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1 **Ranking cows' methane emissions under commercial conditions with sniffers versus**  
2 **respiration chambers**

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19 **ABSTRACT:**

20 The objective of the study was to assess the ranking of dairy cows using individual level  
21 correlations for methane (CH<sub>4</sub>) emission phenotypes on-farm using sniffers and in respiration  
22 chambers. In total 20 lactating dairy cows, ten Danish Holstein and ten Danish Jerseys were  
23 recorded using sniffers installed in milking robots for three weeks of lactation prior to relocation  
24 and acclimation at the respiration chamber (RC) facility where they were each recorded on three  
25 occasions within the RC. Pairwise bivariate linear mixed models were used to determine the  
26 individual level correlations ( $r_1$ ) between sniffer phenotypes and RC phenotypes as proxies for  
27 genetic correlations. Despite differences in feeding and management, the predicted CH<sub>4</sub> production  
28 on farm from sniffers correlated well with CH<sub>4</sub> production in the RC (CH<sub>4</sub>\_RC)  $r_1 = 0.77 \pm 0.18$   
29 and the direct CH<sub>4</sub> breath concentration (CH<sub>4</sub>\_C) correlated nearly as well with CH<sub>4</sub>\_RC  $r_1 = 0.75$   
30  $\pm 0.20$ . The correlations between CH<sub>4</sub> emission phenotypes on-farm from sniffers and CH<sub>4</sub>\_RC  
31 exceeded that of energy corrected milk yield, live weight and dry matter intake demonstrating the  
32 potential of sniffers measurements as large-scale indicator traits for CH<sub>4</sub> emissions in dairy cattle.

33 **Keywords:** Methane, sniffers, breath concentration, respiration chambers

34 **Introduction**

35 Methane (CH<sub>4</sub>) is a potent greenhouse gas produced by dairy cattle and other ruminants as a natural  
36 by-product of fermentation. Research on mitigation strategies such as nutritional additives,  
37 vaccines, and genetic improvement, has gained impetus in recent years (Hill et al., 2016). Whilst a  
38 reliable and accurate measure of CH<sub>4</sub> emission forms the basis of evaluating all of the  
39 aforementioned strategies, they differ in requirements of accuracy and precision as well as the  
40 number, frequency and duration of measurement (Hammond et al., 2016). For instance, genetic  
41 evaluations require measurements on large numbers of animals under the environmental conditions

42 they are expected to perform in order to obtain accurate estimated breeding values (EBV) (Falconer  
43 and Mackay, 1996). The accuracy of EBV is in part conditional on the accuracy and precision of the  
44 phenotypes recorded. However, the accuracy of EBVs can be increased through increasing the  
45 number of records per animal and/or increasing the sample size of related animals recorded (Mrode,  
46 2003), i.e. increasing throughput of less precise phenotypes can still result in accurate EBVs.

47 Indirect calorimetry respiration chambers (RC) are the ‘gold standard’ for CH<sub>4</sub> emission, meaning  
48 RC are regarded as the most accurate and precise measurement from which different instruments or  
49 techniques are benchmarked (Grainger et al., 2007; Hill et al., 2016). The high accuracy and  
50 precision of RC have the benefit of detecting relatively small effects of diets and treatments on CH<sub>4</sub>  
51 production with small numbers of animals (Patra, 2016). However, the capital investment cost for  
52 RC are high and the method is labour intensive, proving to be prohibitive to obtaining  
53 measurements on large numbers of animals (Grainger et al., 2007; Madsen et al., 2010).

54 Furthermore, the effects of confinement within the chamber may alter animal behaviour and are not  
55 necessarily representative of all production systems like extensive grazing systems (Storm et al.,  
56 2012), although promising developments have led to reductions in costs and reduced stress due to  
57 confinement (Hellwing et al., 2012). Thus the merit of genetic selection under RC conditions and  
58 the expectation of reduced CH<sub>4</sub> emissions under environmental conditions in which animals are  
59 expected to perform, have been called into question (Lassen and Løvendahl, 2016).

60 A method that has proven well suited to obtaining large numbers of records on individual animals  
61 under commercial conditions is the high-throughput, cost effective, and non-invasive ‘sniffers’  
62 installed in the feed bin of automated milking stations (AMS) or concentrate feeders (Garnsworthy  
63 et al., 2012; Lassen et al., 2012; Negussie et al., 2016). The non-invasiveness is achieved by  
64 limiting the animal-to-instrument interface, so that the animal is not aware it is being measured and  
65 measurement does not disrupt farm activities. The disadvantages of this are a loss of precision due

66 to variable barn gas dynamics and added noise if head movement of the cow and background barn  
67 gas levels are not accounted for (Huhtanen et al., 2015; Difford et al., 2016; Wu et al., 2018).  
68 Furthermore, sniffer methods record the concentration of gases in the captured breath of the cow  
69 during milking and are thus a spot measure of gas concentrations and not a full 24 hour mass flux  
70 measure (Huhtanen et al., 2015). Rather they utilise the ratio of measures CH<sub>4</sub> to carbon dioxide  
71 (CO<sub>2</sub>) and predicted CO<sub>2</sub> as a tracer gas to approximate CH<sub>4</sub> production (Madsen et al., 2010).  
72 New methods which are cheaper, faster or less invasive are continually under development and are  
73 of value when the gold standard proves expensive or prohibitive to largescale recording. For genetic  
74 evaluations, a new method can replace a gold standard method if the new method is heritable and  
75 their genetic correlation ( $r_G$ ) exceeds 0.80 (Robertson, 1959). If the  $r_G$  is moderate and the new  
76 method is heritable, it can be an indicator trait (Negussie et al., 2017). However, the number of  
77 related animals with simultaneous measurements from both methods required to accurately estimate  
78  $r_G$  with meaningful standard errors, is around  $10^3$ - $10^4$  of animals (Visscher, 1998). If measurements  
79 with both methods are taken on different animals or the same animals at different time points, the  
80 required numbers for accurate estimation of  $r_G$  will be even higher (Bijma and Bastiaansen, 2014).  
81 For CH<sub>4</sub> emission, the RC constitutes a separate environment, as other methods cannot be recorded  
82 simultaneously within the RC. It is of interest to determine the ranking of animals prior to the  
83 investment in thousands of records using both methods, particularly when the gold standard is  
84 expensive. To this end, estimating the repeatability of each method and the individual level  
85 correlations ( $r_I$ ) between methods is of value. The repeatability of a method serves as the upper  
86 threshold for heritability estimates and  $r_I$  serve as proxies for  $r_G$ , respectively (Falconer and  
87 Mackay, 1996; Wolak et al., 2012). The objective of this study was to assess the consistency in  
88 ranking of dairy cows for CH<sub>4</sub> emission using sniffers and respiration chambers.

## 89 **Materials and Methods:**

90 ***Design and Animals***

91 This experiment was designed to compare CH<sub>4</sub> measurements from the non-invasive sniffer method  
92 during AMS milking with the open circuit RC, which is the intensive and traditional method. The  
93 link between the two methods was obtained by having 20 cows (ten Holstein and ten Jersey)  
94 measured first by the sniffer in a commercial setting and then transferred to the RC facility. All  
95 handling of animals was conducted according to a protocol approved by The Animal Experiments  
96 Inspectorate, Ministry of Environment and Food of Denmark (Approval number 2016-15-0201-  
97 00959).

98 ***Sniffer AMS measurement***

99 Data on CH<sub>4</sub> and CO<sub>2</sub> gas concentrations from the breath of individual Holstein and Jersey cows  
100 recorded during milking at Danish Cattle Research Centre (DCRC, Foulum, Denmark), where  
101 sniffer sensors were installed in each of the three AMS (DeLaval International AB, Tumba,  
102 Sweden). The sniffer instrumentation comprises two sensors, the CH<sub>4</sub> sensor (Guardian NG,  
103 Edinburgh Instruments Ltd, Livingston, UK) and the CO<sub>2</sub> sensor (Gascard, Edinburgh Instruments  
104 Ltd, Livingston, UK). The equipment installation, and calibration procedures for the sensors are  
105 described elsewhere (Difford et al., 2016). The DCRC barn is a free-stall housing system with  
106 individual cubicles and cows were offered TMR with an approximate forage to concentrate ratio of  
107 (70:30) ad libitum in individualized feeding troughs (RIC-system, Insentec, Marknesse, The  
108 Netherlands). Cows were provided up to 3 kg of concentrate per day within the feed bin of the  
109 AMS, based on levels of production and thus differences in the forage:concentrate ratio between  
110 cows is expected. Cows had free access to AMS and presented on average  $2.4 \pm 0.86$  visits/d (mean  
111  $\pm$  SD) during the measurement period. Data on live weight is recorded 10 times every second  
112 during AMS milking and processed as described in (Bossen et al., 2009). Milk production from the

113 AMS and fat, protein and lactose percentage estimated from 48 hour periods each week (Løvendahl  
114 and Bjerring, 2006). The estimated milk components were used to correct milk production for fat,  
115 protein, and lactose content (ECM) (Sjaunja et al., 1991).

116 Gas concentrations from the AMS was aligned and merged with the entrance and exit time for each  
117 cow visit to the AMS. Data was omitted when the cow's head was predicted to be outside the feed  
118 bin using the algorithm described in Difford et al. (2016). The CH<sub>4</sub> and CO<sub>2</sub> gas concentrations for  
119 the morning cleaning cycle, when the AMS is empty of any cow, were taken as the ambient barn  
120 concentrations for each day, and deducted from the means of each cow visit. The starting time for  
121 each visit in the AMS was converted to 24 hour angular radians for modelling of diurnal variation  
122 (Lassen et al., 2012). The following model was used on all available DCRC data, within AMS to  
123 obtain daily visits corrected for sensor drift, daily variation, and time of day:

$$124 \quad y_{ijklm} = \mu + d_i + b_j + \sum_{k=1}^3 (f_{1k} \sin \theta + f_{2k} \cos \theta) + C_1 + e_{ijklm} \quad (1)$$

125 where  $y_{ijklm}$  is the natural logarithm of background corrected AMS visit means of CH<sub>4</sub> and CO<sub>2</sub>;  $d_i$   
126 is the effect of test day  $i$  ( $i = 23$  d);  $b_j$  is the effect of the first day after each calibration  $j$  ( $j = 3$ );  $f_{1k}$   
127 and  $f_{2k}$  are regression coefficients of Fourier series linear covariates of the time of day of  
128 measurement, modelled as harmonic pairs. The time of day of visit expressed as 24 hour angular  
129 radians is denoted by  $\theta$ . Term  $C_1$  is the random effect parameter for each cow  $C_1 \sim ND(0, I\sigma^2c)$ , and  
130  $e_{ijklm}$  is the residual  $\sim ND(0, I\sigma^2e)$ . In order to correct daily AMS visit means, the residuals for each  
131 visit are combined with random cow solutions, intercept, calibration day, and the regression  
132 coefficients  $f_{1k}$  and  $f_{2k}$  multiplied by the angular radian corresponding to 12:00:00 a.m. Further, the  
133 CH<sub>4</sub> and CO<sub>2</sub> concentration in ppm was averaged per week of lactation, using an average of visits  
134 weighted by the duration of visits. The average weekly CH<sub>4</sub> and CO<sub>2</sub> concentrations were natural  
135 log transformed, here after defined as CH<sub>4</sub>\_C and CO<sub>2</sub>\_C and combined with the weekly

136 performance data from DCRC for ECM (ECM\_C), LW (LW\_C), and gestation length (GL). The  
137 average daily CH<sub>4</sub> production per week of lactation (CH<sub>4</sub>\_P; L CH<sub>4</sub>/d) was calculated using the  
138 ratio of CH<sub>4</sub>\_C to CO<sub>2</sub>\_C and the equation for CO<sub>2</sub> production from heat production units (HPU)  
139 which utilizes ECM\_C, LW\_C, and GL (CIGR, 2002) and the conversion from HPU to CO<sub>2</sub> to  
140 obtain predicted CO<sub>2</sub> production (CO<sub>2</sub>\_P; L CO<sub>2</sub>/d) (Pedersen et al., 2008) as suggested by Madsen  
141 et al. (2010). Data for the 10 Holstein and 10 Jersey cows from the last three weeks prior to  
142 relocation to the facilities with the RC was retained for further analysis together with RC records.

### 143 *Respiration chamber measurements*

144 The 10 lactating Holstein cows and 10 lactating Jersey cows were entered into a trial containing two  
145 dietary treatments, a control and a high concentrate diet with respective forage to concentrate ratios  
146 of (68:32) and (39:61) in a cross-over design with back-cross (Olijhoek et al., 2018). The trial was  
147 divided into three periods with blocking consisting of four cows per block (five blocks in total per  
148 period) with the same cows in each block over periods. Each cow was recorded for duration of 3 d  
149 in period 1 and 2 d in periods 2 and 3. Methane and CO<sub>2</sub> production in the RC (CH<sub>4</sub>\_RC and  
150 CO<sub>2</sub>\_RC) was calculated from the product of the total flow of outgoing air at standard temperature  
151 and pressure, and the difference between the gas concentrations in the outgoing air and the gas  
152 concentrations in the incoming air from the barn (background). Methane and CO<sub>2</sub> concentration  
153 readings from when the chambers were opened twice daily for milking were omitted before  
154 calculating the average CH<sub>4</sub> and CO<sub>2</sub> production for each cow, during each period. Live weight was  
155 recorded when cows entered and left the RC and the average of these two readings taken from the  
156 RC recording period (LW\_RC) as per Olijhoek et al. (2018). Milk yield was recorded during  
157 milking in the RC and composition determined from two subsequent milkings. The average milk  
158 yield and milk composition was used to determined average energy corrected milk yield in the RC  
159 (ECM\_RC) during RC recording periods (Sjaunja et al., 1991; Olijhoek et al., 2018). The records



160 from these cows were retained for comparison with sniffer measures recorded at DCRC for the  
161 same 20 cows.

## 162 *Statistical Analysis*

163 Pairwise bivariate animal repeatability models were used to estimate variance components  
164 controlling for fixed effects. All analyses were performed using DMU version 6 (Madsen and  
165 Jensen, 2014). The model for the RC traits was as follows:

$$166 y_{ijklmn} = \mu + B_i + P_j + L_k + D_l + C_m + e_{ijklmn} \quad (2)$$

167 Where  $y_{ijklmn}$  is the trait of interest (CH4\_RC, CO2\_RC, LW\_RC and ECM\_RC),  $\mu$  is the intercept,  
168 B is the i'th breed (I = 2 levels), P is the j'th effect of block nested in period (j = 12 levels), L is the  
169 k'th lactation number (k = 3 levels), D is the l'th effect of diet (l = 2 levels),  $C_m$  is the random effect  
170 of the m'th cow  $C_m \sim ND(0, I\sigma^2_c)$ , and e is the residual  $\sim ND(0, I\sigma^2_e)$ .

171 The model for the on-farm traits was as follows:

$$172 y_{ijklm} = \mu + W_i + BR_j + L_k + C_l + e_{ijklm} \quad (3)$$

173 Where  $y_{ijklm}$  is the trait of interest (CH4\_P, CO2\_P, CH4\_C, CO2\_C, LW\_C and ECM\_C),  $\mu$  is the  
174 intercept, W is the i'th week of lactation (I = 2 levels), BR is the j'th breed nested within AMS (j = 3  
175 levels), L is the k'th lactation number effect (k = 3 levels),  $C_l$  is the random effect of the l'th cow  $C_l$   
176  $\sim ND(0, I\sigma^2_c)$ , and e is the residual  $\sim ND(0, I\sigma^2_e)$ .

177 For all pairwise comparisons between RC and on-farm traits it was necessary to restrict residual  
178 covariance to zero as cows were recorded in different environments. Repeatability estimates ( $t$ )  
179 were obtained from the variance components by using the equation:

$$180 t = \frac{\sigma_c^2}{(\sigma_c^2 + \sigma_e^2)}$$

181 Individual level correlations ( $rI$ ), were computed as the correlation between random cow (4)  
182 effects using variance components as show in equation 4:

$$183 \quad rI = \frac{\sigma^2_{c1,c2}}{\sqrt{\sigma^2_{c1}} \cdot \sqrt{\sigma^2_{c2}}} \quad (5)$$

184 The standard errors of the individual level correlations and repeatability estimates where derived  
185 using Taylor series approximations.

## 186 **Results and Discussion**

187 The descriptive statistics for sniffer and RC phenotypes can be found in Table 1. The sniffer  
188 predicted mass flux CH<sub>4</sub>\_P and CO<sub>2</sub>\_P were closer to that of RC mass flux phenotypes CH<sub>4</sub>\_RC  
189 and CO<sub>2</sub>\_RC albeit with lower means, higher variability and consequently higher coefficients of  
190 variation (CV). The sniffer breath concentration phenotypes (CH<sub>4</sub>\_C and CO<sub>2</sub>\_C) were more  
191 different from predicted and measured mass flux phenotypes, with lower means, higher variability  
192 and CV. All CH<sub>4</sub> and CO<sub>2</sub> phenotypes where moderately to highly repeatable ranging from  $t = 0.53$   
193 for CH<sub>4</sub>\_C to  $t = 0.87$  for CO<sub>2</sub>\_P. Live weight and ECM were retained as control variables to  
194 ensure that the RC environment was not considerably different from that of the on-farm  
195 environment for these production traits. The means, SD and CV for LW and ECM were compared  
196 across environments with similar descriptive statistics and repeatability estimates for example  
197 LW\_C and LW\_RC had similar means 568.2 vs 564.5 kg and repeatability  $t=0.93$  and  $t=0.98$ ,  
198 respectively. Recognizing that in the case of the sniffer phenotypes, they are an average of many  
199 measurements from the AMS over a full week of lactation whereas RC phenotypes are the average  
200 of measurements over a 2 – 3 d period in the RC.

201 The individual level correlations between sniffer and RC phenotypes are reported in Table 2. For  
202 ECM and LW the individual level correlations ( $r_i$ ) between on-farm AMS and RC were close to

203 unity  $0.86 \pm 0.15$  and  $0.92 \pm 0.04$ , respectively. These  $r_I$  indicate very similar ranking of cows  
204 across environments, suggesting confinement within the RC had limited effects on ranking for these  
205 traits. Many studies have reported that confinement within RC alters behavior and can induce stress  
206 resulting in a drop in feed intake (Beauchemin and McGinn, 2006; Llonch et al., 2016). However,  
207 the RC in the present study were constructed from transparent polycarbonate to reduce costs and  
208 increase cow welfare, as supported by a study describing that these RC provoked no drop in DMI,  
209 (Hellwing et al., 2012).

210 Sniffer CH4\_P showed the highest  $r_I$  of any on-farm phenotype with CH4\_RC  $0.77 \pm 0.18$ , which  
211 approaches the suggestive threshold of 0.80 for no significant re-ranking, however the standard  
212 errors thereof are large. This finding agrees with that of Hellwing et al (2013), who compared CH4  
213 production calculated from the ratio of CH4 to CO2 measured within the RC and predicted CO2  
214 production to CH4\_RC and CO2\_RC for 157 cow measurements in 8 feeding experiments and  
215 found an R<sup>2</sup> of 55% corresponding to a correlations R= 0.74. The performance of CH4\_P is reliant  
216 on the accuracy of CO2\_P as a predicted tracer gas, the  $r_I$  between CO2\_P and actual measured CO2  
217 production (CO2\_RC) was similarly high  $r_I = 0.79 \pm 0.14$  as that of CH4\_P and CH4\_RC. A  
218 criticism of this CO2 prediction equation is that the metabolizable energy (ME) efficiency and  
219 mobilization of body tissues is not taken into account, running the risk of over predicting CO2  
220 production of efficient cows (increased LW and ECM at a fixed level of intake) and under  
221 predicting CO2 production of inefficient cows (Madsen et al., 2010; Huhtanen et al., 2015). It may  
222 be possible to improve the  $r_I$  between CH4\_P and CH4\_RC through improving the prediction  
223 accuracy of CO2\_RC by taking into account ME utilization. For instance, Negussie et al (2016)  
224 compared CH4\_P in the breath of 20 lactating Nordic Red cattle from concentrate feeders and  
225 CO2\_P predicted from ME intake and found CH4\_P to have a high concordance correlation  
226 coefficient 0.70 and phenotypic correlation 0.80 with CH4\_RC.

227 Since all prediction equations have some level of inherent error and traits used in the prediction of  
228 CO<sub>2</sub>\_P, e.g. ECM\_C, are already in the breeding goal, there is interest in assessing value of directly  
229 measured traits like CH<sub>4</sub>\_C and CO<sub>2</sub>\_C with RC traits. In this instance CH<sub>4</sub>\_C ranked animals  
230 comparatively well with CH<sub>4</sub>\_RC  $r_1 = 0.75 \pm 0.20$  as compared to CH<sub>4</sub>\_P and CH<sub>4</sub>\_RC  $r_1 = 0.77 \pm$   
231 0.18 and exceeded that of commercial control variables ECM\_C, LW\_C and DMI\_C, which are  
232 routinely used to predicted CH<sub>4</sub> production (Ramin and Huhtanen, 2013).

233 A number of authors have labelled breath gas concentration measures as imprecise (Huhtanen et al.,  
234 2015; Goopy et al., 2016; Wu et al., 2018), which is congruent with our results as seen by the lower  
235 repeatability estimates of CH<sub>4</sub>\_C and CO<sub>2</sub>\_C and the increased CV. However, the aforementioned  
236 studies often compare mass flux CH<sub>4</sub> production (g/day) to CH<sub>4</sub> breath concentrations using the  
237 coefficient of determination ( $R^2$ ) and its radicand Pearson's correlation coefficient R, with the  
238 expectation that a deviation of  $R^2$  or R from unity (1.0) indicates imprecision. Recognizing that  
239 CH<sub>4</sub>\_C is a separate, by likely correlated trait from CH<sub>4</sub>\_RC, deviations in  $R^2$  from 1.0 are to be  
240 expected regardless of imprecision. Moreover, these studies often compute R in the presence of  
241 repeated measures per subject without explicitly modelling the random effect of subject, which has  
242 the effect of biasing correlations downwards when one or both of the traits has some imprecision  
243 (i.e  $t < 0.80$ ), known as attenuation of error (Spearman 1904; Adolph & Hardin 2007). Conversely,  
244 these studies often fail to account for non-genetic between subject variation (for instance parity,  
245 lactation stage, breed etc.) which can inflate estimates of R. When repeatability ( $t > 0.80$ ) and  
246 precision is high in one or both phenotypes, and between subject non-genetic factors are accounted  
247 for and single measurement are taken per subject, then the phenotypic correlation ( $r_P$ ) can be a good  
248 predictor of  $r_G$  (Cheverud, 1988; Roff, 1995). In the case of repeated measurements per subject  
249 when one or both traits have some imprecision  $r_P$  is still biased downwards and it is necessary to  
250 partition variation into between subject variation and within subject variation (i.e. residual error or

251 imprecision) and compute  $r_I$  which are one step closer to  $r_G$  (Adolph and Hardin, 2007; Dingemans  
252 and Dochtermann, 2013). Individual level correlations have been used as proxies for  $r_G$  in difficult  
253 or expensive to measure traits in dairy cattle such as DMI (Veerkamp et al., 2013),  $CH_4$  production  
254 (Zetouni et al., 2018) and energy balance (Løvendahl et al., 2010).

255 The  $r_G$  remains the most informative correlation metric for assessing how best to incorporate an  
256 alternative method into a selection index. This point is illustrated in the development of the portable  
257 accumulation chambers (PAC) which are a short-term total  $CH_4$  emission flux method alternative to  
258 RC used in sheep. Goopy et al. (2011) compared the two methods in 39 sheep, by measuring for 22  
259 hours in the RC and then measuring 1 and 2 hours immediately after, in the PAC and found  $r_P =$   
260 0.67. In a subsequent genetic study on 3601 lambs with 4733 records in PAC and 8655 in RC,  
261 Jonker et al. (2018) found  $r_G = 0.67 \pm 0.11$  between the methods. In this case,  $r_P$  was a good proxy  
262 for  $r_G$  but at a fraction of the cost and justified the investment in obtaining the thousands of records  
263 required to accurately define  $r_G$ . Furthermore, the PAC is not genetically equivalent to the RC, but  
264 has promise as a large scale, cost effective indicator trait. Importantly, Jonker et al. (2018) reported  
265  $r_P = 0.27 \pm 0.02$  in the presence of repeated measurements and not  $r_I$  which was a biased predictor  
266 of  $r_G$ . Given  $r_I$  for sniffer  $CH_4$  traits ranged from 0.75-0.77 and were higher than PAC correlations  
267 with RC, should these correlations be validated in a genetic evaluation, sniffers have the potential to  
268 cost effectively generate the large scale recording of thousands of dairy cows for routine genetic  
269 evaluation of  $CH_4$  emissions.

270 These are the first  $r_I$  reported for any  $CH_4$  recording method with the RC method. Method  
271 comparisons to date have made inconsistent use of different correlation metrics. Garnsworthy et al.  
272 (2012) recorded  $CH_4$  production in 12 lactating Holstein cows for 10 days in the AMS using  
273 sniffers and found an  $R^2 = 0.78$  and  $R = 0.88$  with a single record of  $CH_4$  production from RC.  
274 Similarly, Negussie et al. (2016) recorded  $CH_4\_P$  using sniffer sensor installed in automatic

275 concentrate feeders on 22 Finnish Ayrshire cows with subsequent records for CH<sub>4</sub> production in RC  
276 and found ( $R = 0.80$ ), repeated measures were not taken with the RC method nor were between  
277 subject non-genetic effects such as parity or lactation stage accounted for. Conversely, (Muñoz et al.,  
278 2004, 2012) compared CH<sub>4</sub> production from the SF<sub>6</sub> technique with the RC and had repeated  
279 measures per method per cow and controlled for technical factors such as bolus release rate,  
280 recording period etc. but failed to correct for breed, parity or lactation stage or to compute  $r_1$ , instead  
281 reporting  $R^2 = 0.69$  and  $R = 0.83$ . A single study comparing multiple alternative methods has  
282 reported repeated measures correlations ( $r_{RP}$ ) taking into account repeated measures per cow per  
283 method (i.e. similar to  $r_1$  without accounting for non-genetic between subject effects) (Sorg et al.,  
284 2018). Sorg et al. (2018) found an  $r_{RP}$  ranging from 0.57 - 0.74 for CH<sub>4</sub> production between the  
285 laser measuring device, GreenFeed and two sniffer systems in lactating Holstein cows from  
286 northern Europe. Although the different correlations metrics reported maybe be biased predictors of  
287 the  $r_G$  between methods, the  $r_1$  reported herein for sniffer CH<sub>4</sub> phenotypes and CH<sub>4</sub> production in  
288 the RC are promising as indicator traits.

## 289 **Conclusion**

290 Methane emission traits derived from breath gas measurements during milking correlated the  
291 highest with CH<sub>4</sub> production in RC, exceeding that of LW, ECM and DMI. The individual level  
292 correlations with CH<sub>4</sub> production in the RC indicate that sniffer CH<sub>4</sub> traits have the potential to  
293 serve as large scale indicator traits of CH<sub>4</sub> production in the RC. Genetic correlations between RC  
294 phenotypes and breath gas phenotypes are still needed for effective use in genetic selection indices.  
295 Given the difficulties in acquiring suitably large numbers of cows in RC, the most feasible current  
296 way to obtain accurate genetic correlations between alternative methods and the RC is through  
297 international collaborations and incorporation of genomic information. Given the promising

298 individual level correlations between sniffer phenotypes and RC further research into genetic  
299 correlations between sniffers and RC for CH<sub>4</sub> emission is warranted.

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441 **Table 1.** Descriptive statistics for on farm measurements and respiration chamber measurements.

	Unit	Mean	SD	CV (%)	t <sup>1</sup>
<b>On farm<sup>2</sup></b>					
CH4_P	L/d	573	73.9	12.9	0.58 ± 0.11
CO2_P	L/d	6771	578.7	8.5	0.87 ± 0.11
CH4_C	ppm	410	137.0	33.4	0.53 ± 0.11
CO2_C	ppm	5746	1791.7	31.2	0.56 ± 0.12
CH4/CO2_C	ppm/ppm	0.071	0.009	12.7	0.38 ± 0.13
ECM_C	Kg/d	38.1	5.93	18.1	0.71 ± 0.08
LW_C	Kg	568.2	57.3	10.1	0.93 ± 0.02
DMI_C	Kg/d	22.2	4.9	21.5	0.73 ± 0.07
<b>Respiration Chamber<sup>3</sup></b>					

CH4_RC	L/d	521	56	10.7	0.61 ± 0.12
CO2_RC	L/d	6538	702.3	10.7	0.72 ± 0.10
CH4/CO2_RC	L/L	0.081	0.006	7.7	0.57 ± 0.14
ECM_RC	Kg/d	28.3	5.6	19.8	0.65 ± 0.12
LW_RC	Kg	564.5	62.3	11.0	0.98 ± 0.01

442 <sup>1</sup>t = repeatability intraclass correlation coefficient. <sup>2</sup>On farm phenotypes: CH4\_P = predicted  
443 methane production; CO2\_P = predicted carbon dioxide production; CH4\_C = methane breath  
444 concentration; CO2\_C = carbon dioxide breath concentration; CH4/CO2\_C = ratio of methane to  
445 carbon dioxide breath concentration; ECM\_C = Energy corrected milk yield; LW\_C = live weight;  
446 DMI\_C = dry matter intake. <sup>3</sup>Respiration chamber phenotypes: CH4\_RC = methane production;  
447 CO2\_RC = carbon dioxide production; CH4/CO2\_RC = ratio of methane to carbon dioxide  
448 production; ECM\_RC = energy corrected milk yield; LW\_RC = live weight.

449 **Table 2** Individual level correlations between on farm phenotypes and respiration chamber  
450 phenotypes

On farm <sup>1</sup>	Respiration chamber <sup>2</sup>				
	CH4_RC	CO2_RC	CH4/CO2_RC	ECM_RC	LW_RC
CH4_P	0.77 ± 0.18	0.63 ± 0.10	0.70 ± 0.24	0.68 ± 0.21	-0.09 ± 0.29
CO2_P	0.74 ± 0.13	0.79 ± 0.14	0.41 ± 0.29	0.58 ± 0.22	0.20 ± 0.27
CH4_C	0.75 ± 0.20	0.80 ± 0.16	0.03 ± 0.39	0.21 ± 0.35	0.60 ± 0.22
CO2_C	0.62 ± 0.24	0.76 ± 0.18	-0.35 ± 0.38	0.06 ± 0.40	0.69 ± 0.18
CH4/CO2_C	0.60 ± 0.27	0.29 ± 0.37	0.83 ± 0.23	0.68 ± 0.23	-0.66 ± 0.24
ECM_C	0.66 ± 0.20	0.54 ± 0.23	0.52 ± 0.26	0.86 ± 0.15	-0.14 ± 0.27
LW_C	0.54 ± 0.22	0.68 ± 0.16	-0.32 ± 0.33	-0.24 ± 0.28	0.92 ± 0.04
DMI_C	0.70 ± 0.17	0.64 ± 0.18	0.18 ± 0.33	0.33 ± 0.26	0.39 ± 0.22

451 <sup>1</sup>On farm phenotypes: CH4\_P = predicted methane production; CO2\_P = predicted carbon dioxide  
452 production; CH4\_C = methane breath concentration; CO2\_C = carbon dioxide breath concentration;  
453 CH4/CO2\_C = ratio of methane to carbon dioxide breath concentration; ECM\_C = Energy  
454 corrected milk yield; LW\_C = live weight; DMI\_C = dry matter intake. <sup>2</sup>Respiration chamber  
455 phenotypes: CH4\_RC = methane production; CO2\_RC = carbon dioxide production; CH4/CO2\_RC  
456 = ratio of methane to carbon dioxide production; ECM\_RC = energy corrected milk yield; LW\_RC  
457 = live weight.

458