TO THE EDITOR

Dear Associate Editor,
Dear Dr Daniela Famulari,

We are pleased to forward a revised version of our manuscript "A novel injection technique: using a field-based quantum cascade laser for the analysis of gas samples derived from static chambers" to the ATM journal.

Attached you will find our response to the comments made by referee #1 and #2 in the public discussion forum of ATM.

Referee comments are highlighted by yellow captions. Author comments and changes made to the manuscript were written in blue font with *italics* indicating the changes.

We thoroughly enjoyed the review process and believe that the referees' comments have added to the quality of our manuscript.

Kind regards in the name of all authors

Anne Wecking

REFEREE #1

Reviewer comment:
The study "A novel injection technique: using a field-based quantum cascade laser for the analysis of gas samples derived from static chambers" by Wecking et al. describes an experiment in which samples of N2O measured using the static chamber method are then analysed on a QCL and GC instrument for comparison. The study is well written and presented, but there are some over simplifications that should be addressed, in how the complexity of the system is described and the way the data is handled in the study. I advise quite a significant re-write focussing on the actual focus of the study, which is how the instruments compare.

Author comment:
We like to thank Referee #1 for her/his extensive comments.
In response to the above: Our interest was not only to compare two different analytic devices but to further the suitability of a field-based quantum cascade laser (QCL) and associated sampling/measurement procedures to measure chamber derived samples of nitrous oxide (N2O). Of particular interest for us was to develop a technique that is applicable and easy to conduct in field environments, and that can be used in conjunction with eddy covariance (EC). Furthermore, we compared resource efficiency of the whole operation which is critical for decisions that will be made by researchers when considering actual costs of different research approaches and analytical devices.

Reviewer comment:
My first comment is that this study is essentially a comparison of concentrations measured using two instruments. This work could have been carried out with gas standards without the need for any chamber measurements. The real point of the study here is whether gas injected through a QCL following this setup is a valid way to measure gas concentrations. If so, then fluxes calculated from the sample will compare well regardless.

Author comment:
Using gas standards would have allowed for a comparison between GC and QCL. However, this would not have included the whole sampling/analysis approach. Initial lab-based comparisons of QCL to other analytic devices have already been provided in the literature and shown that the suitability of a

QCL device to quantify N2O standards is without doubt (Zellweger et al. (2019); Rosenstock et al. (2013)). We were, therefore, highly interested in whether the lab-based accuracy and precision of a QCL could also be achieved in a field-based "mini-lab" – and under realistic sampling procedures that included N2O sampling from static chambers, sample separation to inject identical sample volumes and concentrations into either of the two analysers, sample storage, sample and data processing.

Reviewer comment:
The novelty of the method is that the authors get past the inability of the QCL to measure actual concentrations of a sample by using standards to integrate peak areas, similar to the way the GC reports measurements, thus reducing the sample volume required.

Author comment:
Agreed, this is one advantage of using the QCL in the field setting.
Further benefits were 1) the process of getting to the point of receiving these peak area data (e.g. developing sampling procedures and the injection set-up in a field environment); and 2) to test whether the QCL could reliably process samples of low, medium and high N2O concentration (that were derived from static chambers in a real scenario including method specific uncertainties).

Reviewer comment:
I'd like to see some examples of the QCL concentration output at 10Hz while measuring low, medium and high concentrations of N2O in the standards to observe the shape of the peaks that are integrated. I think the presentation of the integration of these peaks far outweighs the flux work. By the authors own numbers, I believe a flush rate of the cell is greater than 1 second, so I'd be interested to see how the laser reports concentrations while measuring at 10 Hz, and what the noise looks like.

Author comment:
L. 150: All our injections were conducted at a 10 Hz frequency, which means that all QCL concentration data reported in the manuscript were consistently determined at this particular frequency. Examples of how low to high N2O concentrations were reported by the QCL were provided in the supplementary material of the work, i.e. Figure S1 a) and b). Raw data can also be found in the data repository associated with the manuscript. However, we are in line with the Referee that including a raw 10 Hz time series of injection peaks might be a useful addition to the manuscript/the supplementary material and will be considered.

Changes made to the manuscript:
Supplementary material We addressed the referee's #1 interest in our data processing procedures by introducing a new figure to the supplements. The new Figure (named Figure S1) shows the raw QCL output data (peak areas) that we received after manual injections into the QCL at different low to high N2O concentrations. The figure depicts an example of a time series of injected N2O samples and N2O standards. The shape of the peaks used for later integration is illustrated. We believe, that the new Figure S1 will visualise and complement the content of Section 2.3 nicely and will help to clarify the referees' comments (see comments made by referee #2 below, major comment 2).

Reviewer comment:
As this is the real novelty in the manuscript, much more emphasis should be on the outputs of the instrument itself, and less on the flux measurements.

Author comment:
The focus of our manuscript is on the concentration output of the QCL and GC analysers as represented by the majority of section 2 and section 3.2, 3.3, 3.4. Calculating the resulting N2O fluxes was a necessary exercise in addition to the emphasis on N2O concentrations. We like to point the

Referee to the objective of the bioequivalence analysis applied to both N2O concentration and flux data (see Section 3.4.1). Determining the suitability of a measurement device for a particular purpose has to be evaluated. However, a simple comparison of concentration data based on linear regression does not satisfy this purpose. Consequently, we were led by the intention to not only assess the degree to which the two methods (GC and QCL) would agree (orthogonal regression, Bland Altman). But to further determine whether N2O concentration and flux data, in fact, were statistically speaking "the same" (i.e. bioequivalent). Receiving reliable N2O fluxes when using the QCL injection technique was the ultimate goal of our study. We like to acknowledge that applying statistical tests like bioequivalence describes a concept most N2O flux researchers might be unfamiliar with. However, to assess the comparability of data derived from two different methods these tests are essential as was our evaluation of N2O flux data to discuss the relevance of our results in a real world scenario.

Reviewer comment:

I agree with many of the points the authors make, and I feel the study has value as a reference for people who may want to use the method presented in future studies; however, I don't see it being popular. Most Eddy C sites will value the collection of uninterrupted data over the ability to run the instrument as a makeshift GC.

Author comment:

It is good that we have general agreement about the value of the work. One of the key purposes of this work was to provide information for operators to make informed decisions about how they might operate their eddy covariance sites and consider trade-offs of data loss for short periods of time. How popular this approach becomes remains to be seen but was not really a focus of the current study. Thus, even if not prominent at present, we are convinced that using a QCL in the most cost- and time-efficient way to suit multiple research purposes provides a promising future application.

Changes made to the manuscript:

Our injection method does not greatly interfere with EC measurements. If chamber samples are collectively injected into the QCL, it becomes possible to minimise EC downtime even further. To highlight this capability of our method, we changed the following parts of the manuscript:

L. 384 Clarification of wording to point out that the QCL needs an initial lag time of 10-30 min (assembling of the set-up) before manual injections become possible. *"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.* ***L. 386*** *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."*

L. 474 following We addressed the referee's comment to expand on the idea to minimise EC downtime by adding the following sentences to the paragraph: *"Nonetheless, we here recommend to collectively inject a great number of N2O 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)".*

Reviewer comment:

Choosing manual injections over the more common auto injection systems used by the GC also introduce a bit of a time cost and add room for human error.

Author comment:
This is correct and this human error is captured within the error reported in the paper. Sample loops could be included in our proposed setup to further reduce the error, if desired. The time taken for one injection was less than 10 seconds and accounted for in our time analysis (Table S2) which shows overall a large time saving of, in our case, multiple days (considering transport of samples to the lab and analysis time, see Table 1). We could show that at a given flow rate of 1 L min-1 the delay time between single injections of 1 mL sample volumes was generally short (5 to 8 sec). The return of the N2O concentration in the QCL sample cell to basically zero (= background level of the N2 carrier gas) was straight forward when using visual examination of the real-time curve in TDL-Wintel. Suitable delay times were, thus, easily adjustable if the sample concentration would have exceeded 20 ppm N2O (line 312). In our study, field samples did not exceed a N2O concentration greater 10 ppm. This supported the applicability of our approach. It is to consider that using an auto-injection system would have to account for different delay times after sample injection and, therefore, would likely include longer sample delay and overall sample analysis times.

Reviewer comment:
In terms of running costs, a QCL requires an air conditioned site with mains power to operate in EC mode, which is essentially a lab in itself. This system is expensive to run in terms of power and replacement of parts (pump maintenance, laser lifetime of approx. 7 years etc.). The author's point is that this system is already up and running, so the additional ability to do chamber measurements doesn't add cost.

Author comment:
We agree.

Reviewer comment:
This is true, and although it's also true to say you don't need to take samples off-site, you will still likely have to travel some distance as plot experiments really shouldn't be setup within the footprint range of an eddy C system (ideally more than 1 km away due to the exponential rise in fluxes observed after N fertiliser application and the potential for advection effects which nullify the assumptions made by the eddy c method). The air conditioned site thus becomes a mini-lab, in some cases closer to a field site where chamber measurements are made. Some discussion on the limitations of use is required, as the study seems to suggest N fertiliser plots could be setup next to an EC tower which would be very unwise unless the plots were to mimic the exact conditions of the field of interest to the EC measurements.

Author comment:
We positioned and aligned all static chamber measurements with overall site management conditions and associated research goals. Chambers were placed outside the immediate footprint of the eddy flux tower as determined by Wall et al. (2020). This meant that the chamber trial area was about 100 m (walking distance from the tower) to avoid the Referee´s concerns. Our previous work (Wecking et al., 2020), provided an example of how to conduct chamber measurements near to an EC tower. We demonstrated that the flux signal originating from the chamber plots was not strong enough to impact our EC measurements. To address the reviewers concern, we are going to add in a sentence recommending that care has to be taken when locating chambers near an EC tower to avoid cross-contamination.

Changes made to the manuscript:
L. 94, 95 We made clear that fluxes generated from the chamber plots did not nullify the assumptions made by the EC method. The following sentence was added to the manuscript: *"The physical distance between chamber plots and EC tower ensured that the EC footprint did not experience cross-contamination from chamber N2O fluxes (Wall et al., 2020)."*

Reviewer comment:
In terms of the flux experiment, I find the application of fertilisers to be far larger than is common practice. 300 Kg N ha-1 is very large, and 600 and 900 is beyond realistic. In these cases I assume some kind of saturation of N in the soil and N2O in the chamber during a 45 minute enclosure which would also affect the magnitude of the fluxes observed. In any case, the fluxes reported are of little use other than to compare the instruments.

Author comment:
Applications rates of ammonium nitrate fertiliser were chosen intentionally to trigger different low, medium and high N2O fluxes (line 18, line 95 f.). In this study, we did not intend to mimic realistic fertiliser application scenarios. Having said this, however, the N deposited in a single urine patch of dairy cattle (i.e. a major source of N2O emissions) are comparable to N loading commonly observed at 600 kg N2O-N ha-1 (Selbie et al., 2015, p. 238 and references therein).

Changes made to the manuscript:
We addressed this comment in **L. 116-119** "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 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).*"

Reviewer comment:
In this case, there is no reason to take means from plots. Due to the log-normal nature of N2O emissions, the (arithmetically derived) mean values reported from a small n size (less than 25 chambers) is going to be fairly uncertain. Without accounting for the log-normal nature of these fluxes in both time and space, any uncertainties in cumulative flux estimates are not statistically meaningful.

Author comment:
The study´s intention was not to discuss the effect of different applications rates of ammonium nitrate fertiliser on N2O fluxes. Treatment effects were only of secondary interest. Different rates of fertiliser were only applied to result in a wide range of N2O fluxes (low to high), and thereby to allow for a methodological comparison of GC and QCL data.

Reviewer comment:
I return to my original point that this comparison is of gas concentrations and not of plots. The fluxes derived from both instruments are valuable on a 1:1 basis as presented in Figs 3a and 3b. That's all the paper requires and it's a great result in terms of showing the system works as well as the GC.

Author comment:
Our study is a comparison of two methods, GC and QCL. Validation of this comparison is retrieved from different statistical tests (orthogonal regression, Bland Altman and bioequivalence) that enabled us to use a QCL for the purpose of analysing static chamber derived N2O samples under real field conditions. N2O concentration and flux data were determined and used to verify this objective.

Reviewer comment:
In conclusion, I think the work presented is a well carried out and valid study, but it needs a bit of a re-write to focus on the actual message, and not get distracted by flux comparisons and methods of comparing significance.

Author comment:

Perhaps this is where we disagree, we are focussed on comparing the whole process required to quantify fluxes from a chamber trial using a field-based QCL rather than a laboratory based GC. Even a field based GC would give a very different outcome because of the longer time needed for separating and analysing samples. Our study will provide a broader assessment for researchers considering using QCLs in the field including quantifying precision and accuracy of the approach coupled to time and resource needs.

In addition, testing the suitability of a new method (QCL) compared with a standard method (GC) requires suitable statistical tests that do not only compare the significance but let us evaluate the agreement and bioequivalence of these methods (line 198, we also recommend reading the references quoted in Section 2.5 – Bland and Altman, 1986 and Giavarina, 2015 – that provide powerful insights into the warrant of applying these statistical tests to both N2O concentrations and fluxes in our study).

Yours sincerely,
A. Wecking in the name of all associated Co-Authors
Hamilton, New Zealand – 30-06-2020

REFEREE #2

Major comments

Reviewer comment:
1) I partially agree with reviewer #1 that the overall idea looks a lot like something that could have been achieved in a simple comparison of concentration measurements. If you assume little error in the sampling itself and that the two analysers work with practically identical samples, there would be no reason to do this in the field, to generate the increased N2O concentrations via fertilisation instead of using standards, or to even calculate the fluxes at all (which are of course identical if the concentrations are identical). For an instrument comparison these would all be unwanted potential sources of error and confounding variables in the analysis. That said, I think I see the authors' reasoning, which is to showcase that their idea actually works well in practice and for its intended purpose (measuring fluxes). It is an unfortunate truth that just because something works well in the laboratory doesn't necessarily mean that it must work well in the field. I think this misconception is something that can be remedied quite easily by explicitly discussing early in the manuscript how and why this is much more than just comparing two instruments' ability to measure concentrations. It left me quite puzzled throughout half of the manuscript, because it only really becomes clear after reading and thinking about it for a while.

Author comment:
As pointed out by Referee #2, it was our intended purpose to test whether a QCL analyser could be used for the injection of chamber derived N_2O samples in the fields. We consciously decided to develop a sampling/analytic approach that would reliably work in the fields, instead, of testing a laboratory approach only. Based on own experiences, we found that an applied approach as such would be very useful for other users who conduct measurements with static chambers and eddy covariance but are interested in shortening analysis times, receive immediate measurement results and have an interest in reducing costs by accessing the full potential of the QCL analyser. We acknowledge the Referee's comment and are going to add in a specific acknowledgement of the purpose of our work at the end of the introduction in the revised version of the manuscript.

Changes made to the manuscript:
L. 79 following We clarified the purpose of our research at the end of the introduction more

precisely: "*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 N2O fluxes, was provided by statistical tests: 1) orthogonal regression; 2) Bland Altman and 3) bioequivalence analyses.*"

L. 489 and L. 516 *"practical tool", "rapid field analysis of N_2O samples"* – We adapted our language in the conclusion of the manuscript to tie our final statements back to the last paragraph of the introduction.

Reviewer comment:

2) I don't really get the data workflow. What does the QCL output (shouldn't it directly be ppb?), what is QCL peak area in mV supposed to be and how was it translated into concentrations? Figure S1 also doesn't make a lot of sense to me due to this. What is "N2O calculated"? If you did a calibration with standards in glass syringes and those result in higher peak areas, how can you then accurately calculate concentrations for samples that were obtained with plastic syringes which apparently result in lower peak areas...? Obviously it did work in some way or you wouldn't get so similar results to GC, but you have absolutely lost me somewhere on the way there. Expanding section 2.3 would help a lot. I would like to see basically a recipe to get from the QCL output to whatever you did in Fig. S1 (and further)

Author comment:

*For clarification: The injection of 1 mL sample volumes into the QCL resulted in an output of peak area data (i.e. similar to the output as received after GC analysis). In the subsequent data analysis, we calculated the area under each peak for 1) sample injections of unknown N_2O concentration, and 2) injected N_2O standards of known N_2O concentration. We used the results from 2) to generate quadratic models which we then fitted to the data from 1) to translate outputs into N_2O concentrations (see Section 2.3 of the manuscript). We agree to the referee that explaining the procedure demands additional clarification, which we will implement to L. 159 of the manuscript.

We conducted preliminary tests using glass and insulin syringes in which we applied the above translation from N_2O peak areas to N_2O concentrations. All data presented in the manuscript were purely based on injections made using glass syringes. This applied to both: 1) the injection of N_2O samples and 2) N_2O standards. To avoid confusion about the use of different syringe types, we now intend to remove Fig. S1 from the supplementary material. Instead, we will include additional explanations expanding on Section 2.3 and our work-flow procedures as suggested by the referee.

Changes made to the manuscript:

We expanded on the recommendation of the referee by:

L. 138, 139 Adding a sentence to clarify that all samples were stored in septum-seal Exetainers until the date of their analysis. *"All samples remained in the septum-sealed Exetainers until the date of their analysis."*

L. 155 Making clear that we collectively injected all QCL samples on one day, "*17 September*", only. The sentence in this line was modified to: "The second batch of N2O 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)."

L. 182 We deleted this sentence to avoid further confusion regarding syringe types used. The deleted sentence was*: "Generally, using a 1 mL glass syringe was preferred to commonly used insulin syringes because of its higher accuracy resulting in greater output peak areas (Fig. S1c)."* We also included some new information **L. 190 following**: *"Finally, it was important to keep a record of the injected sample and standard sequence to allow for re-identification in the raw output data of the QCL."*

Supplementary material We re-arranged former Figure S1 and deleted part (c) from Figure S1 to avoid confusion about syringe types. Note Figure S1 is now Figure S2, since we as well added an additional figure to the supplementary material (see comment below).

L. 195 following We added a sentence to the first paragraph of Section 2.3 in order to highlight the importance of the relationship between peak area and N_2O concentration. *"Data processing, therefore, first determined the relationship between peak area and (known) N2O concentration (CN2O) of the injected standards."* Changes in wording were also made to the first and the third sentence of this paragraph as well as to referencing the figures in the supplementary material.

Supplementary material To address the referee's #2 interest in our data processing procedures, we introduced a new figure to the supplements. This **new Figure S1** shows the raw QCL output data (peak areas) from manual injections into the QCL. We believe, that the new Figure S1 will visualise and complement the content of Section 2.3. (Note, former Figure S1 is now labelled as Figure S2 both in the running text and in the supplementary material. The caption of **Figure S2** has changed to: "*Tests conducted prior to the main study showing the calculated normal linear relationship between output peak area and N2O concentration (CN2O) for different scenarios and for different ranges of N2O standards injected: (a) from 0.2 to 10 ppm and (b) from 0.2 to 0.5 ppm; (c) demonstrates the effect of flow rate in L min–1 on the slope of the associated regression lines, output peak area and N2O concentration in ppm.*")

Reviewer comment:

3) This is in regards to L327-339 - I fully admit I'm not overly familiar with bioequivalence statistics, but I have a strong feeling that you are boldly overstating what it can do. From what you wrote and what I could find in your sources, it's still just frequentist inferential statistics. Don't get me wrong, I applaud that you are willing to do solid statistics and think outside the old t-tests-and-scatterplots box. But to me this honestly just looks like another type of null-hypothesis significance testing, with even more 100 % arbitrary (but hopefully consensus-based) thresholds and ranges. I.e. there is nothing objective and certainly nothing that justifies calling something a "proof" about it. I suggest to word pretty much everything about bioequivalence with a bit less praise. It's a good and interesting approach and it makes sense to apply it to fluxes, but that's about it.

Author comment:

*The reviewer is correct that the bioequivalence test performed in our study (as described in Section 3.4.1) is a frequentist hypothesis test. However, what is vitally important here is that the test is designed to assess (e.g. that two products are the same) . In our experiment, we were interested in whether calculated N_2O concentrations/fluxes from QCL (F_{N2O_QCL}) are effectively the same as those determined by a standard method, i.e. in our case laboratory-based GC ($FN_2O_{\_GC}$). The bioequivalence test allowed us to assess this. The equivalence range (i.e. maximum acceptable difference) does need to be specified, and this perhaps could be seen as arbitrary. However, here it is important to be aware of the following: that 1) this is set a-priori to analysis and 2) this explicitly defines what is meant by bioequivalent (as explained in Section 2.5). We have endeavoured to make very clear in the paper what the equivalence range is (e.g. L210, L334, L338, L342, L350, Figure 6) and how this determines the definition of "bioequivalence" (e.g. L205-215). Following the reviewer's advice, we are going to rephrase all sentences regarding bioequivalence so that we don't overstate

what the methodology can do. This includes changing “proof of bioequivalence” to “evidence of bioequivalence” (lines 334, 336, 340, 345, 351).*

Changes made to the manuscript:

L. 26, 27, 422, 424, 428, 431, 432, 496 We changed the word *“bioequivalence” to “equivalence”* to specify our language and indicate that we precisely define what is meant when using the term *“equivalence range”*.

L.420, 426, 429, 434, 437, 496 To satisfy the reviewer, we changed *“proof” to “evidence”* and used a more defined language around the terminology of *“bioequivalence”*.

Reviewer comment:

4) In section 3.4.2 you write about "using a QCL [...] without much disruption of other measurements". I respectfully disagree with that, considering that in section 3.3 you say that you already need an initial lag time of 10 to 30 mins, so I assume you lose at least two half-hourly EC measurements for a single sample (How much is it actually? Please state it in the manuscript!). This is something that I, as someone working at an EC station, would not want to sacrifice at least during daytime and/or after significant management events (but I wouldn’t want to inject gas samples the whole night either). You mention postponing analysis later in section 3.4.2. I would like to ask you to elaborate on this idea. Would it make sense to collect multiple samples and analyse them in one batch? Is this feasible in the field? In Tab. 1 you state that you can inject 200 samples per hour. For how long can the samples be stored on site (e.g. could it wait until the next maintenance of the EC system excl. the QCL)? I would like to see some of your ideas on this and maybe an actual example for a sampling plan that minimises EC downtime. This disruption is a core issue for anyone doing EC, so it should play a much more central role in the discussion.

Author comment:

We acknowledge the Referee’s interest in the capability of the QCL device to provide rapid analysis of chamber derived N_2O samples. As pointed out in L. 133, Section 2.2.3, the conversion of the EC QCL system to the injection mode took less than 30 min. This time comprised the establishment of an operational set-up including: to assemble and mount tubes, gas bottles etc. to the QCL, adjust the flow of N_2 carrier gas and let the temperature-controlled enclosure system of the QCL housing adjust and recover from the disruptions made (e.g. lid-opening, flow-rate change). Afterwards and as identified by the referee, it is recommended to inject as many samples as available and avoid EC down-time. In L. 378, we have already indicated (see quotation of Faust and Liebig, 2018) that storing gas samples in Exetainer vials is possible and allows to minimise disruptions to EC measurements. We will add a couple of sentences to the end of Section 3.4.2 to provide further clarification about the advantages of our injection technique – as indicated by the referee.

Changes made to the manuscript:

We believe that this disagreement was based on misinterpretation of some of the content discussed in Section 3.3. and Section 3.4.2. To clarify that sample analysis after injection into the QCL becomes possible within seconds (not half-hours as interpreted by referee #2) we clarified the following:

L. 138 Adding a sentence to indicate that we stored all our chamber samples in septum-sealed Exetainers*: “All samples remained in the septum-sealed Exetainers until analysis.”*

L. 155 Specification of sentence content: *The second batch of N_2O samples was analysed collectively on the day after the last chamber sampling, 17 September, […].”* *Underlined* words were added to the sentence. **Table S1**: We also added this information to the last column of supplementary Table S1.

Supplementary material We introduced a new figure, Figure S1, that provides an example of the temporal frequency (x-axis) at which we injected individual N2O samples into the QCL. Figure S1 is referenced in the running text of the manuscript in **L. 195, 389**

L. 385 following Clarification of wording to point out that the QCL needs an initial lag time of 10-30 min (assembling of the set-up) before manual injections become possible. *"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."* Underlined words were added to the sentence.

L. 390 new sentence: *"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."*

L. 475 following We addressed the referee's comment to expand on the idea to minimise EC downtime by adding the following sentences to the paragraph: *"Nonetheless, we here recommend to collectively inject a great number of N2O 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)".* Other minor changes were made to the paragraph in order to tidy-up language and wording.

Minor comments of referee #2

- **Reviewer comment:**
  L97: Please give a justification for the very high application rates somewhere around here.
  Changes made to the manuscript: *"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 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)."* This was added to **L. 116-119** to address the comments of referee #2

- **Reviewer comment:**
  Check typographical rules for formulae, etc. Variables should be cursive (but _descriptive_ indices upright).
  Changes made to the manuscript: Equation 1 and 2 were adjusted and font changed to italics, see **L. 211** and **L. 247** of the manuscript

- **Reviewer comment:**
  L163: "Since the quadratic fit suited lower C_N2O better than a linear fit, quadratic models were preferred [...]" The fit will naturally be better (in terms of R2) if you throw more parameters at your model. Am I missing something here?
  Authors' response: We used a quadratic model to build the standard curve for the injected standards of known N2O concentration.
  Changes made to the manuscript: We added a new sentence to **L. 195**: *"Data processing, therefore, first had to determine the relationship between peak area and (known) N_2O concentration (C_{N2O}) of the injected standards."* **L. 196 following** we complemented a few

sentences by adding "*standard curve*" and "C_{N2O}" to **L. 198, 202** in order to clarify our way of calculation. An addition was also made **to L. 204**, *"was between 0.3-10 ppm"* to clarify the minimum and maximum range of real sample N2O concentration.

- **Reviewer comment:**
  L203: Power depends on (among other things) the sample size. You can't just say a 90 % CI corresponds to 80 % statistical power.
  Changes made to the manuscript: We addressed this comment by changing "*corresponding*" to: *"(at a standard power level of 80 %)"*, **L. 263.**

- **Reviewer comment:**
  L268: I think here you can replace "might explain" with "explains". At least to my understanding it's somewhat trivial that you calculate a larger flux if you measure higher concentrations with the QCL, no?
  Changes made to the manuscript: We agree with the referee and changed **L. 339** from "*might explain*" to "*explain*".

- **Reviewer comment:**
  L314-315: Have you tested injecting blanks and see what happens?
  Authors' response: Yes, we have. The injection of blanks did not result in any variation of the baseline flow of N2 carrier gas.
  Changes made to the manuscript: We added this information to **L.390**: "*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.*"

- **Reviewer comment:**
  L520: Typo "TAylor"
  Changes made to the manuscript: Changed to "*Taylor*" **L. 643**

- **Reviewer comment:**
  Figure 3: Panel c and d should have equal scaling on their respective x and y axes (i.e. the 1:1 line should be the diagonal).
  Authors' response: Axis scale and orientation of Figure 3c and 3d meet the prerequisites of using and depicting an orthogonal regression correctly. This means, the slope of the orthogonal estimation line (= major axis) is intermediate between the slope of the Y. X estimation line and the inverse of the slope of the X.Y estimation line.

- **Reviewer comment:**
  Table S3: Where were the soil samples taken?
  Authors' response: As mentioned in **L. 119 following**, Section 2.2.1: *"Separate areas adjacent to the twelve chamber plots were established to collect soil samples for laboratory analyses of soil moisture and soil mineral nitrogen (Nmin). Soil moisture and water-filled pore space (WFPS) were analysed and calculated using the methods described in Wecking et al. (2020a).[…]"*

Additional minor changes:

Dear editor, please, note that all other (very minor) changes made to the manuscript but not specifically mentioned in the above, were implied by the authors. Additional changes made were only to: 1) correct spelling mistakes; 2) support further clarification of the content of the manuscript; and 3) indicate the placement of figures and tables in the running text of the manuscript by: [FIGURE 1 ABOUT HERE]

Also, we hope that the line numbers in the revised version of the manuscript will still match to what we have explained in the above. I.e. that there will not be a miss-match in line numbers due to any difference in the version of Microsoft Word. We used the "**show revisions in balloons**" view.

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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.

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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

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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).

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Orthogonal regression analysis was used to quantify the correlation between F_{N2O_QCL} and F_{N2O_GC}. However, as correlation studies include limitations when assessing the comparability between two methods (i.e. GC and QCL), we also conducted Bland Altman and bioequivalence analyses to determined the degree to which N_2O concentrations and fluxes derived from QCL would be comparable to GC.¶

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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

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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

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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).

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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

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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}.

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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).

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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

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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

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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.

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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.

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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]

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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

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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.

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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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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_2O 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 through the injection tube (inlet 2), the QCL was readily set-up for receiving injections of N_2O 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.

Figure 2: Fluxes of nitrous oxide (F_{N2O}) determined from (a) gas chromatography (F_{N2O_GC}) and (b) quantum cascade laser absorption spectrometry (F_{N2O_QCL}). Symbols depict mean F_{N2O} and marker shading displays the rate of ammonium nitrate (AN) applied: AN_0 (black squares), AN_{300} (dark grey diamonds), AN_{600} (light grey upside-down triangles) and AN_{900} (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 and mineral nitrogen contents associated with flux measurements are provided in the supplementary material, Table S3.

Figure 3: Orthogonal regression analysis of standardised N_2O concentrations (C_{N2O}) and fluxes (F_{N2O}). Data were distinguished by their analytic source of origin, i.e. GC (C_{N2O_GC}, F_{N2O_GC}) and QCL (C_{N2O_QCL}, F_{N2O_QCL}). The regression analysis included all C_{N2O} in (a) but only those C_{N2O} measured at control sites (AN_0) in panel (c). The orthogonal regression analysis was repeated for standardised F_{N2O} with (b) showing all F_{N2O_GC} and F_{N2O_QCL}, and (d) depicting the orthogonal regression for AN_0 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 of the differences between the GC and QCL method expressed as the percentage difference of the standard method A (F_{N2O_GC}) and the new method B (F_{N2O_QCL}) 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_{N2O_GC} and F_{N2O_QCL} from control (AN_0) sites. The percentage mean difference (bias) between F_{N2O_GC} and F_{N2O_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

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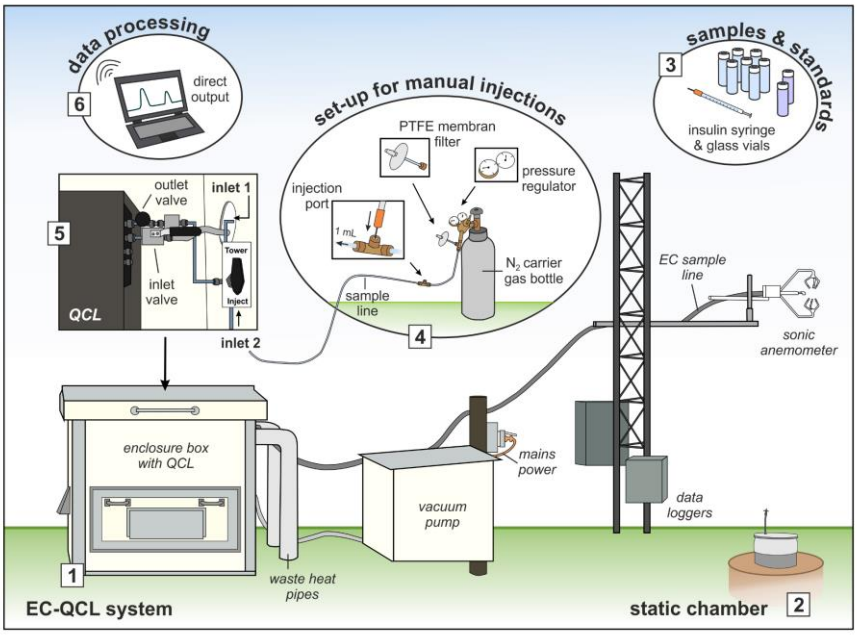
Figure 6: Bioequivalence analysis for N_2O concentrations (C_{N2O}) in (a-d) and N_2O fluxes (F_{N2O}) in (e) with GC defined as the standard method. C_{N2O} and F_{N2O} based on QCL analysis were considered bioequivalent when the 90% confidence interval (CI) of the difference between QCL and GC (x-axis) was completely within the predefined ± 5% bioequivalence range of the difference of the standard method. The bioequivalence analysis was distinguished for C_{N2O} 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 determined for F_{N2O} in panel (e), here distinguished by AN application rate on the y-axis.

List of tables

Table 1: Comparison of the GC and QCL injection methods. Details provided in the below table specifically relate to the application of the two techniques in this study (i.e. have not been generalised). NZD = New Zealand dollars.



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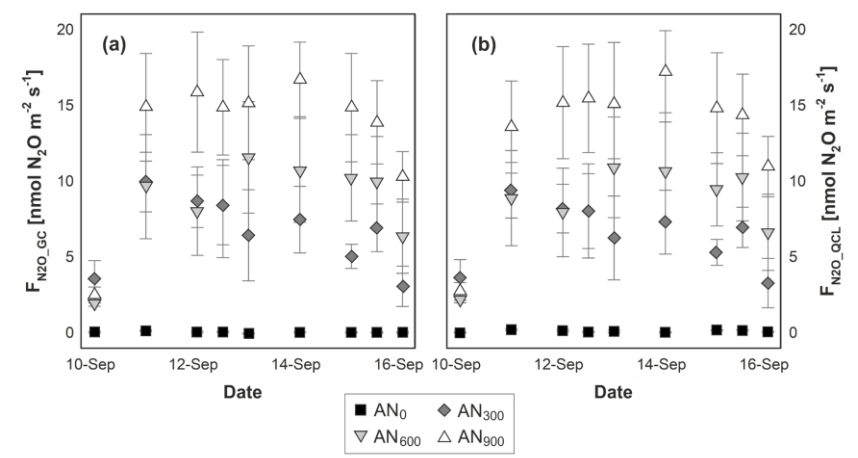

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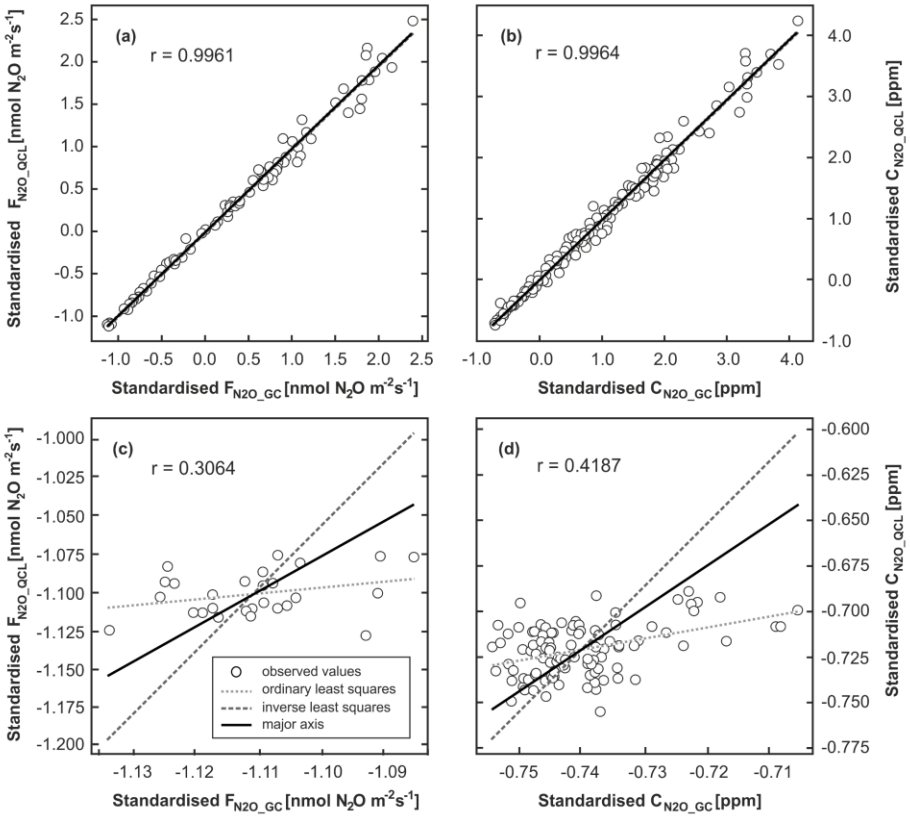

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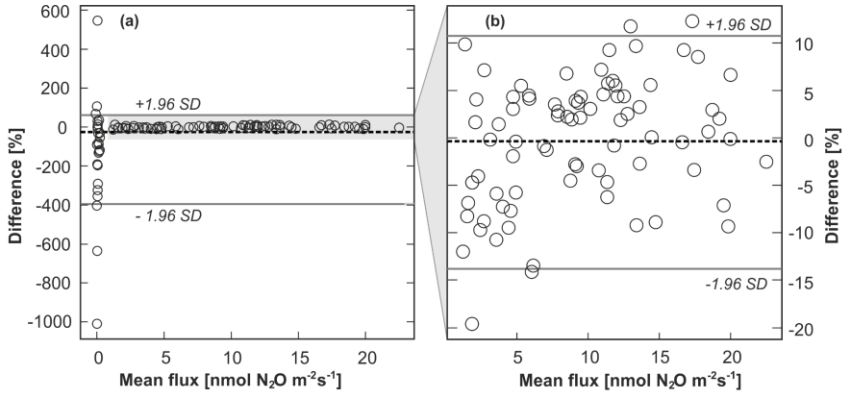

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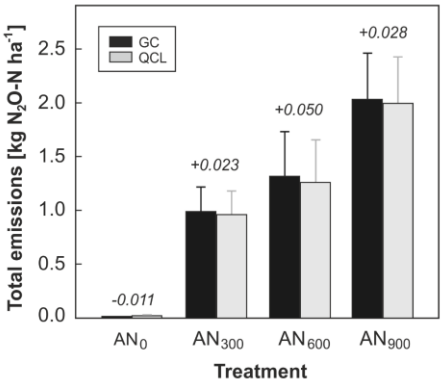

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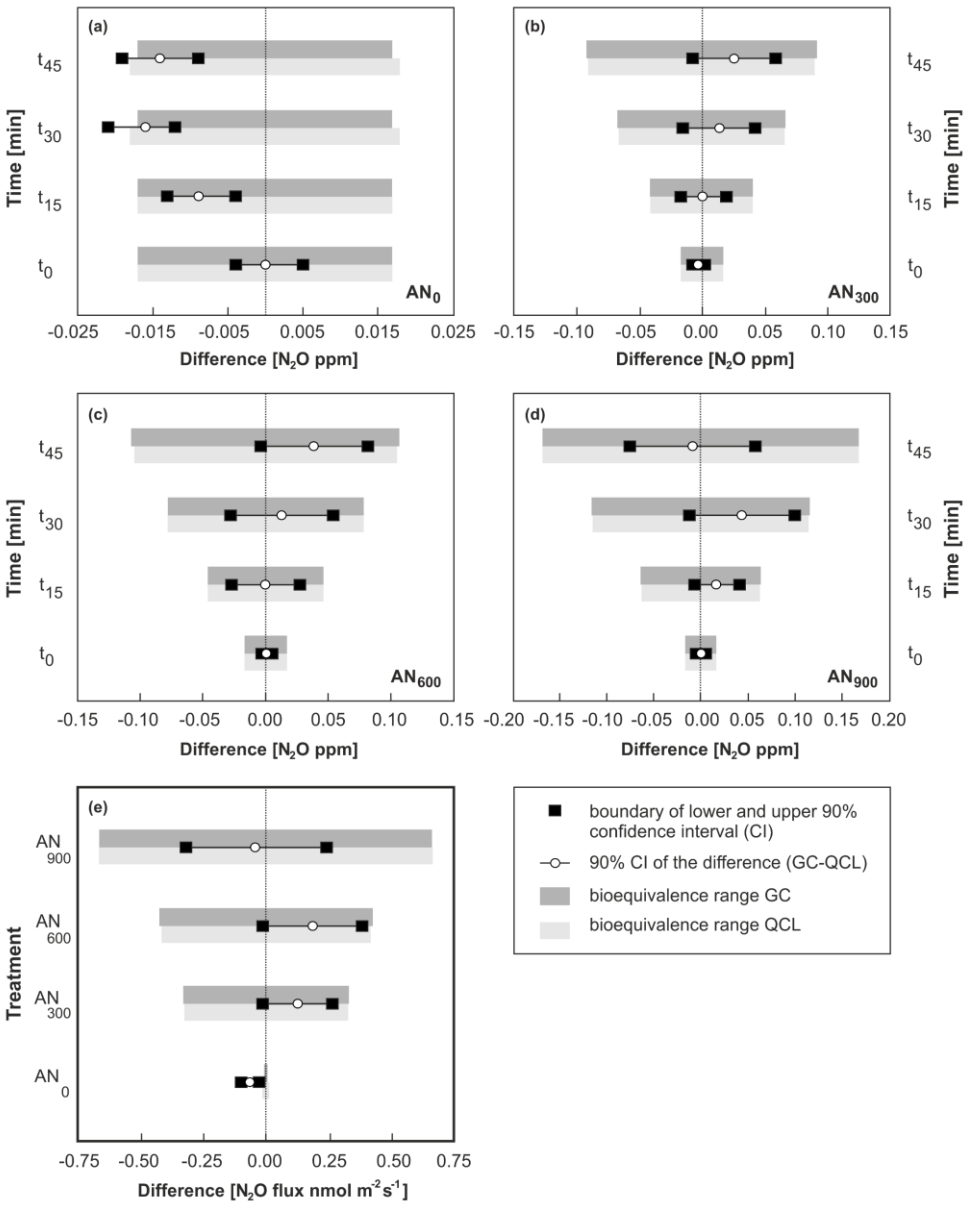
(a)
(b)
(c)
(d)
(e)
Time [min]
t45
t30
t15
t0
AN0
AN300
AN600
AN900
Difference [N2O ppm]
Treatment
AN 900
AN 600
AN 300
AN 0
Difference [N2O flux nmol m-2 s-1]
boundary of lower and upper 90% confidence interval (CI)
90% CI of the difference (GC-QCL)
bioequivalence range GC
bioequivalence range QCL

Figure 6: Bioequivalence analysis for N_2O concentrations (C_{N2O}) in (a-d) and N_2O fluxes (F_{N2O}) in (e) with GC defined as the standard method. C_{N2O} and F_{N2O} 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_{N2O} 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 determined for F_{N2O} in panel (e), here distinguished by AN application rate on the y-axis.

Table 1: Comparison of the GC and QCL injection methods. Details provided in the below table specifically relate to the application of the two techniques in this study (i.e. have not been 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_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

Supplementary material

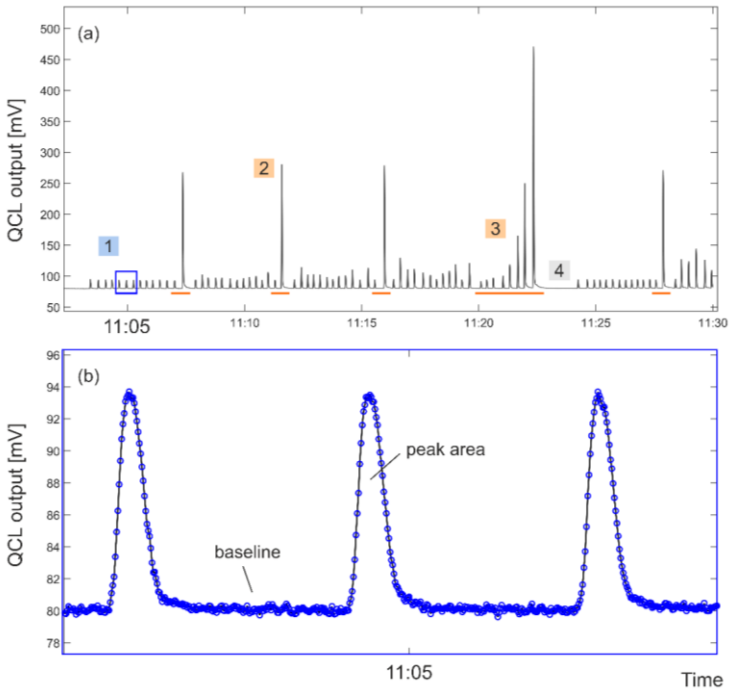

Figure S1: Example of QCL output data depicting how a one half-hourly peak progression sequence looked like. Panel (a) shows the full sequence for injected N_2O samples and standards in a given half hour from 11-11:30 AM, 17 September 2020. Panel (b) captures three individual peaks from within this time period (1) (blue rectangle). Single measurement points are depicted by blue dots with the black line showing an interpolated curvature. Orange bars underneath individual peaks in panel (a) distinguish injected N_2O standards from N_2O samples. (2) identifies 1 ppm and 5 ppm standards injected after every 12 samples, here serving as a running control; (3) shows an example of an injected standard line of known N_2O concentration (range: 0.2–10 ppm); and (4) the lag time that was required to ensure sufficient flushing of the QCL sample cell after injecting a sample or standard (here 10 ppm) of higher N_2O concentration.

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Figure S1

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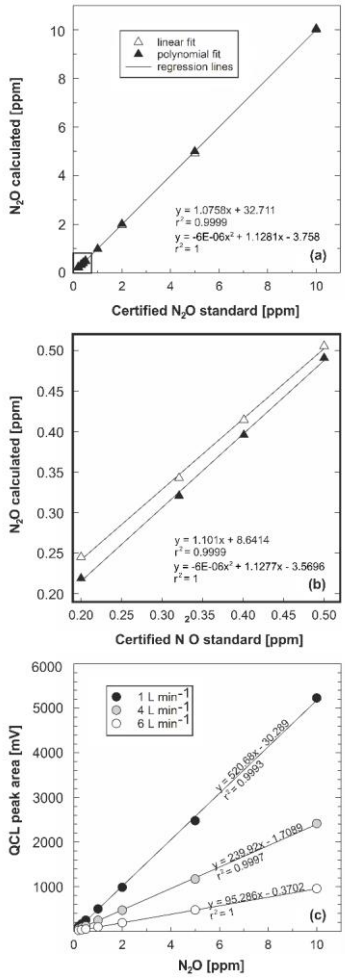

Figure S2: Tests conducted prior to the main study showing the calculated normal linear relationship between output peak area and N_2O concentration (C_{N2O}) for different scenarios and for different ranges of N_2O standards injected: (a) from 0.2 to 10 ppm and (b) from 0.2 to 0.5 ppm; (c) demonstrates the effect of flow rate in L min^{-1} on the slope of the associated regression lines, output peak area and N_2O concentration in ppm.

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Table S1: Chronology of experimental activities.

Date	Activity
15-Aug-19	Trial site fenced off
	Preliminary injection into QCL: testing different syringe types
20-Aug-19	Installation of chamber collars
30-Aug-19	Preliminary injections into QCL: testing different flow rates
10-Sep-19	Treatment application to chamber and soil plots
	Gas and soil sampling – run 1
11-Sep-19	Gas and soil sampling – run 2
12-Sep-19	Gas and soil sampling – run 3 & 4
13-Sep-19	Gas and soil sampling – run 5
14-Sep-19	Gas and soil sampling – run 6
15-Sep-19	Gas and soil sampling – run 7 & 8
16-Sep-19	Gas and soil sampling – run 9
17-Sep-19	Sample injection into QCL

Table S2: Certified N_2O standards used in this study and associated uncertainty levels. Certified N_2O standard concentrations printed in bolt fond were used in quadratic curve models to calculate final sample N_2O concentration.

N_2O [µL L^{-1}] [ppmv]	**Uncertainty** [alpha/beta] [%]	**Background** (gas)	**Company** (name)
0.200	± 0.01	Nitrogen	BOC Ltd.
0.321	± 0.1–0.9%	Cryogenic UltraPure Air	Praxair, Inc.
0.3252	± 0.01	Air	NIWA
0.401	± 0.1–0.9%	Cryogenic UltraPure Air	Praxair, Inc.
0.500	± 0.01	Nitrogen	BOC Ltd.
1.00	± 0.01	Nitrogen	BOC Ltd.
2.00	± 0.02	Nitrogen	BOC Ltd.
5.00	± 0.1	Nitrogen	BOC Ltd.
10.00	± 0.2	Nitrogen	BOC Ltd.
20.00	± 0.2	Nitrogen	BOC Ltd.
50.00	± 1.0	Nitrogen	BOC Ltd.
100.00	± 1.0	Nitrogen	BOC Ltd.

Table S3: This table presents the measured values of nitrous oxide fluxes (F_{N2O}) analysed by GC and QCL, soil water-filled pore space (WFPS), soil ammonium (NH_4^+) and nitrate (NO_3^-) content of the control (AN_0) and across the different treatments of ammonium-nitrate (AN_{300}, AN_{600}, AN_{900}) applied The associated standard error of the mean (SEM) is provided at the right hand side of each control/treatment column.

date	AN_0	SEM	AN_{300}	SEM	AN_{600}	SEM	AN_{900}	SEM
GC nitrous oxide flux [F_{N2O_GC} in nmol N_2O m^{-2} s^{-1}]								
10-Sep-2019	0.04	0.05	3.56	1.20	1.95	0.19	2.49	0.52
11-Sep-2019	0.13	0.04	9.93	1.97	9.63	3.44	14.88	3.55
12-Sep-2019*	0.06	0.05	8.67	1.73	8.02	2.92	15.87	3.96
12-Sep-2019*	0.06	0.01	8.42	2.62	8.19	3.23	14.87	3.15
13-Sep-2019	-0.05	0.03	6.43	3.00	11.57	3.68	15.16	3.76
14-Sep-2019	0.03	0.01	7.46	2.19	10.71	3.43	16.71	2.46
15-Sep-2019*	0.02	0.03	5.03	0.80	10.21	2.84	14.85	3.58
15-Sep-2019*	0.03	0.03	6.92	1.57	9.98	2.96	13.88	2.75
16-Sep-2019	0.02	0.04	3.06	1.33	6.37	2.45	10.29	1.67
QCL nitrous oxide flux [F_{N2O_QCL} in nmol N_2O m^{-2} s^{-1}]								
10-Sep-2019	0.00	0.03	3.65	1.18	2.17	0.19	2.74	0.60
11-Sep-2019	0.21	0.05	9.40	1.83	8.88	3.14	13.57	3.04
12-Sep-2019*	0.14	0.07	8.19	1.60	7.94	2.92	15.17	3.71
12-Sep-2019*	0.06	0.02	8.02	2.47	8.04	3.11	15.46	3.57
13-Sep-2019	0.09	0.08	6.25	2.77	10.91	3.33	15.09	4.05
14-Sep-2019	0.03	0.02	7.30	2.10	10.66	3.24	17.22	2.71
15-Sep-2019*	0.17	0.01	5.30	0.86	9.46	2.42	14.81	3.65
15-Sep-2019*	0.18	0.03	6.95	1.33	10.27	2.89	14.36	2.69
16-Sep-2019	0.06	0.01	3.28	1.63	6.63	2.51	10.97	1.99
Water filled pore space of the soil [%]								
10-Sep-2019	79.43	0.48	78.66	1.82	78.06	1.40	82.30	2.35
11-Sep-2019	81.64	0.59	84.97	1.68	80.16	0.53	82.13	1.79
12-Sep-2019	82.18	1.12	80.63	1.23	79.35	1.05	79.20	1.00
13-Sep-2019	79.62	0.95	79.72	1.87	76.62	2.08	78.13	1.76
14-Sep-2019	79.43	0.56	80.60	2.00	78.37	1.74	77.78	1.19
15-Sep-2019	79.79	0.50	81.70	2.65	77.17	1.49	76.81	0.37
16-Sep-2019	77.92	1.06	81.05	1.98	73.93	1.60	77.41	1.80
Soil ammonium [kg NH_4^+ ha^{-1}]								
10-Sep-2019	1.82	0.50	81.73	5.20	89.36	2.72	264.63	17.19
11-Sep-2019	0.81	0.11	52.26	7.18	141.51	11.08	233.63	33.62
12-Sep-2019	2.15	0.57	44.61	6.52	109.37	6.77	213.76	3.41
13-Sep-2019	2.21	0.33	36.88	6.75	124.48	9.36	194.76	18.88
14-Sep-2019	3.71	0.09	20.31	5.07	59.88	6.05	188.70	18.05
15-Sep-2019	1.84	0.64	9.58	0.99	78.98	12.30	155.84	18.49
16-Sep-2019	1.80	0.29	13.21	3.23	38.50	4.59	124.38	7.64

	Soil nitrate [kg NO_3^- ha^{-1}]							
10-Sep-2019	2.99	0.37	83.67	3.87	104.95	1.33	267.77	15.17
11-Sep-2019	2.46	0.18	69.08	6.54	149.95	8.62	248.89	33.69
12-Sep-2019	2.29	0.07	79.41	6.57	142.52	8.61	230.94	7.36
13-Sep-2019	1.64	0.20	82.21	7.92	149.85	6.25	232.40	13.77
14-Sep-2019	1.84	0.35	73.37	12.71	114.20	8.41	237.77	8.96
15-Sep-2019	2.47	0.31	78.91	1.51	162.60	8.72	231.51	16.94
16-Sep-2019	1.85	0.22	92.49	16.22	134.38	7.60	211.88	18.92

* flux measurements conducted twice daily at 10 AM and 12 PM
SEM = standard error of the mean

Table S4: Results from the linear functional relationship analysis (orthogonal regression). Columns labelled C_{N2O} show results of the regression analysis when using standardised N_2O concentrations. Columns labelled F_{N2O} provide results based on standardised N_2O fluxes. Part of the regression analysis was to characterise both data streams by treatment and control, i.e. first including all data (AN_0, AN_{300}, AN_{600}, AN_{900}) in the analysis and then, separately, only the control (AN_0).

	C_{N2O} all AN	**C_{N2O}** AN_0 only	**F_{N2O}** all AN	**F_{N2O}** AN_0 only
Number of observations	432	108	108	27
Response mean	-0.003164	0.3272	-0.004008	0.3776
Explanatory mean	0.003164	-0.3272	0.004008	-0.3776
Response variance	0.9811	1.238	0.9860	1.139
Explanatory variance	1.021	0.5551	1.023	0.6029
r^2 value	0.9928	0.1753	0.9922	0.0939
r value	0.9964	0.4187	0.9961	0.3064
Angle between Y on X and X on Y	0.2068	42.32	0.2229	54.59
Major eigenvalue	1.999	1.384	2.005	1.241
Minor eigenvalue	0.003606	0.4096	0.003901	0.5017
Bootstrap resampling	200	200	200	200
Ordinary least squares:				
Constant	-0.006253	0.532	-0.007926	0.537
Standard error	0.003914	0.1038	0.007861	0.26
Lower	-0.01331	0.3101	-0.02204	-0.02
Upper	0.001710	0.734	0.006998	1.030
Slope	0.9766	0.625	0.9778	0.421
Inverse least squares:				
Constant	-0.006276	1.49	-0.007957	2.072
Standard error	0.003902	0.6585	0.007902	82.46
Lower	-0.01369	0.9211	-0.02246	-44.95
Upper	0.001786	3.478	0.007118	18.732
Slope	0.9837	3.567	0.9854	4.486
Major axis:				
Constant	-0.006264	1.108	-0.007941	1.326
Standard error	0.003904	0.44	0.007872	40.17
Lower	-0.01349	0.7105	-0.02217	-19.84
Upper	0.001610	2.484	0.006920	9.937
Slope	0.9801	2.387	0.9815	2.511

Table S5: Bland-Altman analysis for F_{N2O_GC} and F_{N2O_QCL} distinguished by treatment in units nmol m^{-2} s^{-1}, if not specified otherwise. This table provides a summary based on mean F_{N2O_GC} and F_{N2O_QCL} across replicates of the same treatment. Fig. 4, instead, illustrates the results of individual F_{N2O_GC} and F_{N2O_QCL} (not depicted in the below table) for each replicate and each treatment as the percentage mean difference between the two methods, i.e. GC (A) and QCL (B).

Sampling	**Treatment**	**GC (A)**	**QCL (B)**	**Mean**	**Difference**	**Difference (%)**
[No.]	[kg N ha^{-1}]	F_{N2O_GC}	F_{N2O_QCL}	(A+B)/2	(A-B)	((A-B)/mean)*100
1	0	0.04	0.00	0.02	0.04	182.48
1	300	3.56	3.65	3.61	-0.09	-2.59
1	600	1.95	2.17	2.06	-0.23	-11.11
1	900	2.49	2.74	2.61	-0.24	-9.24
2	0	0.13	0.21	0.17	-0.08	-44.70
2	300	9.93	9.40	9.67	0.53	5.51
2	600	9.63	8.88	9.26	0.75	8.11
2	900	14.88	13.57	14.22	1.31	9.20
3	0	0.06	0.14	0.10	-0.08	-78.52
3	300	8.67	8.19	8.43	0.48	5.69
3	600	8.02	7.94	7.98	0.08	0.98
3	900	15.87	15.17	15.52	0.70	4.51
4	0	0.06	0.06	0.06	0.00	1.93
4	300	8.42	8.02	8.22	0.39	4.79
4	600	8.19	8.04	8.11	0.15	1.82
4	900	14.87	15.46	15.16	-0.59	-3.89
5	0	-0.05	0.09	0.02	-0.14	-595.36
5	300	6.43	6.25	6.34	0.18	2.88
5	600	11.57	10.91	11.24	0.66	5.88
5	900	15.16	15.09	15.13	0.07	0.49
6	0	0.03	0.03	0.03	0.00	4.14
6	300	7.46	7.30	7.38	0.16	2.19
6	600	10.71	10.66	10.68	0.05	0.47
6	900	16.71	17.22	16.96	-0.51	-3.02
7	0	0.02	0.17	0.09	-0.15	-157.04
7	300	5.03	5.30	5.17	-0.27	-5.22
7	600	10.21	9.46	9.84	0.75	7.67
7	900	14.85	14.81	14.83	0.03	0.22
8	0	0.03	0.18	0.10	-0.15	-149.70
8	300	6.92	6.95	6.94	-0.02	-0.34
8	600	9.98	10.27	10.13	-0.29	-2.86
8	900	13.88	14.36	14.12	-0.48	-3.39
9	0	0.02	0.06	0.04	-0.04	-105.26
9	300	3.06	3.28	3.17	-0.22	-6.86
9	600	6.37	6.63	6.50	-0.26	-4.02
9	900	10.29	10.97	10.63	-0.68	-6.39

Table S6: Bioequivalence analysis for N_2O concentrations (C_{N2O}) and associated fluxes (F_{N2O} in bottom panel of the table). C_{N2O_QCL} and F_{N2O_QCL} were considered bioequivalent when the 90% confidence interval of the difference was completely within the predefined ± 5% bioequivalence range of difference to C_{N2O_GC} and F_{N2O_GC} (corresponding to a test with size 0.05). rep. = replicates, d.f = degrees of freedom, s.e.d = standard error of the difference, LSD = least significant difference

Time/ Treatment	**Mean**		**Standard error of the difference of the mean**			**LSD**	**90% confidence interval**			**Bioequivalence range**			
AN_0	**C_{N2O_GC}** [ppm]	**C_{N2O_QCL}** [ppm]	rep.	d.f	s.e.d	s.e.d	difference (GC-QCL)	lower	upper	GC lower	GC upper	QCL lower	QCL upper
t_0	0.333	0.332	27	26	0.0027	0.0046	0.000	-0.004	0.005	-0.017	0.017	-0.017	0.017
t_{15}	0.333	0.342	27	26	0.0028	0.0048	-0.009	-0.013	-0.004	-0.017	0.017	-0.017	0.017
t_{30}	0.335	0.352	27	26	0.0029	0.0049	-0.016	-0.021	-0.012	-0.017	0.017	-0.018	0.018
t_{45}	0.340	0.354	27	26	0.0027	0.0046	-0.014	-0.019	-0.009	-0.017	0.017	-0.018	0.018
AN_{300}													
t_0	0.333	0.336	27	26	0.0028	0.0048	-0.003	-0.007	0.002	-0.017	0.017	-0.017	0.017
t_{15}	0.822	0.821	27	26	0.1090	0.0186	0.001	-0.017	0.020	-0.041	0.041	-0.041	0.041
t_{30}	1.341	1.327	27	26	0.0168	0.0286	0.014	-0.015	0.042	-0.067	0.067	-0.066	0.066
t_{45}	1.831	1.804	27	26	0.0192	0.0327	0.026	-0.007	0.059	-0.092	0.092	-0.090	0.090
AN_{600}													
t_0	0.336	0.335	27	26	0.0023	0.0042	0.001	-0.003	0.005	-0.017	0.017	-0.017	0.017
t_{15}	0.912	0.912	27	26	0.0160	0.0273	0.000	-0.027	0.027	-0.046	0.046	-0.046	0.046
t_{30}	1.563	1.550	27	26	0.0242	0.0412	0.013	-0.028	0.054	-0.078	0.078	-0.078	0.078
t_{45}	2.143	2.104	27	26	0.0250	0.0427	0.039	-0.004	0.082	-0.107	0.107	-0.105	0.105
AN_{900}													
t_0	0.338	0.337	27	26	0.0028	0.0319	0.001	-0.004	0.005	-0.017	0.017	-0.017	0.017
t_{15}	1.285	1.268	27	26	0.0136	0.1380	0.017	-0.006	0.041	-0.064	0.064	-0.063	0.063
t_{30}	2.338	2.294	27	26	0.0325	0.1959	0.044	-0.012	0.100	-0.117	0.117	-0.115	0.115
t_{45}	3.370	3.379	27	26	0.3900	0.2850	-0.009	-0.076	0.058	-0.169	0.169	-0.169	0.169
Treatment	**F_{N2O_GC}** [nmol N_2O m^{-2} s^{-1}]	**F_{N2O_QCL}**											
AN_0	0.0387	0.1048	27	26	0.0187	0.0319	-0.066	-0.098	-0.034	-0.002	0.002	-0.005	0.005
AN_{300}	6.610	6.483	27	26	0.0809	0.1380	0.127	-0.011	0.265	-0.331	0.331	-0.324	0.324
AN_{600}	8.514	8.329	27	26	0.1149	0.1959	0.185	-0.011	0.381	-0.426	0.426	-0.416	0.416
AN_{900}	13.222	13.265	27	26	0.1671	0.2850	-0.043	-0.328	0.242	-0.661	0.661	-0.663	0.663