

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

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**Abstract.** The development of fast-response analysers for the measurement of nitrous oxide (N<sub>2</sub>O) 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<sub>2</sub>O concentrations in gas samples taken from static chambers has not yet been explored. Gas samples from static chambers are often analysed by GC, a method that 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<sub>2</sub>O concentrations and fluxes, we applied ammonium nitrate (AN) at 0, 300, 600 and 900 kg N ha<sup>-1</sup> (AN<sub>0</sub>, AN<sub>300</sub>, AN<sub>600</sub>, AN<sub>900</sub>) to plots on a pasture soil. After analysis, calculated N<sub>2</sub>O fluxes from QCL (F<sub>N<sub>2</sub>O\_QCL</sub>) were compared with fluxes determined by a standard method, i.e. here laboratory-based GC (F<sub>N<sub>2</sub>O\_GC</sub>). Subsequently, the comparability of QCL and GC data was tested using orthogonal regression, Bland Altman and bioequivalence statistics. For AN treated plots, mean cumulative N<sub>2</sub>O emissions across the seven-day campaign were 0.97 (AN<sub>300</sub>), 1.26 (AN<sub>600</sub>) and 2.00 (AN<sub>900</sub>) kg N<sub>2</sub>O-N ha<sup>-1</sup> for F<sub>N<sub>2</sub>O\_QCL</sub>, and 0.99 (AN<sub>300</sub>), 1.31 (AN<sub>600</sub>) and 2.03 (AN<sub>900</sub>) kg N<sub>2</sub>O-N ha<sup>-1</sup> for F<sub>N<sub>2</sub>O\_GC</sub>. These F<sub>N<sub>2</sub>O\_QCL</sub> and F<sub>N<sub>2</sub>O\_GC</sub> 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<sub>N<sub>2</sub>O\_QCL</sub> and F<sub>N<sub>2</sub>O\_GC</sub> derived under near-zero flux conditions (AN<sub>0</sub>) 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<sub>N<sub>2</sub>O</sub> when in fact the actual flux was below the detection limit of static chambers. Our study demonstrated 1) that the capability of using one QCL to measure N<sub>2</sub>O at different scales, including manual injections, offers a great potential to advance field measurements of N<sub>2</sub>O (and other greenhouse gases) in the 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

35 Accurate measurements of nitrous oxide ( $\text{N}_2\text{O}$ ) 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 ( $\text{CO}_2$ ) over 100 years and is the largest contributor to the depletion of stratospheric ozone (Ravishankara et al., 2009; IPCC, 2013). Agricultural activities on intensively managed soils that receive high inputs of reactive nitrogen ( $\text{N}_r$ ), mostly in the form of animal excreta and nitrogen fertiliser, are the main source of anthropogenic  $\text{N}_2\text{O}$  emissions (Reay et al., 2012). Reactive nitrogen facilitates microbial nitrification and  
40 denitrification in the soil with  $\text{N}_2\text{O}$  being an intermediate of these processes (Firestone and Davidson, 1989; Butterbach-Bahl et al., 2013). The production of  $\text{N}_2\text{O}$  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  $\text{N}_2\text{O}$  fluxes (Flechard et al., 2007; Erisman et al., 2013; Rees et al., 2013). Adequate and precise flux measurements have, therefore, remained challenging (Rapson and Dacres, 2014; Cowan et al., 2020).

45 To date, the common method for measuring fluxes of  $\text{N}_2\text{O}$  ( $F_{\text{N}_2\text{O}}$ ) are closed, non-steady-state 'static chambers' (Lundegard, 1927; Hutchinson and Mosier, 1981); a method used for more than 95 % of all field studies (Rochette and Eriksen-Hamel, 2008; Rochette, 2011; Lammirato et al., 2018). Static chambers are relatively cost-efficient and easy to deploy in the field (Velthof et al., 1996; de Klein et al., 2015). Gas samples are extracted from the chamber headspace during an up to 60-minute enclosure and injected into pre-evacuated glass vials (Rochette and Bertrand, 2003; Luo et al., 2007; van der Weerden et al.,  
50 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  $F_{\text{N}_2\text{O}}$  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 (Denmead, 2008; Christiansen et al., 2011; Chadwick et al., 2014). Through time, different guidelines have been proposed to advance the  
55 standardisation of static chamber techniques (Rochette, 2011; de Klein et al., 2015; Pavelka et al., 2018); but essentially the basic method has remained unchanged for decades (Hutchinson and Mosier, 1981; Chadwick et al., 2014).

Alternative approaches to the static chamber method include the use of (semi-) automated chambers and micrometeorological techniques that allow  $F_{\text{N}_2\text{O}}$  measurements at higher temporal frequency and resolution (Baldocchi, 2014; Rapson and Dacres, 2014; Pavelka et al., 2018). Recent developments in the technology of fast-response analysers have enabled, e.g. tunable diode  
60 laser absorption spectrometers, Fourier transform infrared spectrometers, and, in particular, continuous-wave quantum cascade laser absorption spectrometers (QCL) to be coupled to automated chambers (Cowan et al., 2014; Savage et al., 2014; Brümmer et al., 2017) or eddy covariance (EC) systems (Nicolini et al., 2013; Nemitz et al., 2018). Despite these recent advances in analyser technology, our understanding of the micro- and macro-scale processes that lead to the emission of  $\text{N}_2\text{O}$  has yet remained limited. While chamber measurements help to examine the interaction between soil processes and  $F_{\text{N}_2\text{O}}$  at point-scale  
65 (Luo et al., 2017), EC promotes the understanding of diurnal, seasonal and annual  $F_{\text{N}_2\text{O}}$  dynamics at field to ecosystem levels

(Liáng et al., 2018; Cowan et al., 2020). Some studies have aligned chamber and EC measurements to determine the full range of processes, that drive  $F_{N_2O}$  dynamics across these different scales, but still relied on the use of more than one analyser for measuring  $F_{N_2O}$  (Jones et al., 2011; Tallec et al., 2019; Wecking et al., 2020a).

70 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 near-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  $F_{N_2O}$  determined by field-based QCL ( $F_{N_2O\_QCL}$ ) were equivalent to  $F_{N_2O}$  derived from laboratory GC ( $F_{N_2O\_GC}$ ). 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  
75 devices (QCL and GC) and also discussed the method real-world application. Evidence of concept was provided by statistical tests to assess if the injection method would result in  $F_{N_2O\_QCL}$  equivalent to  $F_{N_2O\_GC}$ , these included: 1) orthogonal regression, 2) Bland Altman, and 3) bioequivalence analyses.

## 2 Methods

### 2.1 Study site

80 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  
85 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 Puinga 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 any chamber  $F_{N_2O}$  (Wall et al., 2020).

### 90 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 ( $C_{N_2O}$ ); 2) analyse these samples on-site using QCL and off-site using GC; 3) quantify and compare resulting  $C_{N_2O}$  and  $F_{N_2O}$ . A thorough description of the QCL operating in EC mode has been provided by Liáng et al. (2018) and Wecking et al. (2020a).

## 95 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  $C_{N_2O}$  in the chamber headspace for subsequent measurements. The three application rates were 300 (AN<sub>300</sub>), 600 (AN<sub>600</sub>) and 900 kg N ha<sup>-1</sup> (AN<sub>900</sub>), while the control plots (AN<sub>0</sub>) did not receive any AN. The rates of AN applied were to match nitrogen loading commonly found in cattle excreta patches, which is the main source of N<sub>2</sub>O 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<sub>min</sub>). Soil moisture and water-filled pore space (WFPS) were analysed and calculated using the methods described in Wecking et al. (2020a). Soil N<sub>min</sub> was derived from field-moist soil samples extracted in 2M KCl (Mulvaney, 1996) and measured colourimetrically using a Skalar SAN++ flow analyser (Skalar Analytical B. V., Breda, Netherlands). Both, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, were expressed in units kg ha<sup>-1</sup> using a site-specific soil dry bulk density of 0.73 g cm<sup>-3</sup> (Wecking et al., 2020a).

Chamber 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; Luo et al., 2008b; de Klein et al., 2015) and was carried out daily at 10 AM (NZDT) (van der Weerden et al., 2013). Additional sampling was also 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 each chamber. Gas samples were taken from this headspace during a 45 min enclosure period at four times – t<sub>0</sub>, t<sub>15</sub>, t<sub>30</sub> and t<sub>45</sub> – 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 analyses would receive 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; 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 <sup>63</sup>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  $0.4 \text{ L min}^{-1}$ . The measurement frequency was set to 1 Hz. Sample Exetainers experienced a storage time of up to two weeks before 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 collectively analysed 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 airflow 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 the overflow of excess carrier gas through a  $0.45 \mu\text{m}$  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  $15 \text{ L min}^{-1}$  used for EC to  $1 \text{ L min}^{-1}$  for manual injections based on Savage et al. (2014), Lebegue et al. (2016) 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.  $35 \pm 0.5 \text{ Torr}$ ,  $33.5 \text{ }^\circ\text{C}$  laser temperature, and a QCL enclosure box temperature of  $30 \pm 0.1 \text{ }^\circ\text{C}$ .

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, 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

160 usage of N<sub>2</sub>O standards were based on preliminary tests conducted in advance of the actual field campaign. Finally, it was important to keep a chronological record of the injected sample sequence to allow for a later re-identification of samples in the raw output data of the QCL.

### 2.3 Data processing

165 GC and QCL analyses resulted in the output of peak area data from the injected N<sub>2</sub>O standards and chamber derived N<sub>2</sub>O samples (Fig. S1). Data processing, therefore, first had to determine the relationship between peak area and (known) N<sub>2</sub>O concentration (C<sub>N<sub>2</sub>O</sub>) of the injected standards. To compute the final but initially unknown C<sub>N<sub>2</sub>O</sub> of chamber N<sub>2</sub>O samples, peak area data from N<sub>2</sub>O standards were fitted to linear and quadratic (second-order-polynomial) models (van der Laan et al., 2009; de Klein et al., 2015). de Klein et al. (2015) recommended the use of quadratic curves models as the standard curve for C<sub>N<sub>2</sub>O</sub> standards measured by GC analysis. However, we found that both linear and quadratic models adequately fitted C<sub>N<sub>2</sub>O</sub> standards derived from QCL. Using a linear fit ultimately resulted in, on average, 3 % smaller F<sub>N<sub>2</sub>O\_QCL</sub> (range -0.5 to -4.3 %) than using a quadratic model. Nonetheless, since the quadratic fit suited lower C<sub>N<sub>2</sub>O</sub> better than a linear fit, quadratic models were applied to represent the standard curves from injected standards of known C<sub>N<sub>2</sub>O</sub> (Fig. S2). The quadratic model used to calculate final C<sub>N<sub>2</sub>O</sub> was based on a selection of standards fitted to the expected minimum and maximum range of real sample C<sub>N<sub>2</sub>O</sub>; which in our study ranged between 0.3–10 ppm (Fig. S1, Table S2). 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) served the processing of data derived from the QCL.

### 2.4 Flux calculation

180 The F<sub>N<sub>2</sub>O</sub> in mg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup> was calculated for both data streams, GC (F<sub>N<sub>2</sub>O\_GC</sub>, n = 108) and QCL (F<sub>N<sub>2</sub>O\_QCL</sub>, n = 108), by applying a linear regression function to the increase in chamber headspace C<sub>N<sub>2</sub>O</sub> between time t<sub>0</sub> and t<sub>45</sub> following Eq. (1) (van der Weerden et al., 2011):

$$F_{N_{2}O\_GC} \text{ and } F_{N_{2}O\_QCL} = \frac{\Delta N_{2}O}{\Delta T} \times \frac{M}{Vm} \times \frac{V}{A} \quad (1)$$

185 where  $\Delta N_{2}O$  is the increase in headspace C<sub>N<sub>2</sub>O</sub> (μL N<sub>2</sub>O L<sup>-1</sup> (ppmv)) with time;  $\Delta T$  is the enclosure period (in hours);  $M$  is the molar weight of nitrogen in N<sub>2</sub>O (44 g mol<sup>-1</sup>);  $Vm$  is the molar volume of gas (L mol<sup>-1</sup>) at the mean air temperature recorded at each sampling occasion;  $V$  is the chamber headspace volume (m<sup>3</sup>); and  $A$  is the area covered by the chamber base, here 0.0415 m<sup>2</sup>. All F<sub>N<sub>2</sub>O</sub> were converted to units of nmol N<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> to allow for comparability between GC and QCL outputs. The integration of F<sub>N<sub>2</sub>O\_GC</sub> (n = 84) and F<sub>N<sub>2</sub>O\_QCL</sub> (n = 84) measured at 10 AM sampling was used to quantify the proportion of applied nitrogen emitted as N<sub>2</sub>O (E<sub>N<sub>2</sub>O</sub>) across the seven day trial in units kg N<sub>2</sub>O-N ha<sup>-1</sup> based on Luo et al. (2007) and Wecking et al. (2020a).

## 2.5 Statistical analyses

190 The statistical analysis for  $C_{N_2O}$  data ( $C_{N_2O\_GC}$  and  $C_{N_2O\_QCL}$ , each  $n = 432$ ) and resulting  $F_{N_2O}$  ( $F_{N_2O\_GC}$  and  $F_{N_2O\_QCL}$ , 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.

195 The orthogonal regression analysis used standardised  $C_{N_2O}$  and  $F_{N_2O}$  data following Eq. (2):

$$\text{standardised } C_{N_2O} \text{ and } F_{N_2O} = \frac{(x - \text{mean})}{\text{standard deviation}} \quad (2)$$

The core of this orthogonal regression was a principal component analysis which, in contrast to ordinary least square regression, allowed for measurements errors in the response and the predictor variable by minimising the squared residuals in a 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 prediction 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  $\pm 1.96$  SD of the mean difference (Giavarina, 2015). The Bland Altman analysis was conducted for individual  $F_{N_2O}$  as well as for mean  $F_{N_2O}$  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 a one-way analysis of variance (ANOVA) for  $F_{N_2O}$  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  $F_{N_2O\_QCL}$  and  $F_{N_2O\_GC}$ . 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. Originating from 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  $F_{N_2O\_QCL}$  had to be as small as possible and within  $\pm 5$  % of the mean difference of the standard method ( $F_{N_2O\_GC}$ ). The null hypothesis ( $F_{N_2O\_QCL}$  is different

from  $F_{N_{2O\_GC}}$  was rejected when the 90 % CI of the difference ( $F_{N_{2O\_QCL}}-F_{N_{2O\_GC}}$ ) was entirely within the predefined equivalence range at a significance level of 5 %. Following the same principles, we conducted a bioequivalence analysis for  $C_{N_{2O\_QCL}}$  and  $C_{N_{2O\_GC}}$ .

## 225 **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 %. The cumulative rainfall in September 2019 was 119 mm of which only 2 mm occurred 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**

#### 235 **3.2.1 Magnitude and general variability**

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

[FIGURE 2 ABOUT HERE]



### 3.2.2 AN treatment flux and concentration data

The correlation between calculated  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  and between  $C_{N_{2O\_GC}}$  and  $C_{N_{2O\_QCL}}$  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  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  and  $C_{N_{2O\_GC}}$  and  $C_{N_{2O\_QCL}}$ , 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  $F_{N_{2O}}$  (i.e.  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  treatment means) of not smaller than -11.2 % and not greater than +9.2 % (Table S5). The percentage difference between individual  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  (not treatment means) was slightly greater but in only less than 3 % of all cases exceeded +10 % and -15 %. This was likely due to the higher variability of  $F_{N_{2O}}$  between individual replicates of the same treatment than across calculated means. For both cases,  $\geq 95$  % of all data points were well within the pre-defined limits of agreement  $\pm 1.96$  SD (Fig. 4b). The overall mean difference (bias) between  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  was  $0.1 \text{ nmol N}_2\text{O m}^{-2} \text{ s}^{-1}$  (Fig. 4b). However, this small bias might be practically irrelevant when compared with the overall detection limit of static chambers and other method-associated uncertainties. Neftel et al. (2007), for instance, quantified the detection limit of static chambers to be  $0.23 \text{ nmol N}_2\text{O m}^{-2} \text{ s}^{-1}$ , and Parkin et al. (2012) reported  $0.03 \text{ nmol N}_2\text{O m}^{-2} \text{ s}^{-1}$ . In contrast, Flechard et al. (2007) and others (e.g. Rochette and Eriksen-Hamel, 2008; Jones et al., 2011) showed that the uncertainty of integrated chamber  $F_{N_{2O}}$  can be as high as 50 % at the annual scale.

[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,  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  measured at control plots ( $AN_0$ ) were only poorly correlated ( $r = 0.3064$ ) (Fig. 3c). The model-fit of the major axis, ordinary and inverse least squares indicated that the regression of  $F_{N_{2O\_GC}}$  on  $F_{N_{2O\_QCL}}$  (and vice versa) was not identical, i.e. differed in the minimisation of squared residuals in a vertical and horizontal direction. Likewise, this also applied to  $C_{N_{2O\_GC}}$  and  $C_{N_{2O\_QCL}}$  (Fig. 3d). Mean  $F_{N_{2O}}$  ranged from a minimum of -0.05 to a maximum of only  $0.21 \text{ nmol N}_2\text{O m}^{-2} \text{ s}^{-1}$  (Table S3). Consequently, Bland Altman statistics determined only small quantitative differences between  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$ . When computing the percentage difference between these  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$ , we found near-zero  $F_{N_{2O}}$  from  $AN_0$  plots were less consistent in relative terms than treatment  $F_{N_{2O}}$  (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  $C_{N_{2O}}$  than GC, which explains why the calculated  $F_{N_{2O\_QCL}}$  at  $AN_0$  plots were higher than  $F_{N_{2O\_GC}}$  (Table S5). However, whether this finding was related to the potentially higher sensitivity of the QCL device or due to other variations in the 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  
285 have resulted in the calculation of very small but apparent positive and negative  $F_{N_2O}$ , when in fact the actual flux was zero  
(*Type I error* as defined by Parkin et al. (2012)). The integration of  $C_{N_2O}$  with time to calculate  $F_{N_2O}$ , therefore, likely included  
this error; rather than being caused by uncertainties associated with the measurement procedures or choice of analytic device  
(Kroon et al., 2008). The deviation between control site ( $AN_0$ ) and treatment  $F_{N_2O}$  ( $AN_{300}$ ,  $AN_{600}$ ,  $AN_{900}$ ) has to be taken into  
account when evaluating the above results and mathematical principles (Sect. 3.2.2). Furthermore, since static chamber  
290 measurements often include near-ambient  $C_{N_2O}$ , and likewise fluxes equal or near-zero,  $F_{N_2O}$  from control plots were kept in  
the manuscript for completeness.

### 3.2.4 Cumulative $N_2O$ emissions

Cumulative  $N_2O$  emissions across the seven-day campaign were quantified slightly greater for the GC ( $E_{N_2O\_GC}$ ) than the QCL  
( $E_{N_2O\_QCL}$ ) method. The mean difference between  $E_{N_2O\_GC}$  and  $E_{N_2O\_QCL}$  for the control ( $AN_0$ ) and each treatment,  $AN_{300}$ ,  $AN_{600}$   
295 and  $AN_{900}$ , was -0.011, +0.0023, +0.050 and +0.028 kg N ha<sup>-1</sup>, 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  
300 et al., 2016; Rapson and Dacres, 2014). Gas chromatographs can be as precise as < 0.5 ppb (van der Laan et al., 2009; Rapson  
and Dacres, 2014) while the precision of a QCL is 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,  
305 Rosenstock et al. (2013) verified the accuracy and precision of different photoacoustic spectrometers based on laboratory QCL.  
However, the analytic precision can also depend on factors other than the technical performance of the analyser itself. Rannik  
et al. (2015) indicated that the performance (and thus the precision of  $F_{N_2O}$ ) of an analyser to measure gas samples from static  
chamber is likely more limited by the precision of the chamber system than by errors related to the analysis or post-processing  
of the data. Imprecisions might be caused by several factors, e.g. chamber type and dimension, experimental set-up,  
310 deployment time and preferred sampling method, all of which can affect the overall 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 1 mL sample volumes when injected into the QCL.  
In practice, these might not have always been equal to 1 mL and, thus, could have resulted in slight variations of output peak  
area. In agreement with our observations, de Klein et al. (2015) found that half the uncertainty of static chamber measurements

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

The QCL analysis of our study was conducted in a temperature- and pressure-controlled environment, where variations in these parameters were unlikely, and the variation in temperature expected to be less than 0.02 ppb °C<sup>-1</sup> (Lebegue et al., 2016). Nonetheless, we recommend a constant baseline flow of N<sub>2</sub> carrier gas at constant pressure (slightly higher than ambient) and temperature for manual injections made into the QCL device to avoid uncertainty affecting output peak areas. Depending on the QCL EC system, an initial lag time of 10 to 30 min before injections might be required 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<sup>-1</sup>, rapid injections into the QCL should become possible shortly afterwards with a delay between single injections of 1 mL sample volumes of not more than 5 to 8 sec. Sample concentrations of the same volume but at N<sub>2</sub>O concentrations > 20 ppm required a longer delay time between individual injections (> 20 sec) to ensure sufficient flushing of the QCL sample cell and avoid cross-contamination (Fig. S1). The identification of suitable delay times was straight forward in our case and could be easily accessed 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<sub>2</sub> carrier gas) did not result in any changes in baseline flow. However, we did not determine the extent to which spontaneous but small variations in the flow rate of N<sub>2</sub> carrier gas would have affected our resulting output peak areas. Further uncertainties might have been associated with processing and curve-fitting procedures applied to the raw dataset in MATLAB, and likely resulted in small underestimations of true output peak areas.

### 3.4 QCL injections

#### 3.4.1 The concept of bioequivalence

335 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<sub>2</sub>O research. Comparisons of different methods for N<sub>2</sub>O analysis made in the literature most commonly used orthogonal (Jones et al., 2011) and linear regression (Cowan et al., 2014; Brümmer et al., 2017; 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 analysis 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.  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  will never be exactly the same (Rani and Pargal, 2004). However, if the difference is sufficiently small for ‘practical purposes’,  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  can be considered effectively the same. Here, accepted evidence of bioequivalence for  $F_{N_{2O\_QCL}}$  was that the 90 % confidence interval of the difference  $F_{N_{2O\_QCL}} - F_{N_{2O\_GC}}$  (corresponding to a test with size 0.05) was within a  $\pm 5$  % difference of  $F_{N_{2O\_GC}}$ . The equivalence range will vary depending on the objective of the research or guidelines provided by a regulatory authority, but commonly does not exceed  $\pm 20$  % (Westlake, 1988; Rani and Pargal, 2004; Ring et al., 2019). In our study, a small equivalence range of  $\pm 5$  % was preferred to test the difference between  $F_{N_{2O\_QCL}}$  and  $F_{N_{2O\_GC}}$  since such recommendations did not exist.

Overall, our results showed that  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  from  $AN_{300}$ ,  $AN_{600}$  and  $AN_{900}$  plots provided evidence of bioequivalence. The 90 % confidence intervals of the difference ( $F_{N_{2O\_GC}} - F_{N_{2O\_QCL}}$ ) were quantified 0.127 ( $AN_{300}$ ), 0.185 ( $AN_{600}$ ) and -0.043 ( $AN_{900}$ )  $\text{nmol N}_2\text{O m}^{-2} \text{s}^{-1}$  and well within the pre-defined equivalence range of  $\pm 5$  % (Fig. 6e, Table S6). At control sites ( $AN_0$ ),  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  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’  $F_{N_{2O}}$ , rather than based on a failure of the statistical principle (Sect. 3.2.3). On the contrary, when tested for  $C_{N_{2O}}$  instead of  $F_{N_{2O}}$ , equivalence was confirmed for  $t_0$  and  $t_{15}$  but did not apply to  $t_{30}$  and  $t_{45}$  (Fig. 6a). Again, failure to establish equivalence was likely related to limitations of the static chamber method which, in this case, were 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  $\pm 5$  % of the mean difference of the standard method (i.e. GC). 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,  $\pm 10$  %. Accepting a greater mean difference between the two methods would have consequently resulted in evidencing bioequivalence for  $C_{N_{2O\_GC}}$  and  $C_{N_{2O\_QCL}}$  even at ambient concentrations. 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  $C_{N_{2O\_GC}}$  and  $F_{N_{2O\_GC}}$ . Negative values, instead, showed that the difference GC-QCL led  $C_{N_{2O\_QCL}}$  and  $F_{N_{2O\_QCL}}$  values to be greater than those from  $C_{N_{2O\_GC}}$  and  $F_{N_{2O\_GC}}$ , but in either case, the overall difference between the two methods did not exceed  $\pm 0.1$  ppm for  $C_{N_{2O}}$  and  $\pm 0.38$   $\text{nmol N}_2\text{O m}^{-2} \text{s}^{-1}$  for  $F_{N_{2O}}$  (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  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  could be considered relevant when using different analytical methods. However, defining the magnitude of  $F_{N_{2O}}$  (e.g. in  $\text{nmol N}_2\text{O m}^{-2} \text{s}^{-1}$ ) at which a unit difference would become relevant is important when using different methods to quantify, compare and, ultimately, upscale  $N_2O$  emissions. We, thus, 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

380 The employment of a QCL analyser proposes an alternative approach for the injection of N<sub>2</sub>O samples taken from static chambers, particularly as  $F_{N_{2O\_QCL}}$  were generally equivalent to  $F_{N_{2O\_GC}}$ . Using a QCL for 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.

385 Once established, a QCL system has relatively low maintenance and offers a straightforward application for manual injections in addition to EC or other measurement tasks. 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, i.e. EC, that were offline during the time of injection into the QCL. To collectively inject a great number of samples turned out to be highly beneficial to minimise the downtime of the EC measurements, in our case, and also helped to

390 reduce 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 (Table 1). Prior to QCL analysis, these 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 a sporadic dilution of our samples might still have occurred due to storage in and potentially insufficient evacuation of Exetainers which, in turn, could have affected subsequent GC and QCL analyses (de Klein et al., 2015). Despite

395 this potential source of uncertainty, storing N<sub>2</sub>O samples in Exetainers enabled repeated injections and allowed to postpone the analysis if EC measurements were of higher importance or if the weather conditions (e.g. precipitation) were unsuitable. Similar to GC, QCL injections required consumables (N<sub>2</sub> carrier gas, N<sub>2</sub>O standards) but, in contrast, time and costs associated with laboratory work were substantially less (Table 1).

[TABLE 1 ABOUT HERE]

## 400 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<sub>2</sub>O samples without major disruption to these other measurement tasks. We found treatment N<sub>2</sub>O concentrations ( $C_{N_{2O\_QCL}}$ ) and fluxes ( $F_{N_{2O\_QCL}}$ ) from QCL agreed with results based on laboratory GC ( $C_{N_{2O\_GC}}$ ,  $F_{N_{2O\_GC}}$ ). The percentage difference between treatment  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$

405 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<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>. A deviation between the GC and QCL methods was determined only for close to zero  $F_{N_{2O}}$  at control plots where  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  values were found outside the predefined equivalence range. However, this was likely due to the calculation of very small but apparent positive and negative  $F_{N_{2O}}$  (when in fact the actual flux was zero), rather than due to uncertainties caused by a weakness of the GC or QCL analysis. Equivalence was evidenced for all other  $F_{N_{2O\_GC}}$  and  $F_{N_{2O\_QCL}}$  and confirmed

410 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  $F_{N_2O}$  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 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 fieldwork. ARW conducted the post-processing of GC and QCL data using MATLAB scripts, which based on the work from 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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## List of figures

**Figure 1:** Schematic illustration of how to use a field-based QCL for EC measurements and manual injections. (1) shows the main components of the QCL EC system; (2) provides an example of a static chamber from which N<sub>2</sub>O samples were taken and stored in (3) pre-evacuated glass vials. Once the set-up for manual injections (4) was assembled and the QCL air-inlet (5) adjusted from drawing ambient air through the EC sample line (inlet 1) to drawing air via the injection tube (inlet 2), the QCL was readily set-up for receiving injections of N<sub>2</sub>O samples and associated standards through the injection port. The data output (6) was immediate allowing processing and data evaluation on the day of chamber sampling.

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**Figure 2:** Fluxes of nitrous oxide (F<sub>N<sub>2</sub>O</sub>) determined from (a) gas chromatography (F<sub>N<sub>2</sub>O\_GC</sub>) and (b) quantum cascade laser absorption spectrometry (F<sub>N<sub>2</sub>O\_QCL</sub>). Symbols depict mean F<sub>N<sub>2</sub>O</sub> and marker shading displays the rate of ammonium nitrate (AN) applied: AN<sub>0</sub> (black squares), AN<sub>300</sub> (dark grey diamonds), AN<sub>600</sub> (light grey upside-down triangles) and AN<sub>900</sub> (white triangles). Error bars illustrate the standard error of the mean (SEM) across the three replicates of the same treatment. Note that flux measurements on 12 and 15 September were conducted twice daily (10 AM and 12 PM) and that the time scale on the x-axis, therefore, is discrete. Soil water-filled pore space (WFPS) and mineral nitrogen (N<sub>min</sub>) contents associated with flux measurements are provided in the supplementary material, Table S3.

**Figure 3:** Orthogonal regression analysis of standardised N<sub>2</sub>O concentrations (C<sub>N<sub>2</sub>O</sub>) and fluxes (F<sub>N<sub>2</sub>O</sub>). Data were distinguished by their analytic source of origin, i.e. GC (C<sub>N<sub>2</sub>O\_GC</sub>, F<sub>N<sub>2</sub>O\_GC</sub>) and QCL (C<sub>N<sub>2</sub>O\_QCL</sub>, F<sub>N<sub>2</sub>O\_QCL</sub>). The regression analysis included all C<sub>N<sub>2</sub>O</sub> in (a) but only those C<sub>N<sub>2</sub>O</sub> measured at control sites (AN<sub>0</sub>) in panel (c). The orthogonal regression analysis was repeated for standardised F<sub>N<sub>2</sub>O</sub> with (b) showing all F<sub>N<sub>2</sub>O\_GC</sub> and F<sub>N<sub>2</sub>O\_QCL</sub>, and (d) depicting the orthogonal regression for AN<sub>0</sub> fluxes only. Ordinary least squares (dotted light grey line) resulted from the regression of Y on X; inverse least squares from the regression of X on Y (long dotted dark grey line). The major axis (black line) based on orthogonal regression of Y and X using a principal component analysis. Here, the squared residuals perpendicular to the line are minimised. Note, for the purpose of illustration axes in panel (c) and (d) have different scales. Table S4 in the supplements provides further results.

**Figure 4:** Bland Altman plots showing the difference between the GC and QCL method expressed as the percentage difference of the standard method A (F<sub>N<sub>2</sub>O\_GC</sub>) and the new method B (F<sub>N<sub>2</sub>O\_QCL</sub>) on the y-axis [((A-B)/mean) × 100] versus the mean of A and B on the x-axis. The limits of agreement are represented by continuous lines at ±1.96 standard deviation (SD) of the percentage difference. The inset (panel b) illustrates the same data but excludes F<sub>N<sub>2</sub>O\_GC</sub> and F<sub>N<sub>2</sub>O\_QCL</sub> from control (AN<sub>0</sub>) sites. The percentage mean difference (bias) between F<sub>N<sub>2</sub>O\_GC</sub> and F<sub>N<sub>2</sub>O\_QCL</sub>, i.e. method A and B, is indicated by the gap between the dashed line (line of equality, which is not at zero) and an imaginary line parallel to the dashed line at y = 0. This figure is

635 based on individual  $F_{N_2O}$  (all treatment replicates). Results for mean  $F_{N_2O}$  across replicates of the same treatment are provided in the supplements, see Table S5.

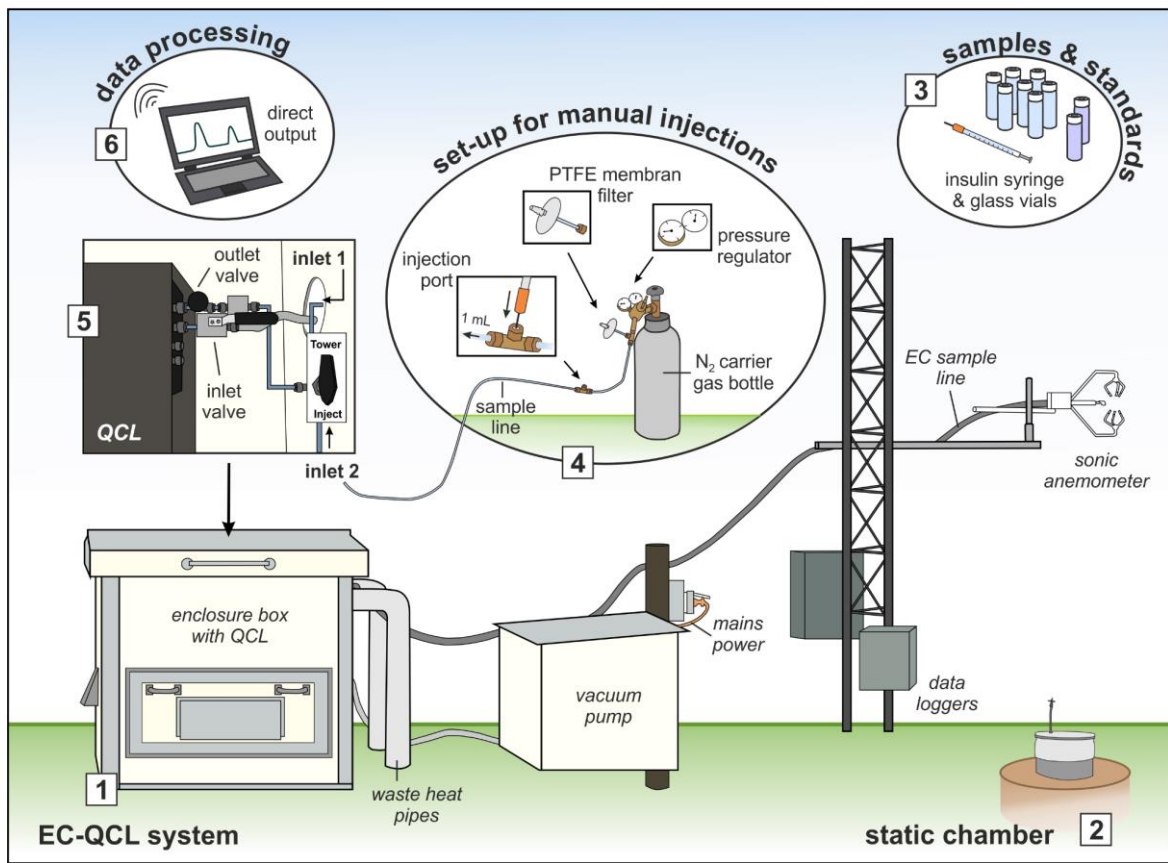
**Figure 5:** Cumulative  $N_2O$  emissions from each treatment ( $AN_{300}$ ,  $AN_{600}$ ,  $AN_{900}$ ) and the control ( $AN_0$ ) in  $kg\ N_2O-N\ ha^{-1}$  at the end of the campaign. Data are distinguished into GC (black bars) and QCL (grey bars) budgets. Error bars quantify the standard error of the mean (SEM). The absolute difference in  $kg\ N_2O-N\ ha^{-1}$  between the two budgets (GC-QCL) is highlighted by the number on the top of each bar-couple.

**Figure 6:** Bioequivalence analysis for  $N_2O$  concentrations ( $C_{N_2O}$ ) in (a-d) and  $N_2O$  fluxes ( $F_{N_2O}$ ) in (e) with GC defined as the standard method.  $C_{N_2O}$  and  $F_{N_2O}$  based on QCL analysis were considered bioequivalent when the 90 % confidence interval (CI) of the difference between QCL and GC (x-axis) was completely within the predefined  $\pm 5\%$  bioequivalence range of the difference of the standard method. The bioequivalence analysis was distinguished for  $C_{N_2O}$  by sampling interval ( $t_0$ ,  $t_{15}$ ,  $t_{30}$ ,  $t_{45}$ ) and treatment with panel (a) showing results for control sites ( $AN_0$ ) and panels (b), (c) and (d) for  $AN_{300}$ ,  $AN_{600}$  and  $AN_{900}$  treatment sites. Similarly, a bioequivalence analysis was conducted for  $F_{N_2O}$  in panel (e) and distinguished by AN application rate on the y-axis.

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## List of tables

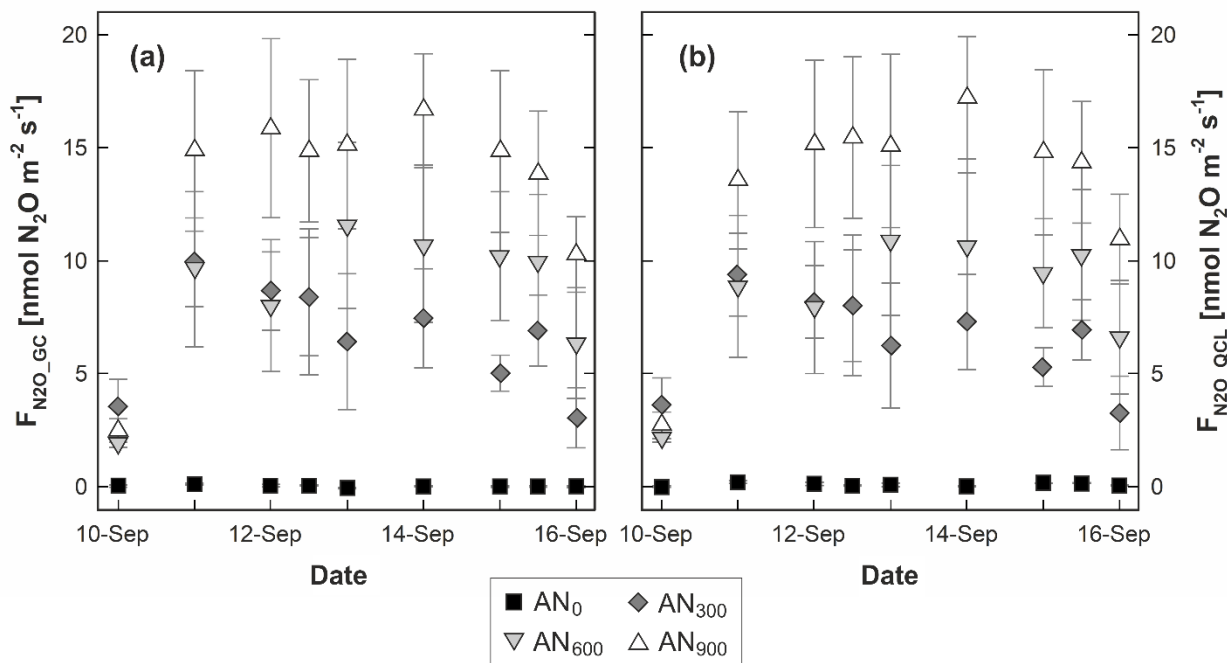
**Table 1:** The GC and QCL methods in comparison: Details provided in the table relate to in this study and information provided were not generalised. NZD = New Zealand dollars.



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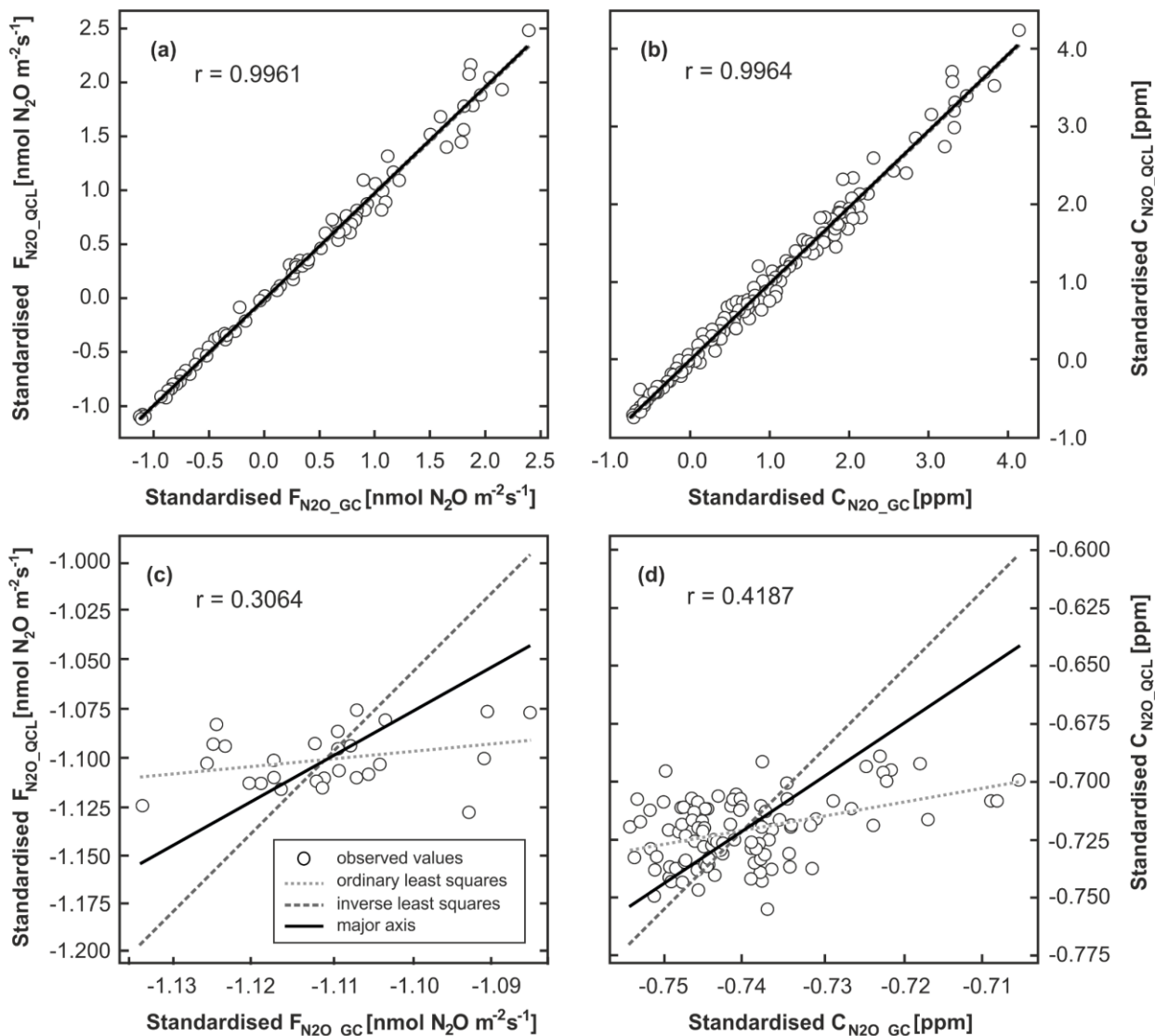
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**Figure 2:** Fluxes of nitrous oxide ( $F_{N_2O}$ ) determined from (a) gas chromatography ( $F_{N_2O\_GC}$ ) and (b) quantum cascade laser absorption spectrometry ( $F_{N_2O\_QCL}$ ). Symbols depict mean  $F_{N_2O}$  and marker shading displays the rate of ammonium nitrate (AN) applied: AN<sub>0</sub> (black squares), AN<sub>300</sub> (dark grey diamonds), AN<sub>600</sub> (light grey upside-down triangles) and AN<sub>900</sub> (white triangles). Error bars illustrate the standard error of the mean (SEM) across the three replicates of the same treatment. Note that flux measurements on 12 and 15 September were conducted twice daily (10 AM and 12 PM) and that the time scale on the x-axis, therefore, is discrete. Soil water-filled pore space (WFPS) and mineral nitrogen ( $N_{min}$ ) contents associated with flux measurements are provided in the supplementary material, Table S3.

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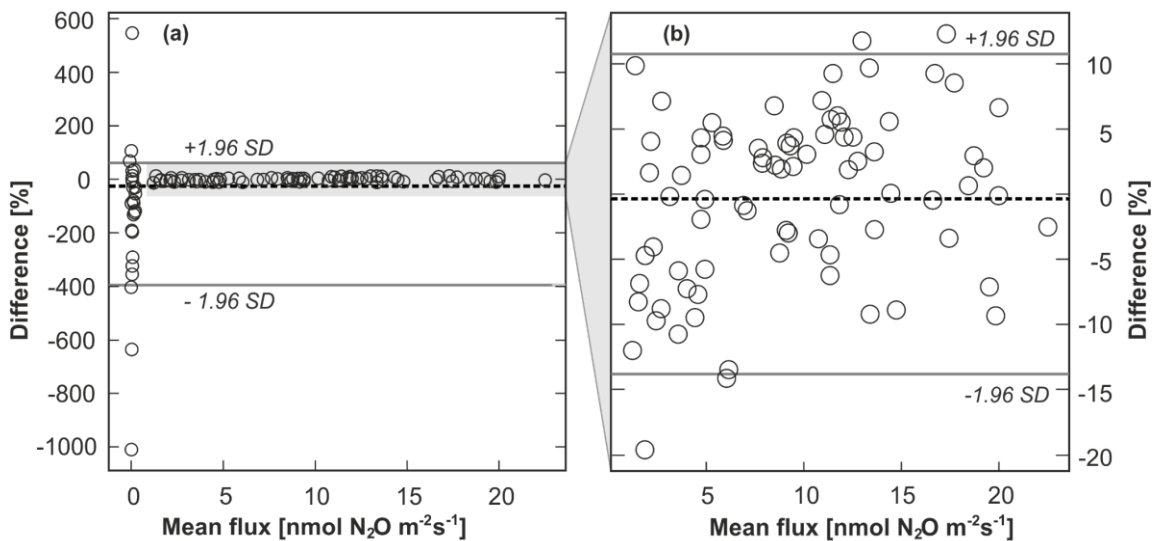


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**Figure 3:** Orthogonal regression analysis of standardised N<sub>2</sub>O concentrations (C<sub>N2O</sub>) and fluxes (F<sub>N2O</sub>). Data were distinguished by their analytic source of origin, i.e. GC (C<sub>N2O\_GC</sub>, F<sub>N2O\_GC</sub>) and QCL (C<sub>N2O\_QCL</sub>, F<sub>N2O\_QCL</sub>). The regression analysis included all C<sub>N2O</sub> in (a) but only those C<sub>N2O</sub> measured at control sites (AN<sub>0</sub>) in panel (c). The orthogonal regression analysis was repeated for standardised F<sub>N2O</sub> with (b) showing all F<sub>N2O\_GC</sub> and F<sub>N2O\_QCL</sub>, and (d) depicting the orthogonal regression for AN<sub>0</sub> fluxes only. Ordinary least squares (dotted light grey line) resulted from the regression of Y on X; inverse least squares from the regression of X on Y (long dotted dark grey line). The major axis (black line) based on orthogonal regression of Y and X using a principal component analysis. Here, the squared residuals perpendicular to the line are minimised. Note, for the purpose of illustration axes in panel (c) and (d) have different scales. Table S4 in the supplements provides further results.

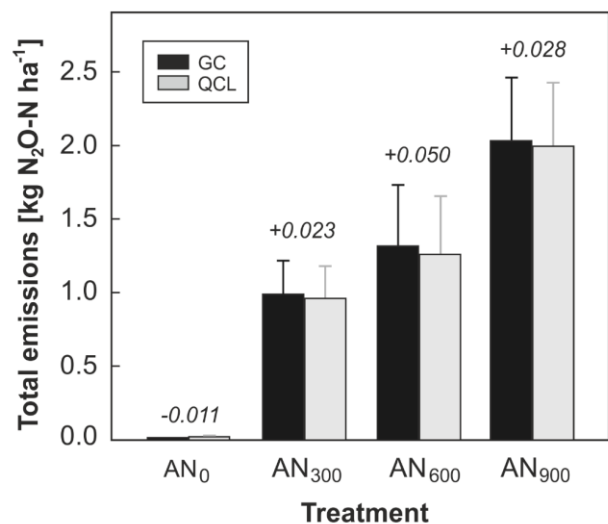




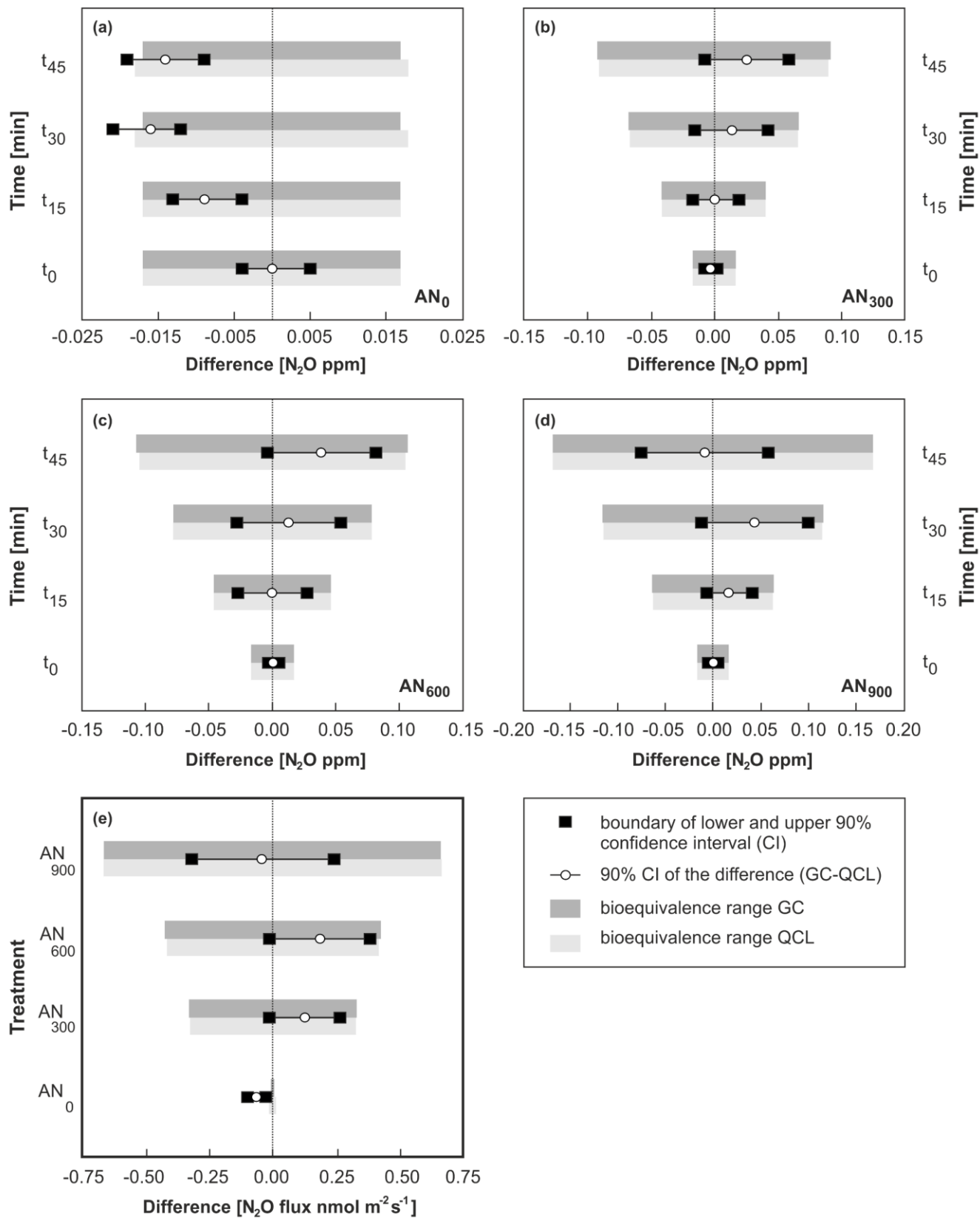
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**Figure 4:** Bland Altman plots showing the difference between the GC and QCL method expressed as the percentage difference of the standard method A ( $F_{N_2O\_GC}$ ) and the new method B ( $F_{N_2O\_QCL}$ ) on the y-axis  $[(A-B)/\text{mean}] \times 100$  versus the mean of A and B on the x-axis. The limits of agreement are represented by continuous lines at  $\pm 1.96$  standard deviation (SD) of the percentage difference. The inset (panel b) illustrates the same data but excludes  $F_{N_2O\_GC}$  and  $F_{N_2O\_QCL}$  from control ( $AN_0$ ) sites. The percentage mean difference (bias) between  $F_{N_2O\_GC}$  and  $F_{N_2O\_QCL}$ , i.e. method A and B, is indicated by the gap between the dashed line (line of equality, which is not at zero) and an imaginary line parallel to the dashed line at  $y = 0$ . This figure is based on individual  $F_{N_2O}$  (all treatment replicates). Results for mean  $F_{N_2O}$  across replicates of the same treatment are provided in the supplements, see Table S5.

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**Figure 5:** Cumulative N<sub>2</sub>O emissions from each treatment (AN<sub>300</sub>, AN<sub>600</sub>, AN<sub>900</sub>) and the control (AN<sub>0</sub>) in kg N<sub>2</sub>O-N ha<sup>-1</sup> at the end of the campaign. Data are distinguished into GC (black bars) and QCL (grey bars) budgets. Error bars quantify the standard error of the mean (SEM). The absolute difference in kg N<sub>2</sub>O-N ha<sup>-1</sup> between the two budgets (GC-QCL) is highlighted by the number on the top of each bar-couple.



**Figure 6:** Bioequivalence analysis for N<sub>2</sub>O concentrations (C<sub>N2O</sub>) in (a-d) and N<sub>2</sub>O fluxes (F<sub>N2O</sub>) in (e) with GC defined as the standard method. C<sub>N2O</sub> and F<sub>N2O</sub> based on QCL analysis were considered bioequivalent when the 90 % confidence interval (CI) of the difference between QCL and GC (x-axis) was completely within the predefined ± 5 % bioequivalence range of the difference of the standard method. The bioequivalence analysis was distinguished for C<sub>N2O</sub> by sampling interval (t<sub>0</sub>, t<sub>15</sub>, t<sub>30</sub>, t<sub>45</sub>) and treatment with panel (a) showing results for control sites (AN<sub>0</sub>) and panels (b), (c) and (d) for AN<sub>300</sub>, AN<sub>600</sub> and AN<sub>900</sub> treatment sites. Similarly, a bioequivalence analysis was conducted for F<sub>N2O</sub> in panel (e) and distinguished by AN application rate on the y-axis.

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**Table 1:** The GC and QCL methods in comparison: Details provided in the table relate to in this study and information provided were not generalised. NZD = New Zealand dollars.

	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 <sub>2</sub> O standards	required	required
Injection volume per sample (mL)	6	1
Carrier gas	N <sub>2</sub>	N <sub>2</sub>
Flow rate (L min <sup>-1</sup> )	0.4	1
Output of result data	post analysis	immediate