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An assessment of factors controlling N₂O and CO₂ emissions from crop residues using different measurement approaches

Badagliacca, G; Ruisi, P; Rees, RM; Saia, S

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1 **Authors: Giuseppe Badagliacca¹, Paolo Ruisi¹, Robert M. Rees^{2*}, Sergio Saia^{1,3}.**

2 **Title:** An assessment of factors controlling N₂O and CO₂ emissions from crop residues using
3 different measurement approaches

4 **Affiliation**

5 ¹ Dipartimento di Scienze Agrarie e Forestali, Università degli Studi di Palermo, Viale delle Scienze
6 90128, Palermo, Italy

7 ² Scotland's Rural College (SRUC), West Mains Road, Edinburgh EH9 3JG, UK

8 ³ Council for Agricultural Research and Economics (CREA) – Cereal Research Centre (CREA-CER),
9 S.S. 673, km 25,200, 71122 Foggia, Italy CREA-CER

10

11

12 **Corresponding Author**

13 **Robert M. Rees** e-mail: bob.rees@sruc.ac.uk Telephone:+44 131 5354365

14 Fax:+441315354144

15

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19 **Abstract**

20 Management of plant residues plays an important role in maintaining soil quality and nutrient
21 availability for plants and microbes. However, there is considerable uncertainty regarding the
22 factors controlling residue decomposition and their effects on greenhouse gas (GHG) emissions
23 from the soil. This uncertainty is created both by the complexity of the processes involved and
24 limitations in the methodologies commonly used to quantify GHG emissions. We therefore
25 investigated the addition of two soil residues (durum wheat and faba bean) with similar C:N ratios
26 but contrasting fibres, lignin and cellulose contents on nutrient dynamics and GHG emission from
27 two contrasting soils: a low-soil organic carbon (SOC), high pH clay soil (Chromic Haploxerert)
28 and a high-SOC, low pH sandy-loam soil (Eutric Cambisol). In addition, we compared the
29 effectiveness of the use of an Infrared Gas Analyzer (IRGA) and Photoacoustic Gas Analyser
30 (PGA) to measure GHG emissions with more conventional gas chromatography (GC). There was a
31 strong correlation between the different measurement techniques which strengthens the case for the
32 use of continuous measurements approaches involving IRGA and PGA analyses in studies of this
33 type. The unamended Cambisol released 286% more CO₂ and 30% more N₂O than the
34 Haploxerert. Addition of plant residues increased CO₂ emissions more in the Haploxerert than
35 Cambisol and N₂O emission more in the Cambisol than in the Haploxerert. This may have been a
36 consequence of the high N stabilization efficiency of the Haploxerert resulting from its high pH and
37 the effect of the clay on mineralization of native organic matter. These results have implications
38 management of plant residues in different soil types.

39

40 **Key words:** crop residues, carbon dioxide, greenhouse gas, nitrous oxide, residue decomposition

41

42 **Introduction**

43 Agriculture forestry and related land uses are responsible for just under 25% of global greenhouse
44 gas emissions (IPCC 2014). Agronomic practices are recognized as key opportunities to reduce
45 GHG emissions (particularly for N₂O and CO₂). The addition of plant residues to the soil from
46 crops and cover crops is of significant importance to crop management strategies to enhance soil
47 organic C (SOC) and soil fertility, and to offset agricultural GHG emissions (Lugato et al. 2014).
48 However, decomposition of residues will also add nitrogen (N) to the soil, and with the default N₂O
49 Emission Factor of 1% of the added N in the IPCC (2006) methodology, a proportional increase in
50 estimated N₂O emissions is predicted. However, IPCC predictions have been developed around
51 limited experimental data and recent studies indicate that default Emission Factors may
52 overestimate residues N₂O emissions (Jeuffroy et al. 2013). GHG emission after crop residue
53 amendment is related to both its decomposition and the microbial activity of soil and depends from
54 several factors linked to the environment, soil properties and crop residue traits (Aulakh et al.
55 1991; Powlson et al. 2011). In particular, the GHG emission from the soil are mediated by soil
56 porosity (Killham et al. 1993), pH (Mörkved et al. 2006), organic C and N content (Hayakawa et al.
57 2009), microbial community (Graf et al. 2016), texture (Chen et al. 2013), soil temperature (Kesik
58 et al. 2006), and moisture content, all of which regulate gas production processes and emission
59 (Skiba and Ball 2002; Rees et al. 2013). Moreover, crop residue addition to the soil can also

60 indirectly affect GHG emissions, providing a source of readily available C and N in the soil,
61 stimulating microbial activity (Aulakh et al. 2001; Huang et al. 2004), promoting the decomposition
62 of native soil organic carbon, and altering soil aeration, water holding capacity, oxidation and
63 denitrification processes in the soil (Fontaine et al. 2004; Derrien et al. 2014).

64 With regard to the crop residue characteristics, the most important property is its C:N ratio
65 that determines organic N dynamics in the soil (Heal et al. 1997; Baggs et al. 2003; Al-Kaisi and
66 Yin 2005; Garcia-Ruiz and Baggs 2007). In particular, it has been shown that higher N₂O emissions
67 occur from soil after the incorporation of residues with low C:N ratio, such as legumes, rather than
68 after cereal straw as a result of mineralization processes (Baggs et al. 2000; Huang et al. 2004;
69 Raiesi 2006). On the contrary, low N₂O emissions were reported following the application of crop
70 residues with high C:N ratios (Gentile et al. 2008). However, it has also been shown that the
71 incorporation of crop residues with high C:N ratios may provide the energy for the denitrification
72 process, and this can increase N₂O emissions (Sarkodie-Addo et al. 2003). Other crop residue
73 properties can play an important role on the decomposition process influencing microbial activity,
74 including lignin (Palm and Rowland 1997), lignin:N ratios (Curtin et al. 1998) polyphenol
75 (Muhammad et al. 2010), water soluble phenolic contents (Palm and Rowland 1997), percentages of
76 soluble C and N (Cogle et al. 1989), neutral detergent fiber (NDF). This understanding of the
77 multiple drivers responsible for GHG emissions from crop residues is helpful in interpreting
78 research findings. Baggs et al. (2000) found an increase of N₂O emissions after lettuce
79 incorporation into the soil due to its low C:N ratio. However, Tanveer et al. (2014) and Zou et al.
80 (2004) observed a reduction of N₂O emission after incorporation of low C:N crop residue of corn
81 and rice straw. This apparent contradiction may be a consequence of the interaction of multiple
82 factors controlling emission. Shan and Yan (2013), in a meta-analysis, reported that the application
83 of canola, bean and lettuce residues increased N₂O emissions more than with other kind of crop
84 residues. With regard to CO₂ emissions Muhammad et al. (2010) observed higher emissions in soil
85 amended with alfalfa than with sugarcane, maize, sorghum and cotton and attributed such result to a
86 release of more easily degradable and soluble C in alfalfa than with other crop residues. Chen et al.
87 (2015) observed a general increase of CO₂ emissions from a soil amended with different types of
88 residues but with higher cumulative emissions in peanuts, soybean and maize than in other cereals
89 due to their higher N and lower neutral detergent fiber (NDF) content.

90 An accurate quantification of CO₂ and N₂O emission following return of crop residues to
91 soils is required to develop efficient strategies to reduce the environmental impact of farming
92 practices. Presently, static chamber methods coupled with gas chromatography (GC) analysis are
93 the most widely technique used to quantify GHG losses in field and laboratory experiments.
94 However, the method is time consuming and entails a wide series of operations from the manual
95 sampling to the laboratory analysis, introducing errors, and making difficult to implement high
96 resolution monitoring over time (Tirol-Padre et al. 2014). In order to obtain high resolution
97 temporal data, InfraRed Gas Analyzer (IRGA) and Photoacoustic Gas Analyzer (PGA) has been used
98 in agricultural GHG emissions studies (Luo and Zhou 2006; Lawrence et al. 2009; Stackhouse et al.
99 2011). IRGA allows to measure CO₂ fluxes using an infra-red sensor and PGA is a photo-acoustic
100 infrared multi-gas monitoring system that allows to measure simultaneously CO₂, N₂O and CH₄.
101 Measurement of CO₂ efflux by IRGA systems are usually based on different methodology proposed
102 by the manufacturing companies and there isn't an internationally recognized protocol creating

103 uncertainties in the comparison between different instruments (Mills et al. 2011). PGA has been
104 widely used in field experiments and several authors found a high correlation between CO₂ and
105 N₂O measurements made with PGA and GC (Klein et al. 2008; Iqbal et al. 2013). Other authors
106 reported an overestimation of emission on the data obtained with PGA than GC (Yamulki and
107 Jarvis 1999). Furthermore, the precision of measurement may also depend from the soil type and
108 soil cover, which can affect the assessment of emission spatial variability. The precision of the
109 various instruments (IRGA and PGA comparing to the widely used CG) in measuring GHGs
110 emission has never been measured. However, in contrast to the GC-based methodology, these
111 systems are able to provide a continuous measurement of the GHGs emission, thus allowing to
112 better study the trend of the emission from the soil and its relationship with agronomical
113 management techniques and environmental variability. In addition, IRGA and PGA has not been
114 previously directly compared.

115 Soil GHG emissions from Cambisols which occur widely in cool temperate climates have
116 widely studied in the past whereas the effect of soil characteristics typical of the Mediterranean
117 such as vertisols, with their high clay content high pH and low organic carbon content, on crop
118 residue decomposition and gaseous emissions are less known. The aims of the present study were:
119 (i) to evaluate the short-term emissions of N₂O and CO₂ after the addition of two crop residues
120 with different structural fibre composition (either faba bean and wheat), in two soils with
121 contrasting properties, a Chromic Haploxerert with a high clay content and a Eutric Cambisol with
122 a sandy-loam texture; (ii) assess the flexibility of two systems for the high temporal resolution
123 measurements (IRGA and PGA), to measure soil GHG emissions from soils with different emission
124 levels in controlled conditions. Experiments were undertaken in a controlled pot setup over a short
125 period and in the absence of plants in order to simulate the effects of crop residues between
126 cropping cycles. These conditions avoided strong time-related variation in the emission due to the
127 impoverishment of the ready available N pool and living plant C inputs to and mineral uptake from
128 soil, which could have altered the emission rates.

129

130 **Materials and Methods**

131 An experiment was established during 2014 in controlled environment conditions at
132 Scotland's Rural College (SRUC) Edinburgh. A complete randomized factorial design with three
133 replicates was adopted. Treatments were soil: Eutric Cambisol and Chromic Haploxerert (Vertisol);
134 and kind of plant residue added: faba bean residue, durum wheat residue or unamended control. The
135 Cambisol was collected at nine locations per plot from the top 20 cm at Bush Estate (lat, 55° 51' N,
136 long, 3° 12' W; 199 m a.s.l.) near Edinburgh (Scotland), the Haploxerert (Vertisol) was collected at
137 the Pietranera Farm (37°30' N, 13°31' E; 178 m a.s.l.) in Santo Stefano Quisquina (Sicily). Both
138 soils were sampled in early October 2014. Soil was collected from conventional tilled experimental
139 plots at the Bush Estate in Scotland and from conventionally tilled plots at Pietranera farm in Sicily
140 (Table 1). At both sites the soil was collected in plots previously cultivated with cereals (wheat in
141 Sicily and barley in Scotland). Further information regarding the soil sampling sites are available in
142 Vinten et al. (1992) and Amato et al. (2013), respectively. Before establishing the experiment, soil
143 was air-dried and passed through a 2 mm mesh and visible roots and organic residues were
144 removed, and then mixed thoroughly before use; water hold capacity of both soils were measured
145 on a weight basis. Oven-dried crop biomass of wheat (cv. Simeto) and faba bean (cv. Gemini) (see

146 Table 2 residues traits), cultivated at Pietranera farm, were ground to pass a 1 mm screen, mixed,
147 and used as crop residues.

148 Pots were 10 cm in diameter and 25 cm high, and were filled with 1.5 kg of soil to achieve
149 a bulk density of 1.25 g cm^{-3} . Crop residues were mixed with the soil at a rate of 5 g crop residue
150 per kg of soil. The bottom part of the pot (15-25cm depth) was filled with sand. Then, pots were
151 brought to 60-70% of the water holding capacity. After each sampling an amount of water
152 corresponding to the evaporation losses was added to each pot and the pots were randomized inside
153 the greenhouse. During the experiment, soil temperature was recorded using a temperature data
154 logger (EL-USB-3, Lascar Electronics, United Kingdom).

155 Both CO_2 and N_2O soil emissions were measured three times per week, on 22 sampling
156 occasions, by means of two different methods: an online Infrared Gas Analyzer (IRGA, EGM-4
157 CO_2 , PP system, USA) and a Photoacoustic Gas Analyser (PGA, INNOVA 1412, LumaSense
158 Technologies A/S, USA). Measurements were always taken between the 9:00 and the 15:00 and
159 each time the equipment order was reversed. The IRGA was equipped with a SRC-1 Soil
160 Respiration Chamber equipped with a fan, with a diameter of 10 cm and 15 cm height, sealed on
161 top of the pot by an airtight rubber. The air from the chamber was sent to the analyser at flow rate
162 of 0.1 l min^{-1} . After 15 seconds of flushing, the chamber was placed above the pot, equilibrated for
163 15 seconds, then the CO_2 concentration was measured every 5 seconds and the flux was calculated
164 from the concentration increase over time until a good linear fit was obtained.

165 The PGA was equipped with a PVC chamber with a diameter of 10 cm and 10 cm height,
166 connected to the equipment by two small rubber pipes on the chamber top, and sealed above the pot
167 by a rubber seal. The analyser automatically pumped $\sim 0.1 \text{ l min}^{-1}$ of air from inside the chambers
168 and performed the analysis with a 5-second sampling integration time and a fixed flushing time: 8
169 seconds for the chamber and 3 s for the tubing. The PGA instrument was calibrated in the lab for
170 CO_2 and N_2O by the LumaSense technologies company, with a gas concentration of 3496.8 ppm for
171 CO_2 and 51.32 ppm for N_2O , and its detection limits were of 1.5 ppm for CO_2 and 0.03 ppm for
172 N_2O . The equipment performed a built-in compensation for water and cross interferences. Before
173 the flux measurements, the instrument analyzed ambient air for about 30 min until readings for CO_2
174 and N_2O were stable. The overall time for sampling and measurement of CO_2 and N_2O
175 concentration and dew-point temperature was approximately 70 seconds; each measurement was
176 made every two minutes.

177 Gas flux measurement (CO_2 from both IRGA and PGA, and N_2O from PGA), in two
178 different periods during the experiment, were compared with analyses by gas chromatography in
179 order to confirm the reliability of the instruments. CO_2 and N_2O emissions were measured using the
180 static closed chamber technique (Hutchinson and Mosier 1981). A chamber of polyvinyl chloride
181 (PVC), with a diameter of 10 cm and 15 cm height and a lid with a gas sampling port was sealed above
182 each pot for 60 min. Before and after this period gas samples were collected in portable evacuated
183 glass vials (Chadwick et al. 2014), transported to the lab and analyzed by a gas chromatography
184 (Agilent 7890a, Agilent Technologies Ltd, Stockport, UK) equipped with a thermal conductivity
185 detector (TCD, detection limit for CO_2 of 23.9 ppm) and an electron capture detector (ECD,
186 detection limit for N_2O of 0.074 ppm). Fluxes of CO_2 and N_2O were calculated from the increase in

187 concentration in the chamber corrected for the chamber air temperature using the following relation
188 (Jantalia et al. 2008):
189

$$f = \frac{\Delta C}{\Delta t} \times \frac{V}{A} \times \frac{m}{Vm}$$

190
191 where $\Delta C/\Delta t$ is the gas increment during the chamber closure time, V is the volume of the chamber,
192 A is the soil area, m is the molecular weight of the gases and Vm is the gas molar volume corrected
193 for the ambient temperature.

194 The total amount of N_2O and CO_2 emissions were calculated by linear interpolation between
195 consecutive using the following equation (Cai et al. 2012):
196

$$\text{Cumulative emission of } N_2O \text{ or } CO_2 = \sum_{i=1}^n (F_i + F_{i+1})/2 \times (t_{i+1} - t_i) \times 24$$

197 where F are the emission flow of N_2O and CO_2 at the i^{th} measurement, $(t_{i+1}-t_i)$ is the time length
198 between two adjacent measurements and n is the total measurement number.

199 Plant dry matter (oven drying), ether extract (Method 920.39, diethyl ether, traditional
200 Soxhlet extraction), total N (Kjeldahl) and crude protein (calculated from the total N by standard
201 Jones factor, $N \times 6.25$) were analyzed following methods described by AOAC (1995). Neutral
202 detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), cellulose and
203 hemicellulose were analysed following the sequential method proposed by Van Soest et al. (1991)
204 and using a Fibertec System M 1020 extractor (Foss, Höganäs). The soluble fraction was obtained
205 by boiling 1 g of ground residues in deionized water (100°C) for 30 min followed by extraction
206 with a neutral detergent (EDTA and Na lauryl sulphate at 100°C) for 60 min to obtain the NDF
207 fraction. ADF extraction was performed by boiling the sample for 60 minutes in an acid detergent
208 solution (Cetyltrimethylammonium (CTAB) in H_2SO_4). Then, the residual detergent was removed
209 by washing the sample with hot water. Finally, the ADF was then treated with 72% H_2SO_4 (w/w) for
210 3 hours at ambient temperature and the final mass of the non-extractable fraction was considered as
211 lignin (ADL). Cellulose was calculated as the difference between ADF and ADL while
212 hemicellulose as the difference between NDF and ADF. Ash and ADL ash measurements were
213 performed at 550°C for 4 h. For each residue type the analyses were performed in triplicate. Total
214 C of biomasses and soils were analysed by an automated analyser (Flash 2000, Thermo-Finnigan,
215 Glasgow, UK).

216 At the end of the experiment, two soil samples from each pot were collected: one from the
217 top to 5 cm depth and the other from 5 to 15 cm depth. Soil pH was measured in a 1:5 (v/v)
218 suspension of soil in water. Dissolved organic C (DOC) content in the soil was determined by a
219 total organic C analyser (DC-80, Rosemount Analytical, Inc. Dohrmann Division, USA) after the
220 removal of inorganic C by acidifying the sample. Concentrations of NH_4^+ -N and NO_3^- -N were
221 determined from 10 g of soil extracted with 100 ml of 2M KCl (1:5 ratio); then the filtered extract
222 NH_4^+ -N and NO_3^- -N concentrations were measured by a continuous flow analysis autoanalyser
223 (SAN SYSTEM, Skalar Analytical B.V., Netherland).

224 Analysis of variance (ANOVA) was undertaken using a Mixed model according to the
225 statistical design in SAS environment (SAS Institute 2008). Treatment means were separated using
226 *p* differences of the LSMEANS.

227 Regressions between GC and IRGA, and GC and PGA, for CO₂, and for CO₂ and N₂O,
228 respectively, were computed. Soil CO₂ emission rate measurements from IRGA and PGA were
229 compared on the 22 sampling occasions. Comparisons were made by a regression analysis and the
230 index of agreement (IoAd) (SAS Institute 2008; Bennett et al. 2013).

231 **Results**

232 The temperature inside the greenhouse during the experiment ranged from a minimum of
233 17°C to the maximum of 28.5°C, with an average of 20.5°C, while soil temperature ranged from a
234 maximum of 27°C to a minimum of 20°C with a slight decreasing trend from the start to the end of
235 the experiment (Fig. 1). The chemical composition of the plant residues used in the present study,
236 expressed as percentages, are reported in Table 2. The N content of faba bean and durum wheat
237 were comparable (1.4% vs 1.3%, respectively). With regards to the other constituents, marked
238 differences were found between the plant residues. In particular, faba bean had higher ADF (+66
239 %), ADL (+186%), cellulose (+60 %), and NDF (+19%) than wheat, and a lower content of
240 hemicellulose (-51 %) (Table 2).

241

242 **Carbon and nitrogen dynamics**

243 The Haploxerert used in the present study had a high pH (8.1) and high clay and low total C
244 content (1.39%), whereas the Cambisol had a near neutral (6.6), low clay and high C content
245 (2.48%). Interaction between soil and residue type for these soil properties by the end of the
246 experiment was strong and significant (*p*<0.05) (Table 3). As expected, the addition of organic
247 residues mostly increased DOC in both the top- and sub-soil layers of the Haploxerert (on average
248 by 52.5% compared to unamended control), whereas there was no significant effect on the
249 Cambisol.

250 The soil incubation, either with or without plant residues incorporation, decreased soil pH by
251 0.86 in the Cambisol and 0.33 in the Haploxerert. The effect of the addition of organic residues to
252 the soil pH varied with both the soil and kind of biomass incorporated: in the Cambisol, addition of
253 wheat residues significantly decreased pH in the top- and sub-layers when compared with the
254 unamended control whereas addition of faba bean residues did not influence soil pH. In the
255 Haploxerert, no effect of the addition of organic residues on soil pH were found in both soil layers.

256 The concentration of NH₄⁺-N was higher in the Haploxerert than Cambisol, and this
257 particularly apparent in the sub-layer. The role of the addition of organic residues on soil NH₄⁺-N
258 depended on the soil and kind of biomass added: addition of durum wheat residues increased soil
259 ammonium-N in top-layer of both soils (+40% in the Cambisol and +102% in the Haploxerert),
260 whereas NH₄⁺-N in the soils amended with faba bean residues was similar to those of the controls.
261 In the sub-layer of the Cambisol, the effect of the addition of the organic residues was similar to
262 that observed in the top-layer, whereas addition of both residues strongly increased the NH₄⁺-N of

263 Haploxerert comparing to the unamended control (+133% in faba bean and +454% in wheat
264 residues).

265 The concentration of NO_3^- -N in both layers was significantly higher in the Cambisol when
266 compared with the Haploxerert and this occurred irrespective of the addition of organic residues. In
267 the Cambisol, addition of faba bean residues reduced NO_3^- -N more than wheat residues, especially
268 in the sub-layer, when compared with the unamended control. In the Haploxerert, NO_3^- -N in both
269 layers did not vary with the addition of plant residues.

270 NH_4^+ -N: NO_3^- -N ratio differed considerably in the different soil types: in the unamended
271 controls, it was 6.467 in the Haploxerert and 0.006 in the Cambisol. In the latter, addition of organic
272 residues to the soil did not influence the NH_4^+ -N: NO_3^- -N of either the top- or sub-layer. In the top-
273 layer of Haploxerert, the addition of organic residues reduced the NH_4^+ -N: NO_3^- -N ratio, especially
274 when faba bean residues were added. In the sub-layer, an opposite result was found and thus
275 addition of organic residues increased the NH_4^+ -N: NO_3^- -N ratio, especially when wheat residues
276 were added.

277 **Greenhouse gas emissions**

278 Carbon dioxide fluxes, measured with IRGA, ranged from a minimum value of $0.11 \text{ g m}^{-2} \text{ h}^{-1}$
279 ¹ to a maximum value of $3.64 \text{ g m}^{-2} \text{ h}^{-1}$ (Fig. 2). For almost the entire experimental period, the
280 Cambisol had a higher CO_2 emission flux than the Haploxerert. At the beginning of the experiment
281 the two soil reached the maximum emission flux at the first and second day of measurement with
282 fluxes of $3.58 \text{ g m}^{-2} \text{ h}^{-1}$ for the Cambisol and $1.42 \text{ g m}^{-2} \text{ h}^{-1}$ for the Haploxerert.

283 The highest CO_2 fluxes were recorded in both soils amended with wheat straw whereas the
284 lowest in the unamended controls. The differences in emission between the two soils were strong in
285 the first two weeks of measurement, where the 53.8% and 46.2% of total CO_2 were emitted from
286 the Cambisol and the Haploxerert, respectively. After the first two weeks of measurement, the
287 differences between the two soils reduced and the emission decreased until the end of the
288 experimental period.

289 The CO_2 emissions measured with PGA showed a similar trend to those acquired by IRGA.
290 However, in the first part of the experimental period, PGA emissions were slightly higher than
291 those observed by the IRGA, especially from the Cambisol. In the second part of the experiment, no
292 differences between the techniques were found (Fig 2).

293 Total CO_2 emissions were 74% lower in the unamended Haploxerert ($198 \text{ g CO}_2 \text{ m}^{-2}$)
294 compared to the Cambisol ($765 \text{ g CO}_2 \text{ m}^{-2}$). Addition of plant residues to the soil increased total
295 emission to a different extent depending on the soil under study (interaction Soil x Residue Type
296 significant $p < 0.001$): in the Cambisol, addition of faba bean and wheat resulted in an increase of
297 24% and 88%, respectively, of the total CO_2 emissions. In the Haploxerert, no differences were
298 found between the kind of biomass incorporated, which, on average, increased total CO_2 emission
299 by 171% compared to the unamended control (Fig 4).

300 Emissions of N_2O during the experiment ranged from 0.022 to $0.348 \text{ mg m}^{-2} \text{ h}^{-1}$ (Fig 5).
301 However there were large differences between soils with emissions of $0.024 \text{ mg m}^{-2} \text{ h}^{-1}$ to 0.117 mg

302 $\text{m}^{-2} \text{h}^{-1}$ and from $0.022 \text{ mg m}^{-2} \text{h}^{-1}$ to $0.348 \text{ mg m}^{-2} \text{h}^{-1}$ in the Haploxerert and Cambisol,
303 respectively. The Cambisol reached a N_2O emission peak at 7 days after the beginning of the
304 experiment, whereas the Haploxerert soil showed a continuous and constant reduction of the N_2O
305 emission from the beginning of the experiment until the end of the trial. In addition, marked
306 differences between amended and unamended soil were observed in Cambisol during the first half
307 of experiment. The highest fluxes were measured in both soils amended with wheat straw.
308 Cumulative N_2O emission in the unamended controls of the Cambisol soil was 30% higher than in
309 Haploxerert soil (85.1 and $59.9 \text{ mg N}_2\text{O m}^{-2}$, respectively). Crop residue addition had a different
310 effect in each soil (interaction Soil x Residue Type significant $p < 0.001$). In the Cambisol, N_2O
311 emissions in the pots amended with wheat was $159.8 \text{ mg N}_2\text{O m}^{-2}$, (+88% more than the control)
312 and that of the pots amended with faba bean was $127.0 \text{ mg N}_2\text{O m}^{-2}$, (+49% than the control). In the
313 Haploxerert, faba-bean added pots emitted in total $80.8 \text{ mg N}_2\text{O m}^{-2}$ (+35% than the control) and
314 that added with wheat $67.2 \text{ mg N}_2\text{O m}^{-2}$ (+12% than the control; Fig 6).

315 **Comparisons of gas measurement techniques**

316 Few differences were found for the IRGA and PGA in CO_2 measurement when compared
317 with that from the GC. The determination factor was 0.937
318 ($y_{GC} = 1.0534x_{IRGA} - 0.0221 \text{ g CO}_2\text{m}^{-2} \text{h}^{-1}$) and 0.925 ($y_{GC} = 0.9887x_{PGA} - 0.0095 \text{ g CO}_2\text{m}^{-2} \text{h}^{-1}$) for
319 IRGA and PGA, respectively and index of agreement was 0.998 for both instruments.

320 With regards to the N_2O measurement, the linear regression between GC and PGA showed a
321 relatively high relationship between the results ($R^2 = 0.90$;
322 ($y_{GC} = 0.8993x_{PGA} - 0.0063 \text{ mg N}_2\text{O m}^{-2} \text{h}^{-1}$)), although PGA- N_2O were, on average, 5.2 % higher
323 than the GC- N_2O measurements. However, in this case, the index of agreement was also 0.998 .

324 The comparison between CO_2 measurements obtained by IRGA and PGA across the entire
325 experimental period (more than 600 measurements) showed a high correlation between the two
326 instruments ($R^2 = 0.95$; $\text{IoAd} = 0.996$; ($y_{IRGA} = 1.0118x_{PGA} - 0.0003 \text{ g CO}_2\text{m}^{-2} \text{h}^{-1}$). However, the
327 cumulative CO_2 emissions measured by PGA were on average 9% higher than those measured by
328 IRGA. Differences in CO_2 fluxes from the two soil were apparent from the different measurement
329 techniques. Thus although the overall CO_2 fluxes measured by PGA were 6% higher than IRGA,
330 such differences were up to 10% greater when the comparison was limited to the Haploxerert soil,
331 and up to 17% when only the control plots were considered. In the Cambisol the differences between
332 the instruments were lower at around 5%.

333

334 **Discussion**

335 **N_2O and CO_2 emission and soil properties**

336 This study evaluated the effect of soil incorporation of two different plant residues on N_2O
337 and CO_2 emissions. The characteristics of two soils were distinctly different, with the Cambisol
338 having a low pH and high SOC while the Haploxerert had a high pH and low SOC. Emissions and
339 soil parameters varied according to both the kind of residue added and the soil type. The total CO_2
340 and N_2O emissions, (measured by PGA), from the unamended Cambisol were 249% and 40%
341 higher than the unamended Haploxerert, respectively, suggesting large differences in biochemical
342 and microbial activity between both soils driven by differences in soil physical and chemical

343 properties. Moreover, the differences in CO₂ emissions between the two soils followed the
344 differences in stable-C (TOC was 78% higher in the Cambisol than in Haploxerert) and readily
345 available-C (DOC in the Cambisol was double that in the Haploxerert). This latter form, although it
346 may be preferentially utilized by soil microorganisms, can be protected by soil aggregates or
347 adsorbed by mineral particles (Majumder and Kuzyakov 2010; Steinbeiss et al. 2008; Shi et al.
348 2014). The higher CO₂ emissions (per unit of carbon present in the soil) from Cambisol were
349 nevertheless a reflection of differences in the carbon pools. Such a differences suggest that the
350 Haploxerert had a relatively low respiration rate, which may have been a consequence of protection
351 by the higher clay content in the Haploxerert of SOC pools (Baldock and Skjemstad 2000; Krull et
352 al. 2003; Lutzow et al. 2006; Alluvione et al. 2013; Six and Paustian 2014), and coupled with
353 relatively low soil microbial activity due to a low free substrate availability. Another important
354 aspect related to the clay content is its mineralogy; the Haploxerert is characterized by prominent
355 swelling-shrinkage behaviour, which suggests that a high content of montmorillonite, can slow
356 down organic matter decomposition by absorption, interacting with soil microbes and their external
357 enzyme activity or limiting oxygen diffusion (Vogel et al. 2015). In addition, a recent highly
358 reliable model on SOC on the region the Haploxerert in the present study came from confirmed that
359 these kind of soil (along with other vertisols) have a high ability to stabilize the soil organic matter
360 (Schillaci et al, 2017; Saia et al. 2017). CO₂ and N₂O fluxes reached a peak in the within the first
361 week of incubation, and were higher in the Cambisol than in the Haploxerert. The transient effects
362 of the CO₂ and N₂O emission rates were likely to have resulted from increased gas diffusivity due
363 to the soil disturbance in the establishment of the experiment and the rapid decomposition of the
364 highly-labile free organic fraction (either added or not) (Magid et al. 1999; Baggs et al. 2006). Crop
365 residue distribution within the soil, as reported by several authors (Curtin et al. 1998; Jacinthe et al.
366 2002; Lian et al. 2016) stimulated and increased CO₂ emissions but with different magnitudes in the
367 two soils. In particular, the difference in CO₂ emissions between soils was reduced when an organic
368 residue (either faba bean or wheat) was added. The Cambisol emitted +88% and +152% more CO₂
369 than the Haploxerert when faba bean and wheat residues were added, respectively. Similar
370 differences were found for N₂O emission between soils amended with organic residues. These
371 findings are supported by research by An et al. (2015) where straw C input to the soil was more
372 effective at stimulating microbial activity and extractable organic carbon in a low fertility soil, than
373 in a high fertility soil, probably as a consequence of the starvation of the soil microbial community
374 (Bastida et al. 2013) and also a possible effect of clay which increases the contact between the
375 substrate and microorganisms. However, their experiment used a soil with a lower clay content
376 (24.9%), and we expect that in the soil used in our study which was more rich in clay (52.5%), this
377 effect was less important due to the absorption effects described above. Other studies have shown
378 that an increasing clay content (achieved by making artificial soils) accelerated the decomposition
379 rate of added organic matter supporting the concept that clay can have a primary role in influencing
380 decomposition-stabilization processes in the soil regulating the nutrient available for
381 microorganisms, emissions and organic carbon stabilization and sequestration (Velthof et al. 2002;
382 Six and Paustian 2014; Wei et al. 2014; Bajgai et al. 2014). Nitrous oxide emissions from the
383 Haploxerert were affected also by soil clay content and it's direct action on N immobilization
384 processes, as observed also by Begum et al. (2014) in an experiment conducted in a same type of
385 soil (Vertisol) with a comparable clay content (62%), closely linked to the stabilization of the
386 organic matter and confirmed by the high NH₄⁺-N:NO₃⁻-N observed. Furthermore, as result of the

387 the high cation exchangeable capacity of this soil (35 cmol kg^{-1}) the addition of organic matter had
388 a no effect on the pH, whilst in the Cambisol the wheat straw significantly reduced pH, most
389 probably as a consequence of the nitrification process which may acidify soil due to the release of
390 H^+ ions (Van Miegroet and Cole 1983). This would have been promoted by the high degradability
391 of wheat residues, that produced a higher nitrate content in the soil and promoted gaseous emissions
392 (both CO_2 and N_2O) compared to the soil where faba bean was added. In another experiment Aye et
393 al. (2016) using wheat and field-pea, with a different C:N ratio, as residues in a soil with 29% clay
394 found an increase in the decomposition process up to pH 7.4. However, in our experiment, although
395 the pH of the Haploxerert was slightly higher (7.8), the lower DOC concentration, CO_2 and N_2O
396 fluxes in Haploxerert, suggest that the lower decomposition rates that can be linked to the much
397 higher clay content (52.5%) confirming the dominant influence of clay as key factor in determining
398 nutrient turnover and emissions in this soil. The original pH of the soil may have played a role in
399 determining the magnitude of N_2O emissions by the soil microbial community. As reported from
400 Rousk et al. (2009), an acid pH at around 6 can stimulate fungal growth; fungi are recognized for
401 not having the ability to synthesise nitrous oxide reductase and their denitrification end product is
402 therefore N_2O . Other studies have reported that fungi could contribute up to 18% of potential
403 denitrification (Herold et al. 2012). Thus pH differences may also have contributed to differences in
404 N_2O emissions from soils.

405 There was a clear correlation between CO_2 and N_2O emissions in both soils, although this
406 was greater in the Cambisol, where oxygen depletion and CO_2 emissions could have helped create
407 anaerobic microsites in the soil increasing denitrification and N_2O production (Gök and Ottow
408 1988; Aulakh et al. 1991; Begum et al. 2014; Nett et al. 2015). The mineralization rate of an organic
409 residue added to the soil mostly depends on its C:N ratio and to a lesser extent to its lignin:N ratio
410 and fibre content (Trinsoutrot et al. 2000; Nguyen and Marschner 2016; Cheng et al. 2015).
411 However, in the present study, the difference in the C:N ratio of the residues used (38.6 in faba bean
412 and 40.7 in wheat) does not explain the difference in soil mineral N concentration and CO_2 and N_2O
413 emissions between the crop residues. Thus, it is more likely that mineralization rate of faba bean
414 residues was lower than wheat residues due to the different lignin, acid detergent, and neutral
415 detergent fibre contents (+188%, +66%, +19%, respectively in faba bean comparing to wheat).

416 The incorporation of plant residues, either of wheat or faba bean, introduced contrasting
417 effects on the $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations on each of the soils. The addition of plant
418 residues increased the $\text{NH}_4^+\text{-N}$ concentration of the Haploxerert, but not that of the Cambisol, and
419 such an increase was more evident when wheat residues were added. At the same time, addition of
420 plant residues reduced the total $\text{NO}_3^-\text{-N}$ content of the Cambisol, but not that of the Haploxerert, and
421 such an effect was more evident when faba bean residues were added. Such a result points to a net
422 immobilization process in the soil due to consumption of N in order to decompose organic C
423 (Corbeels et al. 2000; Jin et al. 2013). In the Haploxerert, a similar quantity of total CO_2 was
424 emitted after the addition of both crop residues, but the faba-bean addition showed a slightly higher
425 N_2O emission than wheat addition treatment coupled with lower $\text{NO}_3^-\text{-N}$ content at the end of the
426 experiment. Thus, it is likely that in this soil, which was characterized by a lower soil microbial
427 activity, the lower mineralization of faba bean residues led to a more constant availability of labile
428 C and N, due stimulating bacterial and fungal activity along the experiment until the end, and as
429 consequence, denitrification in soil microsites as reported from other authors (Deenik 2006; Shah et

430 al. 2016). By contrast, wheat residues produced a rapid flush in emission in the initial phase of the
431 experiment and shown at the end of the experiment higher $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentration in to
432 the soil suggesting other limitation. This selective activity of microbes induced by the residue
433 composition results in readily available straw C being used more rapidly while more recalcitrant
434 and stable compounds are decomposed more slowly (Majumder and Kuzyakov 2010). In the
435 Cambisol both crop residues showed the same trend in gas emissions, (CO_2 and N_2O), due to a
436 direct effect of residue characteristics on decomposition and N availability. The rapid
437 mineralization of wheat resulted lower DOC and higher $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations and a
438 reduction in pH, as described above. In the case of faba bean the higher presence of recalcitrant
439 compounds, in particular lignin, slowed down nutrient release and decreased emissions.

440 **Comparison between gas flux measurement techniques**

441 This study has clearly demonstrated that IRGA and PGA methodologies used to measure
442 CO_2 and N_2O emissions provided data consistent with that measured by GC. The comparison of
443 CO_2 and N_2O emission rates measured by IRGA and PGA was very strong correlated with GC
444 measurements, an observation also reported by other authors (Pumpanen et al. 2004; Iqbal et al.
445 2013; Nicoloso et al. 2013; Tirol-Padre et al. 2014). In particular, the same trend was observed for
446 both gas fluxes measured in the Cambisol and Haploxerert, which were characterized by different
447 patterns of CO_2 and N_2O emissions. Similar results to those observed in the present experiment
448 were found for N_2O fluxes by Iqbal et al. (2013), who reported a slightly higher emissions with
449 PGA than with GC (+5%), However, by contrast, we didn't find any difference in CO_2 flux
450 measurements when comparing PGA and GC. Nicoloso et al. (2013) observed an overestimation of
451 18.6% and 13.6% comparing PGA to GC, for CO_2 and N_2O respectively; we did not find any
452 differences between the techniques, which may have been due to the lower gas concentrations
453 measured during our experiment. That also defined, the positive effect of the compensation against
454 water vapor and cross interference, the two main sources of interference on measurement, during
455 the experiments.

456 With regard to the accuracy of CO_2 emission data recorded by IRGA, if comparing our
457 performance with those obtained from Pumpanen et al. (2004), the latter of which are based on CO_2
458 concentration measurements, we obtained better results with very similar fluxes between IRGA and
459 GC. The quality of data obtained from EGM-4 IRGA used in the present study was also confirmed
460 by Mills et al. (2011) who found good similarity in soil respiration flux with a different IRGA type.
461 However, PGA was found to have some limitations in reporting CO_2 fluxes measured by IRGA in
462 the first part of the experiment and monitoring the emissions of Haploxerert control in the later part
463 of the experiment, showing some difficult on measure low and high peak of emission producing a
464 slight overestimation on data. At medium and low emission rates the instruments performances
465 were similar and this was also confirmed by GC. Taking into account the reliability of data, together
466 with the speed of measurement, and the capacity to obtain high resolution temporal data, this study
467 highlights the benefits of using online IRGA and PGA measurements in studies of residue
468 decomposition. When applied in the field experiment, the short time required from IRGA and PGA
469 to take a measurement of emission provides an opportunity to make more measurements permitting
470 a higher spatial and temporal resolution. In the case of the PGA, the results produced had a

471 considerable importance due to the possibility of this instrument to measure two or more gaseous
472 compounds simultaneously Horsley et al. 2014.

473 Finally, although the chamber techniques coupled with GC is considered the reference
474 technique for the GHG monitoring, direct measurement by these devices eliminates many of the
475 risks resulting from sampling pitfalls and sample storage that can negatively affect the
476 measurements (Cowan et al. 2014; Tirol-Padre et al. 2014). For the specific application to GHG
477 studies, the initial cost and maintenance can be lower than GC systems, requiring also less
478 specialized staff to operate. The comparison of CO₂ emissions rates measured by IRGA and PGA
479 across the entire experimental period revealed, overall, that there were small differences between
480 both methods.

481 **Conclusions**

482 Soil plays a major role in controlling GHG emissions to the atmosphere and are a key
483 determinant of emissions originating from plant residues. Our study demonstrated, when
484 comparing two different soils, how specific properties, such as clay content and pH, can
485 significantly alter decomposition, immobilization and gaseous emissions. These results have
486 implications for developing low-C management practices, especially under organic farming systems
487 where residue management could be a strategy to replace mineral fertilizers and limit C footprint. In
488 Vertisols, which are widespread, but less well understood, CO₂ and N₂O emissions were strongly
489 controlled by clay content limiting emissions, promoting C sequestration and N transfer to next crop
490 cycle. Although many studies on the decomposition of residues have focused on C/N ratios, this
491 study highlights the importance of fibre compounds, often referred to as secondary, on determining
492 soil CO₂ and N₂O emissions and as their effect can change in relation to the soil characteristics. In
493 particular, in soil with high organic carbon contents and microbial activity such as a Cambisol, the
494 crop residue type determined the total emission. There was a unique trend for higher emissions of
495 both gases (CO₂ and N₂O) in the presence of more decomposable wheat than with recalcitrant faba
496 bean. In Haploxerert, by contrast, the slower decomposition of crop residues resulted in a similar
497 CO₂ release from the different residues, but slightly higher N₂O emissions from faba bean.

498 The direct comparison between IRGA and PGA and their validation with GC confirmed that
499 these two techniques are equivalent in providing reliable data for long-term monitoring, and this
500 occurred under various conditions (differing soil type residue addition). This result is important
501 when considering that GC-based methodologies need of a number of sample steps from gas
502 collection, transport, sample storage, and analysis, each of which can potentially add error to the
503 measurement. In addition, GC-based methodologies are not able to provide a continuous
504 measurement of the GHG emissions and thus are poor at quantifying temporal variability. By
505 contrast, the high sensitivity of IRGA and PGA, range and ease of application, number of gases
506 analyzed (including water vapor) allow a better monitoring of the radiative force of the soil while
507 eliminating many of the risks of the GC-based methodologies.

508

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767 temperature. *Adv. Atmos. Sci.* 21:691–698
- 768

769 **Tables and Figures**770 **Table 1** Main properties of soils.

Soil Properties	Scotland Bush Estate	Sicily Pietranera
Soil classification	Eutric Cambisol	Chromic Haploxerert (Vertisol)
Soil series	Macmerry	Gessoso-solfifera (sulphurous-chalky)
Texture	Sandy-loam	Clay-loam
Coordinates	55.9 N, 3.2 W	37.3 N, 13.3 W
Altitude	199	178
Slope [%]	6	7
Clay [%]	12.7	52.5
Silt [%]	15.7	21.6
Sand [%]	71.6	25.9
pH	6.6	8.1
Field capacity (pF 2.5) [%]	36	38
Permanent wilting point (pF 4.5) [%]	20	16
Organic matter [%]	4.3	2.4
Total N [%]	0.21	0.13

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774 **Table 2** Composition of crop residues.

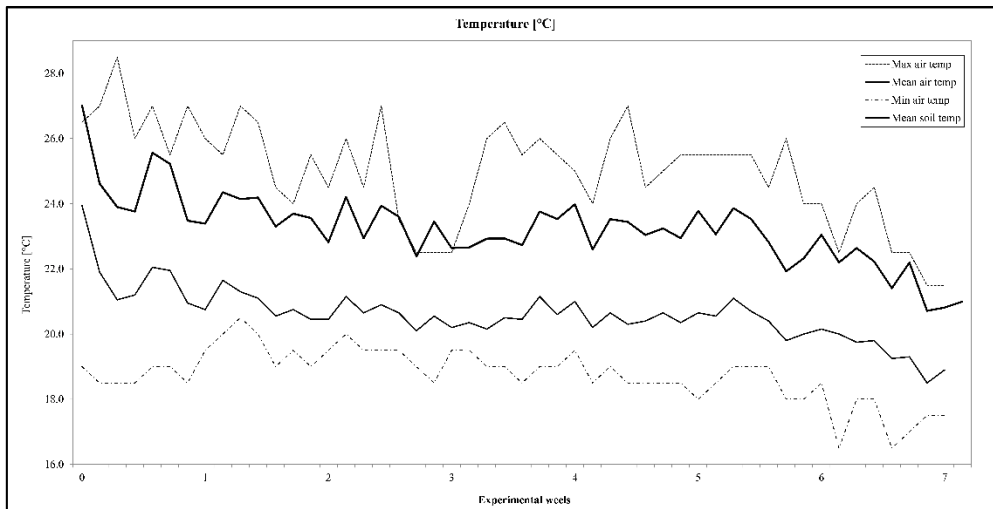
Chemical properties of crop residues	Faba Bean	Durum Wheat
Organic Matter	91.8	92.1
N content	1.4	1.3
Crude protein	8.8	8.1
Ether extract	1.1	1.7
Acid detergent fibre (ADF)	48.0	28.8
Acid detergent lignin (ADL)	10.0	3.5
Cellulose	38	25.3
Neutral detergent fibre (NDF)	54.0	45.4
Hemicellulose	6	16.6
Ash	8.2	7.9
ADL Ash	0.4	3.2

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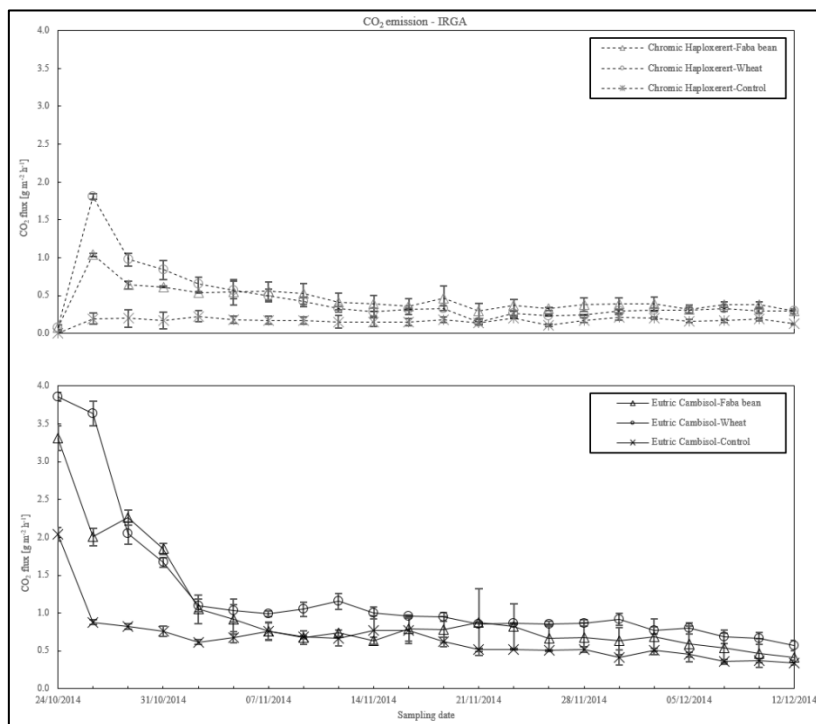
Table 3 Effect of the addition of two crop residues (Durum wheat or fababean, and unamended control) on Dissolved Organic C (DOC), pH, NH₄⁺-N and NO₃⁻-N content and NH₄⁺-N: NO₃⁻-N ratios in 0-5 cm and 5-15 cm soil layers of a Chromic Haploxerert and Eutric Cambisol soils.

		Chromic Haploxerert			Eutric Cambisol			<i>P</i>		
		Faba bean	Durum wheat	No addition	Faba bean	Durum wheat	No addition	<i>Soil</i>	<i>Residue Type</i>	<i>S × T</i>
0-5 cm soil layer										
DOC	mg C kg ⁻¹ soil	42.5	43.2	33.6	73.5	67.6	67.2	<.001	0.000	0.007
pH	-	7.7	7.8	7.8	5.8	5.4	5.9	<.001	0.009	0.019
NH ₄ ⁺ -N	mg N kg ⁻¹ soil	1.6	3.3	1.7	0.9	1.3	0.9	<.001	<.001	<.001
NO ₃ ⁻ -N	mg N kg ⁻¹ soil	0.4	2.4	0.3	104.6	149.6	164.5	<.001	0.001	0.001
NH ₄ ⁺ -N: NO ₃ ⁻ -N	-	4.3	1.4	6.5	0.009	0.008	0.006	<.001	0.001	0.001
5-15 cm soil layer										
DOC	mg C kg ⁻¹ soil	75.9	83.0	48.1	86.4	93.1	91.8	<.001	<.001	<.001
pH	-	7.7	7.7	7.8	5.9	5.6	5.8	<.001	0.037	0.043
NH ₄ ⁺ -N	mg N kg ⁻¹ soil	13.5	32.0	5.8	1.1	1.5	0.9	<.001	<.001	<.001
NO ₃ ⁻ -N	mg N kg ⁻¹ soil	0.5	0.5	0.8	36.9	43.3	66.4	<.001	<.001	<.001
NH ₄ ⁺ -N: NO ₃ ⁻ -N	-	25.7	62.9	7.3	0.030	0.034	0.014	<.001	<.001	<.001



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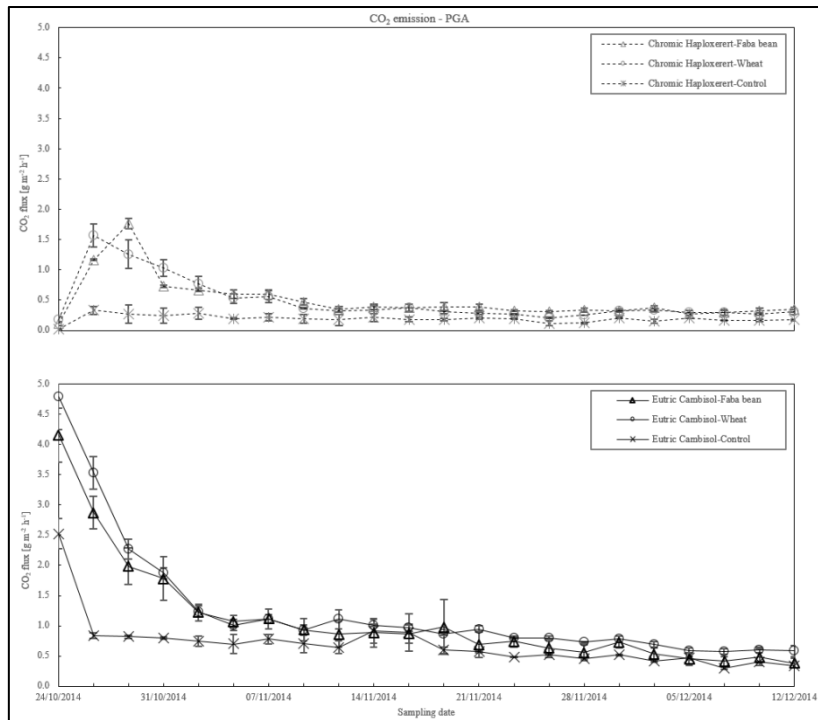
2 **Fig. 1** Daily minimum, maximum, mean air temperature in the greenhouse and mean soil temperature during the
 3 experiment



4

5 **Fig. 2** CO₂ emission course from Chromic Haploxerert and Eutric Cambisol amended with faba bean and wheat
 6 residues, or unamended (control), measured with IRGA during the experimental period. Data are means±S.E (n=3)

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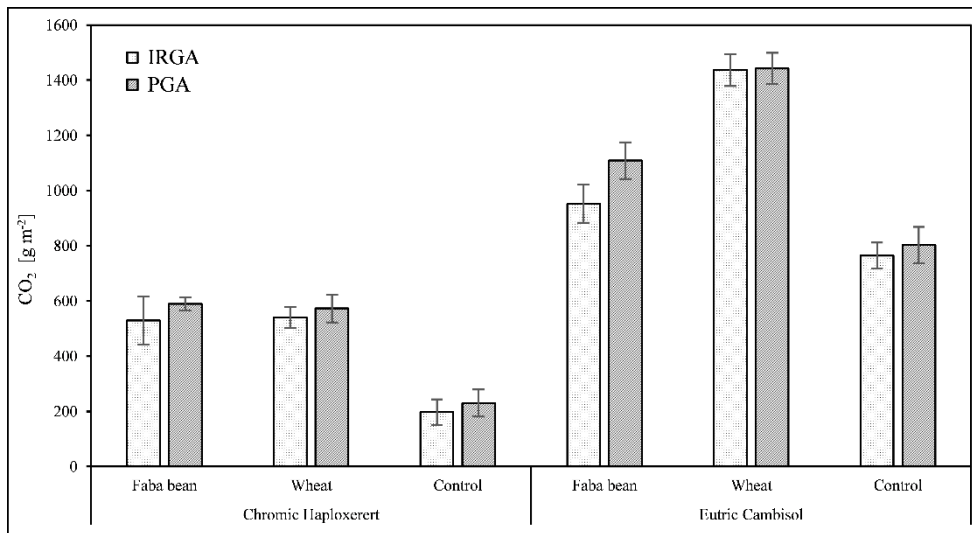


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9 **Fig. 3** CO₂ emission course from the Chromic Haploxerert and Eutric Cambisol soils amended with faba bean and wheat biomass,
 10 or unamended (control), measured with PGA during the experimental period. Data are means±S.E (n=3)

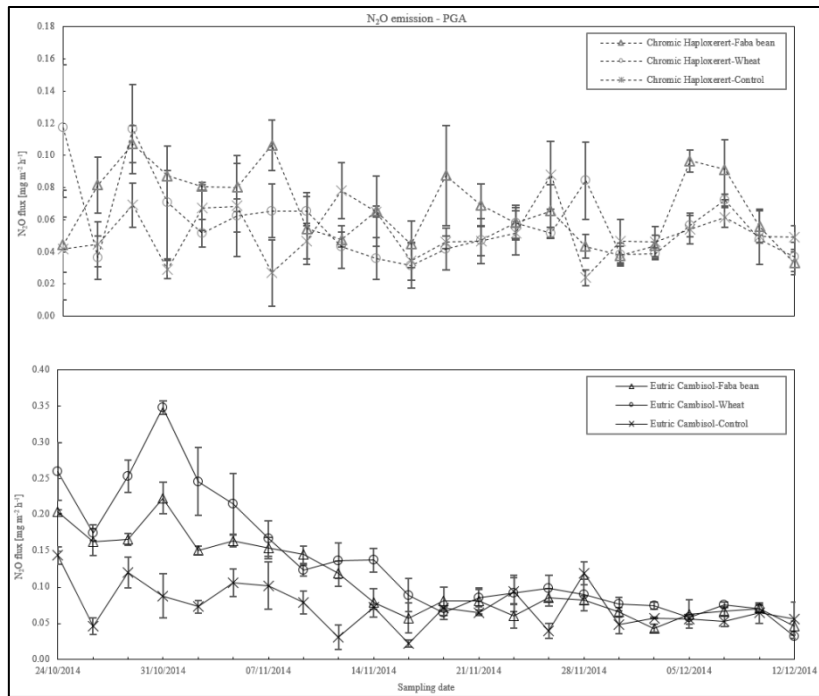
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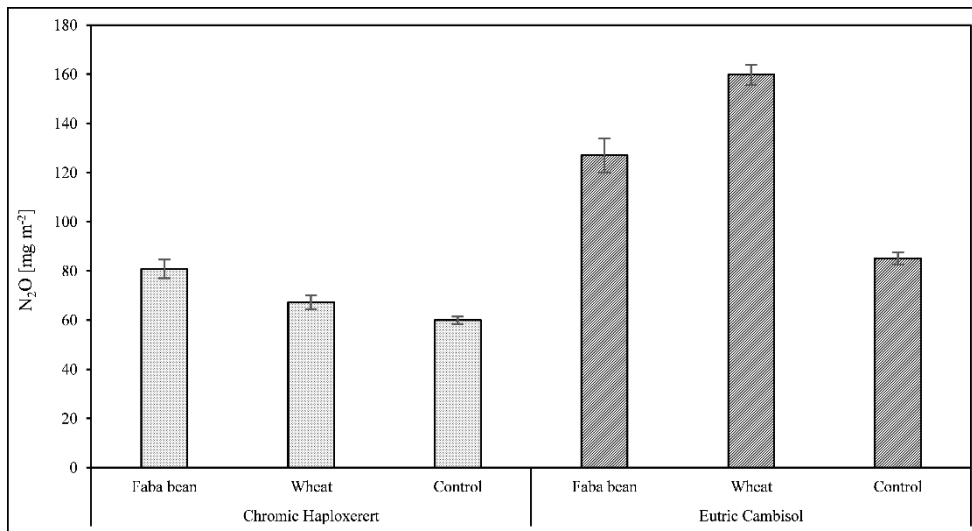
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14 **Fig. 4** Total CO₂ emission from the Chromic Haploxerert and Eutric Cambisol amended with faba bean and wheat
 15 biomass, or unamended (control), measured with IRGA and PGA. Data are means±S.E (n=3)



16
 17 **Fig. 5** N₂O emission course from the Chromic Haploxerert and Eutric Cambisol amended with faba bean and wheat
 18 biomass, or unamended (control), measured with PGA during the experimental period. Data are means±S.E (n=3)

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20
 21 **Fig. 6** Total N₂O emission from the Chromic Haploxerert and Eutric Cambisol soils amended with faba bean and wheat
 22 biomass, or unamended (control), measured with PGA. Data are means±S.E (n=3)