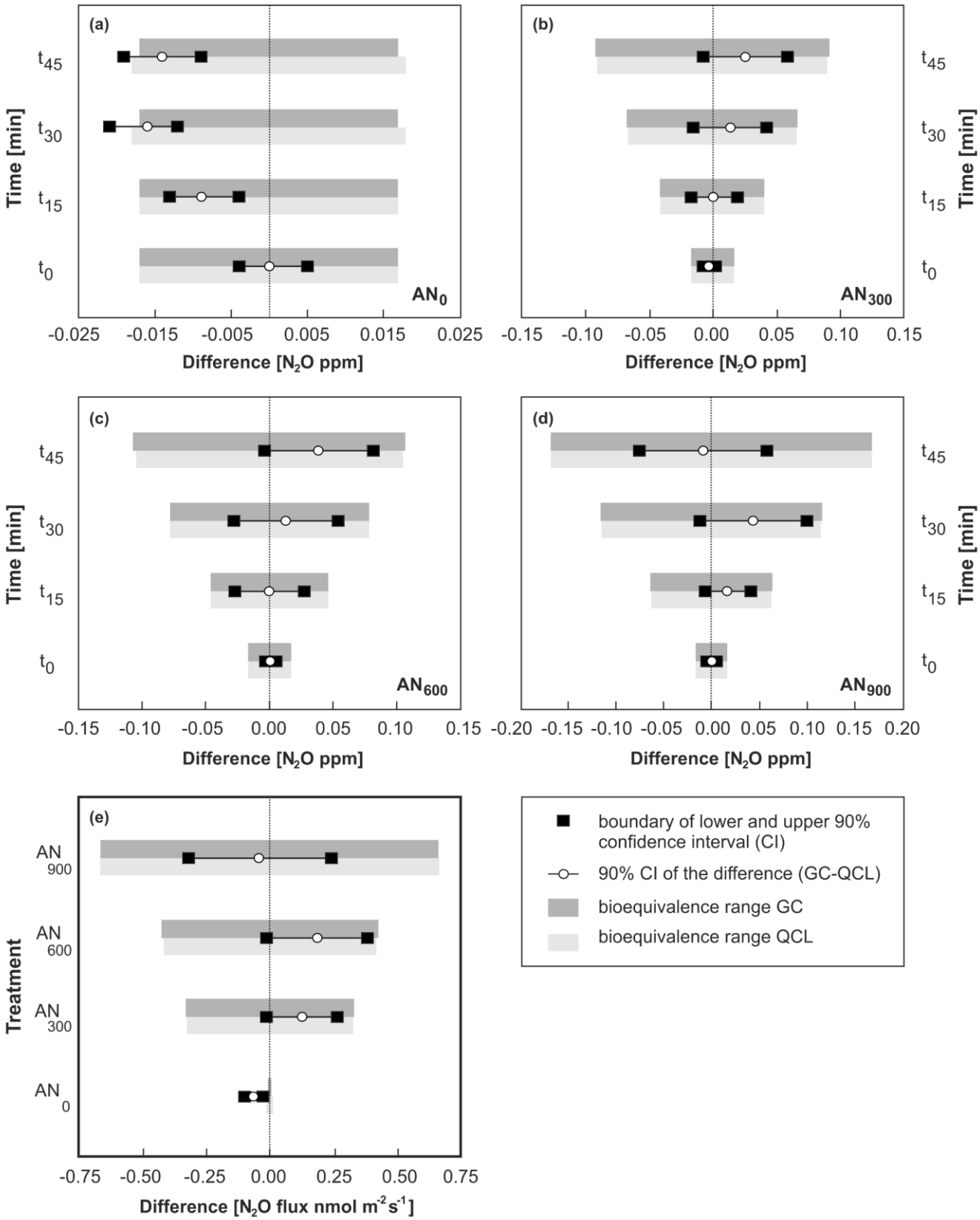
(a)
Time [min]
t45
t30
t15
t0
AN0
-0.025 -0.015 -0.005 0.005 0.015 0.025
Difference [N2O ppm]
(b)
AN300
-0.15 -0.10 -0.05 0.00 0.05 0.10 0.15
Difference [N2O ppm]
(c)
AN600
-0.15 -0.10 -0.05 0.00 0.05 0.10 0.15
Difference [N2O ppm]
(d)
AN900
-0.20 -0.15 -0.10 -0.05 0.00 0.05 0.10 0.15 0.20
Difference [N2O ppm]
(e)
Treatment
AN 900
AN 600
AN 300
AN 0
-0.75 -0.50 -0.25 0.00 0.25 0.50 0.75
Difference [N2O flux nmol m-2 s-1]
boundary of lower and upper 90% confidence interval (CI)
90% CI of the difference (GC-QCL)
bioequivalence range GC
bioequivalence range QCL

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	GC	**QCL**
Capital cost per device (NZD)	40,000	160,000
Labour effort for preparation and data processing of 100 samples (hours)	2 to 3	< 1
Transport of samples	required	not required
Storage of samples	required	optional
Analysis location	lab-based	field-based
Analysis time (days)	multiple days	immediate
Analysis cost per sample (NZD)	3.5	< 0.5
Possible injections (per hour)	7.5	~200
Lag time between injections (sec)	480	< 10
Injection procedure	manual/automated	manual
Injection of N_2O standards	required	required
Injection volume per sample (mL)	6	1
Carrier gas	N_2	N_2
Flow rate (L min^{-1})	0.4	1
Output of result data	post analysis	immediate