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Soil management effects on greenhouse gases production at the macroaggregate scale

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17 **Abstract**

18 Agricultural management practices play an important role in greenhouse gases (GHG)

19 emissions due to their impact on the soil microenvironment. In this study, two

20 experiments were performed to investigate the influence of tillage and N fertilization on

21 GHG production at the macroaggregate scale. In the first experiment, soil

22 macroaggregates collected from a field experiment comparing various soil management

23 systems (CT, conventional tillage; NT, no-tillage) and N fertilization types (a control

24 treatment without N and mineral N and organic N with pig slurry treatments both at 150

25 kg N ha^{-1}) were incubated for 35 days. Methane (CH_4), carbon dioxide (CO_2) and

26 nitrous oxide (N_2O) production was quantified at regular time intervals by gas

27 chromatography. In the second experiment, the effects of fertilization type and soil

28 moisture on the relative importance of nitrification and denitrification processes in N_2O

29 emission from soil macroaggregates were quantified. Nitrate ammonium,

30 macroaggregate-C concentration, macroaggregate water-stability, microbial biomass-C

31 and N (MBC and MBN, respectively) and water-soluble C (WSC) were determined.

32 While NT macroaggregates showed methanotrophic activity, CT macroaggregates acted

33 as net CH_4 producers. However, no significant differences were found between tillage

34 systems on the fluxes and cumulative emissions of CO_2 and N_2O. Greatest cumulative

35 CO_2 emissions, macroaggregate-C concentration and WSC were found in the organic N

36 fertilization treatment and the lowest in the control treatment. Moreover, a tillage and N

37 fertilization interactive effect was found in macroaggregate CO_2 production: while the

38 different types of N fertilizers had no effects on the emission of CO_2 in the NT

39 macroaggregates, a greater CO_2 production in the CT macroaggregates was observed for

40 the organic fertilization treatment compared with the mineral and control treatments.

41 The highest N_2O losses due to nitrification were found in the mineral N treatment while

42   denitrification was the main factor affecting N_2O losses in the organic N treatment. Our

43   results suggest that agricultural management practices such as tillage and N fertilization

44   regulate GHG production in macroaggregates through changes in the proportion of C

45   and N substrates and in microbial activity.

46

47   **Keywords**

48   Carbon dioxide, denitrification, fertilization, macroaggregate, methane, nitrification,

49   nitrous oxide, tillage.

Introduction

The production and consumption of soil greenhouse gases (GHG) is mediated by several microbial processes (Conrad, 1996). For instance, soil carbon dioxide (CO_2) emissions are the result of microbial heterotrophic respiration while methane (CH_4) is normally oxidized by methanotrophic bacteria in aerobic soils (Goulding et al. 1995). Furthermore, soil nitrous oxide (N_2O) production is the result of nitrification and denitrification processes (Blackmer et al., 1980; Firestone et al., 1980; Poth and Focht, 1985). Those microbial processes are regulated by the physical protective capacity of aggregates that limit decomposition of organic C and N compounds (Elliott, 1986). Soil aggregates not only protect C and N, but they also regulate both the structure and the activity of the soil microbial community (Gupta and Germida, 1988; Miller et al., 2009). The intra-aggregate distribution of pores plays a major role in microbial access to oxygen, substrates and water. As Young and Ritz (2000) pointed out, soil structure regulates oxygen diffusion to habitat sites, depending on the connectivity and tortuosity of pore pathways. The aggregate architecture also controls the distribution of water films within soil matrix, affecting microbial microhabitats. Thus, the diffusion of oxygen to the center of aggregates will depend on the spatial arrangement of water films (Young and Ritz, 2000). The last factors affect the importance of denitrification and respiration activities and demonstrate the role played by soil aggregates regulating them (Beare et al., 1994; Estavillo et al., 2002). Moreover, due to their physical protective capacity, soil aggregates also regulate the microbial accessibility to substrates.

In a recent experiment, Lenka and Lal (2013) have suggested that the aggregate hierarchy theory of Tisdall and Oades (1982) could be extended to describe the effect of soil aggregation on GHG emission from soil. That theory postulates that the nature of the organic binding agents (transient, temporary and persistent) regulates different

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hierarchical stages of aggregation. Microaggregates are formed by the joining of primary particles and silt-sized aggregates and persistent organic binding agents, while these microaggregates are bound together into macroaggregates by temporary and transient organic binding agents. These organic materials are protected by the heterogeneity of the soil microenvironment which limits the access of decomposers and their enzymes (Schmidt et al., 2011; Ananyeva et al., 2013).

The agricultural practices play an important role in GHG emissions due to their effects on the soil microenvironment. Tillage breaks soil aggregates leading to enhanced organic matter decomposition (Álvaro-Fuentes et al., 2008; Beare et al., 1994) and reduced C and N concentration (Plaza-Bonilla et al., 2010). Contrarily, the use and maintenance of no-tillage (NT) increases the stability of soil macroaggregates (Plaza-Bonilla et al, 2013b), a fact that could lead to a reduction in heterotrophic respiration due to a greater substrate protection, thus limiting the emissions of CO_2. Likewise, CH_4 production is also affected by tillage management. For instance, in a wheat-fallow rotation, Kessavalou et al. (1998) reported higher CH_4 uptake rates under NT when compared with a plough treatment. Also, Hütsch (1998a) reported 4.5-11 times greater CH_4 oxidation rates under NT than under conventional tillage (CT). Ball et al. (1999) hypothesized that the reduction in CH_4 oxidation usually found when tillage is performed could be due to the disturbance of the methanotrophic microbes by tillage, the changes in gas diffusivity or a long-term damage to methanotrophs due to disruption of soil structure. Tillage also has an impact on N_2O emissions. Estavillo et al. (2002), studying the effects of ploughing a permanent pasture on the emissions of this gas, observed an increase in both soil organic N mineralization and N_2O production rates from nitrification and denitrification processes after the breakage of soil aggregates by tillage.

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100 Nitrogen fertilization has a strong impact on soil aggregation and C and N protection.

101 The application of organic fertilizers such as pig slurry enhances the proportion of

102 easily-decomposable C fractions (Morvan and Nicolardot, 2009) that could act as

103 substrates for the denitrification process and the concomitant soil N_2O emissions to the

104 atmosphere (Burford and Bremnner, 1975). Sexstone et al. (1985) quantified the

105 diffusion of oxygen within soil aggregates establishing a relationship between their size

106 and their potential to act as denitrifying microsites within soil. Nitrogen fertilization

107 also plays a major role in methane oxidation. Different authors (Hütsch et al. 1993;

108 Mosier et al. 1991; Steudler et al. 1989), working with incubated soil cores from

109 agricultural, grassland and forest experiments, observed a decrease in CH_4 uptake when

110 applying inorganic N to soil. Contrarily, recent findings suggest that ammonium-based

111 fertilizers could stimulate the activity of methanotrophs (Bodelier and Landbroek,

112 2004).

113 In recent years, different experiments have been performed to analyze the effects of

114 aggregate size on CO_2, CH_4 and N_2O production (Diba et al., 2011; Drury et al., 2004;

115 Kimura et al., 2012). However, inconsistent results have been observed in the literature

116 due to the simultaneous diverse microbial processes that soil aggregates can hold (Sey

117 et al., 2008). For instance, Parkin (1987) related the spatial heterogeneity in the N_2O

118 emissions usually observed in most experiments with the presence of particulate organic

119 matter within soil aggregates. Those studies demonstrate that different aggregate

120 attributes such as size or C fractions within them regulate GHG production processes.

121 However, few experiments have studied the effects of agricultural management

122 practices on soil GHG production at the aggregate scale.

123 Thus, the objectives of this study were: (i) to analyze the effect of the use of different

124 types of tillage and N fertilization on the production of GHG by soil macroaggregates

125   and, (ii) to quantify the relative importance of the nitrification and denitrification

126   processes on the macroaggregate emissions of N_2O depending on the type of fertilizer

127   used. We hypothesized that (i) CT macroaggregates would emit a greater amount of

128   GHG due to their lower protection of the organic C and N compounds when compared

129   to NT macroaggregates and (ii) the application of pig slurry and mineral N would result

130   in different rates of GHG production provided by soil macroaggregates.

Materials and Methods

131

132 Soil samples were collected from an experimental field established in 2010 in Senés de

133 Alcubierre, NE Spain (41º 54' 12'' N, 0º 30' 15'' W), in an area with a temperate

134 continental Mediterranean climate. This field experiment has a randomized block design

135 with three replications comparing different tillage systems and N fertilization

136 treatments. Two tillage systems (CT, conventional tillage with disk ploughing and NT,

137 no-tillage) and two types of N fertilizers (mineral N with ammonium nitrate and

138 ammonium sulphate and organic N with pig slurry), with three N doses (0, 75 and 150

139 kg N ha^{-1}), were compared. Each year, in the CT treatment, tillage is performed right

140 before the seeding of barley (*Hordeum vulgare L.*) with one pass of a disk plough to 20

141 cm depth in October, after the application of organic and mineral fertilizers. The NT

142 treatment consisted of a total herbicide application (1.5 L 36% glyphosate per hectare)

143 for controlling weeds before sowing. Mineral N fertilizer was manually applied. The

144 treatment with 150 kg N ha^{-1} was split into two applications: half of the dose before

145 tillage as ammonium sulphate (21% N) and the other half at the beginning of tillering,

146 in February, as ammonium nitrate (33.5% N). For the 75 kg N ha^{-1} treatment the entire

147 dose was applied at tillering as ammonium nitrate. Equally, in the treatments with

148 organic fertilization, the 75 kg N ha^{-1} rate was applied entirely at tillering and the 150 kg

149 N ha^{-1} one was split into two applications, one half before tillage and the other half at

150 tillering. The organic fertilization treatment consisted of the application of pig (*Sus*

151 *scrofa*) slurry from a commercial farm in the area. The slurry was conventionally

152 surface-spread using a commercial vacuum tanker fitted with a splashplate. The

153 machinery was previously calibrated to apply the precise dose after analyzing the pig

154 slurry. The main edaphoclimatic characteristics of the experimental site are listed in

155 Table 1. Prior to the establishment of the experiment the field was conventionally tilled

156 and fertilized with mineral N for four decades until 2008. Then, the whole field was

157 transformed to no-tillage. Finally, as commented before, when the experiment started in

158 2010, the CT plots were added. The cropping system is a continuous barley

159 monoculture.

160 *Experiment 1: GHG production from soil macroaggregates under different tillage*

161 *and N fertilization treatments.*

162 Soil samples were obtained from both tillage treatments (CT and NT) and the lower (0

163 kg N ha^{-1}, Control) and the higher (150 kg mineral N ha^{-1}, Mineral, and 150 kg organic

164 N ha^{-} with pig slurry, Organic) fertilization treatments of the field experiment. Soil

165 sampling was performed in March 2012 during the late tillering stage of the crop, three

166 weeks after the top-dressing application of fertilizers. In each plot (i.e., tillage and N

167 fertilization treatments), soil samples were collected from four areas that correspond to

168 the four replications. From each sampling area, a composite sample of approximately

169 500 g was taken from the 0-5 cm soil depth using a flat spade and outside the wheel

170 tracks areas. Afterwards, the samples were stored in crush-resistant airtight plastic

171 containers for 3-4 hours. Once in the laboratory, the samples were gently passed

172 through an 8mm sieve and air-dried at room temperature. Soil macroaggregates (0.250-

173 8 mm) were obtained placing the 8-mm soil sieved sample on the top of a 0.250 mm

174 sieve in an electromagnetic sieve apparatus (Filtra FTL-0200, Badalona, Spain). A

175 sieving time of 1 min and the lowest power program of the device were used to avoid

176 macroaggregate breakage. The dry-sieved macroaggregates (0.250-8 mm) obtained

177 were stored in aluminium trays taking care to avoid any breakage until further analyses.

178 Samples of 40-g each of dry-sieved macroaggregates (0.250-8 mm) were placed in 500

179 ml Mason jars. Four jars were built for each tillage and N fertilization combination. A

180   stainless steel fitting turned to accommodate two silicon-Teflon septa was inserted in

181   the lid of each jar to ensure air tightness. A volume of 12.8 ml of distilled water was

182   added to each macroaggregate sample using a micropipette in order to avoid the

183   breakage of the macroaggregates when adding the water, and also to obtain a

184   gravimetric moisture content of about 32%. This value corresponds to the field capacity

185   of the bulk soil of our experiment according to Saxton and Rawls (2006). All the jars

186   were covered with a layer of parafilm which was pinpricked to ensure air exchange and

187   avoid sample desiccation during the incubation process. The weight of the jars with the

188   wet macroaggregate samples was recorded and then every 48 hours to check for water

189   evaporation. Distilled water was added when needed. Air samples were withdrawn at

190   0(0), 4(0.17), 12(0.5), 24(1), 48(2), 72(3), 192(8), 384(16), 504(21), 672(28) and

191   840(35) hours(days) after the beginning of the incubation process. The parafilm layer of

192   each jar was removed 15 min prior to each gas sampling. Then all the lids were tightly

193   closed and a 15 ml headspace gas sample was withdrawn with the use of a gas-tight

194   syringe, pumping twice before the extraction to ensure a total mixing of the gas in the

195   jar (0 min sampling). Afterwards, 15 ml of ambient air were injected in the jars to

196   compensate for the volume previously withdrawn. A second gas sampling was

197   performed 60 min later. The gas samples obtained were injected in 12 ml Exetainer

198   borosilicate glass vials (model 038W, Labco, High Wycombe, UK) until their analysis.

199   Once the samplings were made (i.e., after 60 min) the lids were opened and the jars

200   were covered with a parafilm layer until the next sampling event. Also, the jars were

201   covered during the incubation to avoid light exposure. As explained in the next section,

202   the difference in GHG concentration between 0 and 60 min samplings was used to

203   calculate the GHG fluxes.

204   *Gas and soil analysis*

205 The gas samples were analyzed with an Agilent 7890A gas chromatography system

206 equipped with an electron capture detector (ECD) and a flame ionization detector (FID)

207 plus methanizer, and three automated valves to obtain the three gases of interest (i.e.,

208 CH_4, CO_2 and N_2O) for each gas sample injection. A HP-Plot Q column (30 m long,

209 0.32 mm of section and 20 µm) was used along with a 15 m long pre-column of the

210 same material. The injector and the oven temperature were set to 50ºC. The temperature

211 of the FID and ECD detectors was set to 250ºC and 300ºC, respectively. The methanizer

212 temperature was set to 375ºC. For the FID detector, H_2 was used as a carrier gas and N_2

213 as a make-up gas at 35 and 25 ml min^{-1}, respectively. In the case of the ECD detector,

214 5% methane in Argon was used as a make-up gas at 30 ml min^{-1}. The volume of sample

215 injected was 1 ml. The system was calibrated using analytical grade standards (Carburos

216 Metálicos, Barcelona, Spain). Soil CH_4, CO_2 and N_2O production in the jar headspace

217 was calculated according to Holland et al. (1999). Gas concentrations (ppm) obtained

218 with the chromatography system were converted to mass units with the ideal gas

219 equation:

220 $C_m = (C_v \times M \times P)/(R \times T)$

221 where C_m is the mass/volume concentration (e.g., mg CO_2-C m^{-3} incubation jar

222 headspace), C_v is the volume/volume concentration (ppm of each GHG obtained with

223 the chromatography system), M is the molecular weight of each GHG (e.g., 12 g CO_2-C

224 mol^{-1} or 28 g N_2O-N mol^{-1}), P is atmospheric pressure, R is the universal gas constant

225 and T is the incubation temperature (298 K). C_m was multiplied by the headspace

226 volume of the incubation jars (5 x 10^{-4} m^{-3}) to obtain the mass of CH_4-C, CO_2-C or

227 N_2O-N accumulated during the incubation. Thus, the mass of GHG produced (e.g., mg

228 CH_4-C kg^{-1} macroaggregates h^{-1}) is calculated as follows:

229    $f = ((C_1 - C_0)/(m \times t)) \times 1000$

230   where f is the mass of gas produced per unit of time, C_1 and C_0 are the mass of C or N

231   produced at the end and at the beginning of two consecutive samplings, respectively, m

232   is the mass of air-dried macroaggregates in each jar (0.04 kg) and t is the incubation

233   period (1 h). Finally, the cumulative production of CH_4-C, CO_2-C and N_2O-N was

234   calculated using the trapezoid rule by linear interpolation between two consecutive

235   samplings.

236   Additionally, the initial mineral N (i.e., nitrate and ammonia), the C concentration, and

237   the proportion of water-stable macroaggregates were quantified for each experimental

238   unit. Once the incubation was finished, the microbial biomass-C and microbial biomass-

239   N (MBC and MBN, respectively), the nitrate and ammonia content, the water-soluble C

240   (WSC) and the C concentration of each 40 g macroaggregates sample were also

241   quantified. Soil nitrate (NO_3^-) and ammonium (NH_4^+) were determined extracting 10 g

242   of macroaggregates with 80 ml of 1 M KCl and using a continuous flow autoanalizer

243   (Seal Autoanalyzer 3). The macroaggregate-C concentration was quantified by the wet

244   oxidation method of Walkley-Black described by Nelson and Sommers (1996), with a

245   modification to increase the digestion of soil organic carbon (SOC), which consisted in

246   boiling the sample and the extraction solution at 150ºC for 30 min (Mebius, 1960). The

247   proportion of water-stable macroaggregates and their sand content were determined

248   following a modification of the method of Elliott (1986) as described in Plaza-Bonilla et

249   al. (2013a). The microbial biomass-C and microbial biomass-N were determined with

250   the chloroform-fumigation and direct extraction method of Vance et al. (1987). The

251   extracts were analyzed for organic C and N with a multi C/N TOC-TNB analyzer 3100

252   (Analytik Jena, Jena, Germany). The extraction coefficient applied for both C and N

253   was 0.38 (Sparling and Zhu, 1993; Vance et al., 1987). The WSC was extracted by

254 shaking 10 g of macroaggregates in 40 ml of distilled water with 0.5 g potassium

255 sulphate in a centrifuge tube for 30 min, centrifuging for 5 min at 5000 rpm and filtering

256 all supernatant solution through a Whatman no.42 filter. The organic C in the filtrate

257 was determined by the same device used for the MBC-MBN determination.

258 *Data analysis*

259 Cumulative GHG data were log-transformed and analyzed using the SAS statistical

260 software (SAS Institute Inc., 1990). To compare the effects of tillage, fertilizer

261 treatments and sampling time on cumulative GHG emissions, a repeated measures

262 analysis of variance for a bifactorial design was performed for each gas. When

263 significant, differences among treatments were identified at the 0.05 probability level of

264 significance using an LSD test. For each sampling time, the linear relationship between

265 CO_2 and N_2O production in the CT and NT macroaggregates was determined with the

266 statistical package JMP 10 (SAS Institute Inc, 2012). To analyse the relationship

267 between CO_2 production, proportion of water-stable macroaggregates and their C

268 concentration, a stepwise regression was performed using the statistical package JMP

269 10 (SAS Institute Inc, 2012).

270

271 ***Experiment 2: Relative importance of nitrification and denitrification in N₂O***

272 ***production from soil macroaggregates under different N fertilization types.***

273 Soil samples from the 0-5 cm soil depth were collected in the same fertilization

274 treatments as in Experiment 1 (i.e., 0 and 150 kg N ha^{-1} as mineral N and pig slurry),

275 only under NT. However, for this experiment soil sampling was performed on

276 December 2012, three weeks after the pre-seeding fertilization of the crop. In each plot

277 (i.e., N treatment), six areas that would correspond to the six replications of the

278 experiment were defined.

279 From each area, a 500 g composite soil sample was taken from the 0-5 cm soil depth

280 using a flat spade, taking care to avoid the wheel track areas. Dry-sieved

281 macroaggregates fractionation was analogous to Experiment 1. The experimental set up

282 consisted of three N fertilization types (0 kg N ha^{-1}, mineral N at 150 kg ha^{-1} and

283 organic N with pig slurry at 150 kg ha^{-1}), two soil moisture treatments (15% and 30 %

284 gravimetric water content) and three levels of acetylene (0%, 0.01% and 5%, v v^{-1}).

285 Each combination of the three factors was repeated six times according to the

286 experimental replications. Therefore, the total number of observations was 108. To

287 achieve this number of observations, the dry-sieved macroaggregates from each

288 experimental replication was divided in six subsamples of 40 g that were placed in

289 Mason jars. These six subsamples were divided in two groups. In the first group, three

290 subsamples were moistened with distilled water to 15% gravimetric water content. The

291 other three subsamples were moistened to 30% gravimetric water content. The lids of

292 the jars were closed and a 15 ml headspace gas sample was taken for every jar (0 min

293 sampling). Afterwards, for each soil moisture treatment three acetylene (C_2H_2)

294 treatments were applied: 0%, 0.01% and 5% (v v^{-1}) corresponding to partial pressures of

295 0 Pa, 10 Pa and 5000 Pa, respectively) following the method proposed by Klemedtsson

296 et al. (1988) to differentiate the relative contribution of the nitrification and

297 denitrification processes in N_2O emissions. Different drawbacks of the method have

298 been reported in the literature. Among them, Baggs (2008) enumerates (i) a possible

299 underestimation of denitrification by preventing the supply of nitrifier-NO_3^-, mainly in

300 aquatic systems (Groffman et al., 2006), (ii) acetylene could be used as a C-substrate for

301 denitrification, and (iii) a limited diffusion of acetylene into fine textured soils.

302　However, acetylene-based methods still have a role in systems with high NO_3^-

303　concentrations (Groffman et al., 2006), such as the agricultural soil of our experiment,

304　and are useful for comparative purposes between different treatments (Estavillo et al.,

305　2002). According to each treatment, different volumes of ambient air were injected to

306　equilibrate the pressure into the jars. The jars with the macroaggregates were incubated

307　at 25ºC for 24 hours. After that, another 15 ml gas sample was withdrawn to calculate

308　the accumulation of N_2O in the 24 hours period for each jar. Air gas samples were

309　stored and analyzed following the same methodology as in Experiment 1. It was

310　assumed that the N_2O measured in the treatment without acetylene (i.e., 0% C_2H_2)

311　corresponded to the N_2O produced by the nitrification and denitrification processes. In

312　turn, the N_2O measured in the treatment with 0.01% C_2H_2 corresponded only to that

313　produced during the denitrification process (Davidson et al., 1986) and, finally, the N_2O

314　measured in the treatment with a C_2H_2 concentration of 5% corresponded to the N_2O

315　produced due to a complete denitrification (Yoshinari et al., 1977). The production of

316　N_2O by the nitrification process was calculated from the difference between the N_2O

317　measured in the 0% and the 0.01% C_2H_2 treatments, while the production of N_2O by the

318　denitrification process corresponded to the amount of N_2O measured in the 0.01% C_2H_2

319　treatment, and complete denitrification (i.e., N_2O that would be reduced to N_2) was

320　calculated as the difference between the N_2O measured in the 5% and the 0.01% C_2H_2

321　treatments. The gas samples were analyzed with an Agilent 7890A gas chromatography

322　system equipped with an ECD detector with the same parameters as in Experiment 1.

323　Moreover, the mineral N content as nitrate and ammonium and the WSC were also

324　determined prior to the incubation following the methodology described above.

325　*Data analysis*

326   The N_2O production data were transformed using the Box-Cox procedure and analyzed

327   using the SAS statistical software (SAS Institute Inc., 1990). To compare the effects of

328   fertilizer treatments and soil moisture on N_2O production an analysis of variance was

329   performed. When significant, differences among treatments were identified at the 0.1

330   probability level of significance using an LSD test. Furthermore, the linear relationship

331   between WSC and N_2O production was determined with the statistical package JMP 10

332   (SAS Institute Inc., 2012)

333   **Results**

334   *Experiment 1: GHG production from soil macroaggregates under different tillage and*

335   *N fertilization treatments.*

336   Tillage significantly affected the fluxes of CH_4 produced by soil macroaggregates. As an

337   average of all the samplings performed during the incubation period, macroaggregates

338   of the CT treatment acted as emitters of CH_4 while those under NT acted as a CH_4 sink

339   (Table 2). Also, significant differences on cumulative CH_4 fluxes were observed

340   between CT and NT (Fig. 1a). According to the data, the methanotrophic activity in the

341   NT treatment began after the first 72 hours of macroaggregate incubation (Fig. 1a). In

342   contrast to CH_4, no significant differences were found between tillage systems on

343   neither the fluxes nor the cumulative emissions of CO_2 and N_2O (Table 2, Fig. 1b and

344   c).

345   Nitrogen fertilization treatments did not affect the fluxes of CH_4 and N_2O (Table 2).

346   Also, cumulative emissions of CH_4 and N_2O did not differ between N fertilization

347   treatments (Fig. 2a and c). CO_2 followed a different trend, with greater average fluxes in

348   the organic treatment (1669.4 µg CO_2-C kg macroaggregates^{-1} h^{-1}) when compared with

349   the control (1217.5 µg CO_2-C kg macroaggregates^{-1} h^{-1}) and the mineral (1199.4 µg

350   CO_2-C kg macroaggregates^{-1} h^{-1}) treatments (Table 2). Also, cumulative CO_2 emissions

351   were the greatest under the organic fertilization treatment in the first 48 hours of the

352   incubation, without differences between the control and mineral treatments (Fig 2b).

353   When the incubation was finished (i.e., after 840 hours), the organic treatment presented

354   a greater cumulative CO_2 emission when compared with the mineral treatment, while

355   the control presented intermediate values (Fig. 2b).

356 The interaction between tillage and N fertilization significantly affected the fluxes of

357 CO_2 (Table 2). The different N fertilization treatments did not show different CO_2

358 fluxes for the NT macroaggregates, whereas the CT macroaggregates under organic

359 fertilization emitted greater amount of CO_2 (1824.5 µg CO_2-C kg macroaggregates^{-1} h^{-1})

360 compared with the control (1021.4 µg CO_2-C kg macroaggregates^{-1} h^{-1}) and mineral

361 (1155.1 µg CO_2-C kg macroaggregates^{-1} h^{-1}) fertilization treatments (Table 2).

362 No differences between tillage systems were found in the organic carbon (OC)

363 concentration of dry-sieved macroaggregates before or after 840 hour incubation (Table

364 3). However, different results arose when analyzing the OC concentration in the soil

365 macroaggregates under different N fertilization treatments. In this case, greater

366 macroaggregate-C concentration was found in the organic fertilization treatment both

367 before and after the incubation period when compared with the control and mineral

368 treatments (Table 3). Nevertheless, the decrease in the OC concentration during the

369 incubation was not statistically different between N fertilization treatments. Significant

370 differences between tillage and N fertilization treatments were found on the initial NO_3^-

371 concentration of the macroaggregates (Table 3). A greater initial NO_3^- concentration

372 was found in the CT treatment than in the NT treatment, with 110.4 and 92.6 mg NO_3^--

373 N kg^{-1} dry-sieved macroaggregates, respectively. In the case of N fertilization, the

374 mineral treatment showed the greatest initial NO_3^- concentration while the control

375 presented the smallest one and the organic treatment intermediate values. After the

376 incubation period (i.e., 840 hours) the mineral and organic fertilization treatments

377 showed greater NO_3^- concentration when compared with the control. Also, a significant

378 interaction between tillage and N fertilization was found on this variable: while the

379 macroaggregates of the control and organic fertilization treatments presented no

380 significant differences between CT and NT, the macroaggregates of the CT treatment

381   fertilized with mineral N presented a greater amount of initial NO_3^- when compared to

382   the ones of the NT treatment (Table 3). Significant differences between tillage and N

383   fertilization treatments were also found on the NO_3^- concentration variation (0 vs. 840

384   hours). In this case, the NT-control treatment presented the greatest increase in the NO_3^-

385   concentration in the macroaggregates, followed by the CT-control treatment (Table 3).

386   Differences between N fertilization treatments were also found on the initial NH_4^+-N

387   concentration and its variation during the incubation process. The organic treatment

388   presented the greatest values, followed by the mineral and the control treatments (Table

389   3). The reduction of the NH_4^+-N concentration during the incubation period was higher

390   in the fertilized treatments (about 87% and 93% reduction in the NH_4^+ concentration in

391   the mineral and the organic treatments, respectively) compared with the control (about

392   32% reduction) (Table 3). Furthermore, no differences between treatments were found

393   on the MBC content. However, a greater MBN content was found in the organic

394   treatment compared with the mineral and the control treatments (Table 3). The WSC

395   content after the incubation process was significantly affected by both tillage and N

396   fertilization treatments. Thus, a greater WSC content was found under NT than under

397   CT and in the organic N treatment compared with the mineral and control ones (Table

398   3).

399   A highly significant polynomial relationship (r^2= 0.72; p<0.001) was observed between

400   the initial NH_4^+ concentration in the macroaggregates and the cumulative N_2O-N

401   emission during the first 48 hours of incubation (Fig. 3). Furthermore, significant linear

402   relationships were observed between CO_2 and N_2O production in six samplings in CT

403   and in three samplings in NT (Table 4).

404 At the end of the incubation period, a greater proportion of water-stable

405 macroaggregates was quantified under NT compared with CT (Fig. 4). Moreover, a

406 significant interaction (P<0.05) between tillage and N fertilization was found on the

407 water-stability of macroaggregates. While under NT no differences between fertilization

408 treatments were observed in the proportion of water-stable macroaggregates, under CT a

409 greater proportion of water-stable aggregates was found in the organic treatment when

410 compared with the mineral treatment, with intermediate values in the control (Fig. 4).

411 *Experiment 2: Relative importance of nitrification and denitrification on N_2O*

412 *production from soil macroaggregates under different N fertilization types.*

413 No differences between N fertilization treatments were found on NH_4^+ concentration of

414 the macroaggregates before the incubation (Table 5). In contrast, before the incubation

415 process, the fertilized treatments (mineral and organic) presented a greater NO_3^-

416 concentration in the macroaggregates when compared with the control (Table 5).

417 Moreover, significant differences between N fertilization treatments were found on the

418 WSC concentration with greater values in the organic fertilization treatment when

419 compared with the mineral and the control ones (Table 5).

420 At the 15% moisture level, total losses of N as N_2O and N_2 during the incubation of the

421 macroaggregates resulted in 173, 254 and 139 mg N kg^{-1} h^{-1} for the control, mineral and

422 organic treatments, respectively (Table 6). In turn, at the 30% moisture level, N losses

423 reached 4751, 5552 and 4922 mg N kg^{-1} h^{-1} for the control, mineral and organic

424 treatments, respectively (Table 6). Both N fertilization and soil moisture content

425 significantly affected the amount of N_2O-N produced due to the nitrification and

426 denitrification processes (Table 6). The production of N_2O due to the nitrification

427 process was greater in the mineral treatment when compared with the control, with

428   intermediate values in the organic treatment. Different results were obtained in the

429   production of N_2O due to the denitrification process. In this case, the organic treatment

430   showed greater values than the control, while intermediate values were found in the

431   mineral treatment (Table 6). Nevertheless, the production of N_2 due to a complete

432   denitrification process was only affected by soil moisture, with the greatest values in the

433   30% moisture treatment when compared with the 15% moisture treatment (Table 6).

434   Moreover, the production of N_2O due to nitrification and denitrification processes was

435   4.3 and 7.3 times greater in the 30% than in the 15% moisture treatment, respectively

436   (Table 6). No significant relationship was found between WSC and the amount of N_2O

437   produced during the denitrification process (data not shown).

Discussion

Effects of tillage and N fertilization on GHG production from soil macroaggregates

440   CH_4 was the only greenhouse gas produced by the macroaggregates that presented

441   significant differences between tillage treatments. In the CT treatment macroaggregates

442   acted as CH_4 producers, whereas macroaggregates of the NT treatment oxidized CH_4

443   mainly from the first 72 hours until the end of the incubation. Methanotrophic activity is

444   reduced by anoxic conditions. In our experiment, an equal amount of water was added

445   to the macroaggregates of both tillage treatments to bring them to the field capacity of

446   undisturbed soil in our field experiment. Therefore, it could be hypothesized that

447   differences in the intra-aggregate pore architecture and connectivity could have

448   maintained a higher amount of aerobic microsites within the NT macroaggregates, thus

449   facilitating the oxidation of CH_4. This hypothesis is in line with the findings of

450   Kravchenko et al. (2013) who studied the effects of tillage on the intra-aggregate

451   porosity of macroaggregates and observed higher intra-aggregate porosity >100 µm in

452   NT macroaggregates when compared with CT macroaggregates. Another hypothesis

453   could be the influence of the different types of tillage on the diversity of

454   microorganisms within macroaggregates, which could have maintained a greater

455   amount of methanotrophs in the NT treatment.

456   According to our results, no differences between tillage systems were found on the

457   amount of macroaggregate-C mineralized as CO_2. That result could be related to the

458   absence of differences in macroaggregate-C concentration prior and after the

459   incubation. Different results were obtained by Fernández et al. (2010) when using soil

460   of a long-term (14-yr) experiment. These authors observed higher production of CO_2 by

461   NT macroaggregates when compared with CT macroaggregates and related this finding

462   to the higher amount of organic C in the NT macroaggregates. Thus, in our experiment,

463   the similar macroaggregate-C concentration found in the CT and NT treatments would

464   have influenced the lack of differences in CO_2 production by macroaggregates.

465   Similarly to CO_2 production, no differences between tillage treatments were found on

466   the fluxes and cumulative emissions of N_2O by soil macroaggregates. Although a higher

467   initial NO_3^- concentration susceptible to denitrification and a greater reduction in the

468   concentration of NH_4^+ during the incubation were found under CT, no greater MBN

469   content was found in this treatment when compared with NT. For that reason, the

470   hypothesis of a greater N immobilization under CT was not supported by our data.

471   Another hypothesis could be a greater or complete denitrification under CT, in which

472   the mineral N would be emitted as N_2. It is known that the $N_2/(N_2 + N_2O)$ ratio

473   increases with decreasing O_2 concentration (Tiedje, 1988). That hypothesis would be in

474   line with the smaller intra-aggregate porosity described above and the related higher

475   anaerobic conditions in CT macroaggregates.

476   Wrage et al. (2001) suggested that a greater soil organic matter content and better

477   aggregate structure could facilitate O_2 diffusion, thus reducing the production of N_2O in

478   NT soils. In our experiment, we found a greater water-stability of macroaggregates

479   under the NT treatment when compared with the CT treatment as it has also been

480   observed in other studies in the Mediterranean area (Álvaro-Fuentes et al., 2009; Plaza-

481   Bonilla 2010, 2013b). However, the greater macroaggregate water-stability under NT

482   was not followed by a lower production of N_2O as suggested by Wrage et al. (2001).

483   Extrapolating our results to a structured soil, the greater macroaggregate water-stability

484   found under NT could imply a more interconnected porous space in the soil matrix. This

485   could lead to a greater aeration and reduced N_2O emissions in NT when compared with

486   CT.

Macroaggregate CO_2 emissions were influenced by fertilization type: when the incubation was finished (i.e., after 840 hours), the macroaggregate CO_2 losses under the organic fertilization treatment were higher than under the mineral fertilization treatment. Moreover, the interaction between tillage and N fertilization types also affected the CO_2 produced by macroaggregates. Thus, under CT the CO_2 emissions were greater with the use of organic fertilizer compared with either the use of mineral fertilizers or in absence of fertilization. The application of pig slurry usually enhances the amount of readily decomposable C compounds in the soil (Arcara et al., 1999; Sánchez-Martín et al., 2008; Yang et al., 2003). This fact was observed in our experiment, in which a higher WSC content was measured in the macroaggregates of the pig slurry treatment. A similar trend was observed in macroaggregate water-stability. In this case, unlike the CT treatment, the NT treatment did not show an interaction with the type of fertilizer on macroaggregate water-stability. Contrarily, the application of organic fertilizer under CT led to greater proportion of water-stable macroaggregates than the control treatment. These findings suggest that the use of NT buffers the effects of the application of organic fertilizers on the increase of macroaggregate stability (Plaza-Bonilla et al., 2013a).

We found significant linear relationships between the cumulative CO_2 production and (i) the macroaggregate-C concentration (R^2: 0.21; P: 0.016) and (ii) the proportion of water-stable macroaggregates (R^2: 0.19; P: 0.021). However, when both variables (i.e., macroaggregate-C concentration and proportion of water-stable macroaggregates) were included in a stepwise procedure in order to analyze their relationship with CO_2 production, no statistical significance was found. This finding suggests that the relationship found between CO_2 production and macroaggregate stability was due to a

511   greater C concentration in those macroaggregates that are more water-stable resulting in

512   a greater production of CO_2.

513   In contrast to tillage, the different N fertilization treatments had no significant effects on

514   CH_4 emission. However, a trend (not significant) to CH_4 uptake could be observed in

515   Figure 2a in the control treatment and near zero emissions in the organic treatment.

516   Ammonium has been reported to be a competitive inhibitor of CH_4 oxidation

517   (Whittenbury et al., 1970). Interestingly, the uptake of CH_4 that we observed in the NT

518   and control treatments began after the first 72 hours of incubation and coincided with

519   the reduction in the rate of N_2O emissions. Hütsch (1998b) pointed out that CH_4

520   metabolism only begins when the nitrification process is almost completed. That

521   conclusion would explain the time-lapse that we found until the CH_4 uptake began in

522   the NT and control treatments.

523   In our experiment, fertilization type did not lead to differences in the N_2O produced by

524   soil macroaggregates. However, a trend to lower emissions under the control treatment

525   and higher emissions under the organic fertilization with pig slurry was observed (Fig.

526   2c). It is already known that the denitrification process is intensified under the presence

527   of easily decomposable C fractions such as WSC (Arcara et al., 1999). Thus, the

528   application of organic wastes, such as animal manure, usually enhances N_2O emissions

529   when compared with inorganic fertilizers (Heller et al., 2010) due to their easily

530   decomposable C content and sufficient mineral N to activate the population of

531   denitrifiers in soil (Johnson et al., 2007; Sánchez-Martín et al., 2008). Our results show

532   the relationship between the initial ammonium concentration in soil macroaggregates

533   and their N_2O production. That relationship could be explained by the role played by the

534   NH_4^+ ion in the nitrification and denitrification processes. Ammonium oxidation is the

535   first step in the nitrification process that produces NO_3^-, which in turn is the most

536   important ion involved in the denitrification process.

537   *N_2O production by soil macroaggregates as affected by the type of N fertilization*

538   At 15% soil moisture, the nitrification process was the predominant N_2O producer,

539   while at 30% soil moisture the denitrification process emitted nine times more N (as the

540   sum of N_2O and N_2) than nitrification. These results agree with the conceptual model

541   developed by Bouwman (1998) about N_2O emissions fractionation from nitrification

542   and denitrification processes as a function of water-filled pore space. Although in small

543   amounts, the denitrification process lead to N_2O production in the macroaggregates

544   incubated at 15% soil moisture content. This could be related with the presence of

545   anaerobic microsites within the macroaggregates. Sexstone et al. (1985) quantified

546   oxygen profiles in wet aggregates and found anaerobic centers in all the aggregates that

547   denitrified. However, aerobic denitrification could have also occurred. As in other

548   studies (Bandibas et al., 1994; Diba et al., 2011; Liu et al., 2007), we observed an

549   important increase of the N_2O evolved when doubling soil moisture. This finding

550   demonstrates the role played by the absence of oxygen as electron acceptor on

551   nitrification and denitrification processes (Bouwman, 1998).

552   The combination of higher NO_3^- concentration and greater WSC in the organic N

553   treatment would explain the greater amount of N_2O evolved due to the denitrification

554   process when compared to the control treatment (Burford and Bremner, 1975;

555   Mulvaney et al., 1997). Contrarily, the greater N_2O loss from nitrification in the mineral

556   N treatment cannot be explained by a higher NH_4^+ concentration before the incubation,

557   a fact that could be related to a greater organic N mineralization during the incubation

558   that could have increased the amount of mineral N susceptible of being nitrified.

559 Different authors have observed a greater mineralization in N fertilized soils compared

560 with soils without N fertilization (Hatch et al., 2000; Zhang et al., 2012). Another

561 hypothesis could be a more efficient nitrification process in the control treatment in

562 comparison with the mineral treatment that would explain the lower N_2O emissions

563 found in the control. Nemergut et al. (2008) and Ramirez et al. (2012) found changes in

564 soil microbial community when using repeated application of mineral fertilizer when

565 compared to unfertilized soils. Thus, it could be hypothesized that the mineral fertilizer

566 applications in our field experiment could have led to changes in the microbial

567 community structure with higher nitrification efficiency in the unfertilized treatment.

568 Furthermore, our data shows a similar N_2O emission in the mineral and the organic

569 fertilization treatments. This suggests that readily decomposable C was not a limiting

570 factor to denitrification in the mineral treatment. The lack of differences between

571 fertilization treatments on macroaggregate N_2O production after the first 24 hours of

572 incubation in Experiment 1 corroborates this hypothesis.

Conclusions

Tillage and N fertilization treatments affected the production of GHG at the soil macroaggregate scale due to changes in C and N substrates within macroaggregates. Moreover, the different methanogenic and methanotrophic activities found in the tillage treatments suggest changes in porosity and anaerobic conditions within soil macroaggregates when either conventional tillage or no-tillage are used. Easily decomposable C compounds associated with the organic fertilization together with the presence of nitrate stimulated the denitrifying activity. The use of mineral and organic fertilizers leads to differences in the relative importance of the nitrification and the denitrification processes in the production of N_2O by soil macroaggregates: while N_2O losses due to the nitrification process were preponderant in the mineral fertilization treatment, denitrification N_2O losses had a higher importance under organic fertilization due to a higher presence of C-labile compounds. A significant effect of the interaction between tillage and N fertilization treatments on CO_2 production, with higher emissions under CT when applying organic fertilizers and no differences between types of fertilizers on CO_2 emissions under NT, demonstrated the capacity of NT aggregates to protect C. Our study shows that tillage and N fertilization and their interaction play a major role in GHG production from soil macroaggregates due to their impact on the soil mineral and organic substrates that regulate the microbial activity.

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Figure captions

Fig. 1 Cumulative CH_4 (A), CO_2 (B) and N_2O (C) production from dry-sieved macroaggregates (0.250-8 mm) as affected by tillage (CT, conventional tillage; NT, no-tillage). * For each sampling time, values are significantly different at $P<0.05$.

Fig. 2 Cumulative CH_4 (A), CO_2 (B) and N_2O (C) production from dry-sieved macroaggregates (0.250-8 mm) as affected by N fertilization (0, control; mineral N at 150 kg N ha^{-1} and organic N with pig slurry at 150 kg N ha^{-1}). For each sampling time different letters indicate significant differences between N fertilization treatments at $P<0.05$.

Fig. 3 Regression analysis between the initial concentration of NH_4^+-N and the cumulative N_2O-N emissions after 48 hours of incubation of dry-sieved macroaggregates (0.250-8 mm).

Fig. 4 Proportion of sand-free water-stable macroaggregates (0.250-8 mm) as affected by tillage (CT, conventional tillage; NT, no-tillage) and N fertilization treatments (control without fertilization; mineral at 150 kg N ha^{-1}; organic at 150 kg N ha^{-1}) at the end of the incubation period (after 840 hours). Different letters indicate significant differences between tillage and fertilization treatments at $P<0.05$. * Indicate significant differences between tillage treatments at $P<0.05$.

813   **Table 1** General characteristics of the experimental site. Soil properties were measured

814   in the Ap horizon (0-30 cm depth) at the beginning of the experiment.

Elevation (masl)	395
Mean air temperature (ºC)	13.4
Annual precipitation (mm)	327
Annual ETo (mm)	1197
Soil classification[†]	Typic calcixerept
pH (H_2O, 1:2.5)	8.0
Organic C (g kg^{-1})	15.6
Organic N (g kg^{-1})	1.4
EC 1:5 (dS m^{-1})	1.0
CaCO3 eq. (%)	28.9
Particle size distribution (%)	
Sand (2000-50 µm)	6.2
Silt (50-2 µm)	63.3
Clay (<2 µm)	30.5

815

816   [†]According to the USDA classification (Soil Survey Staff, 1994).

40

817 **Table 2** Analysis of variance of the fluxes of CH_4, CO_2 and N_2O from dry-sieved

818 macroaggregates (μg CH_4-C, CO_2-C and N_2O-N kg^{-1} macroaggregates h^{-1}, respectively)

819 as affected by tillage (CT, conventional tillage; NT, no-tillage), N fertilization

820 treatments (0, control; mineral N at 150 kg N ha^{-1} and organic N with pig slurry at 150

821 kg N ha^{-1}), sampling time, and their interactions. Values are the means of all samplings

822 (0, 4, 12, 24, 48, 72, 192, 384, 504, 672 and 840 hours after the beginning of the

823 incubation).

	CH_4 fluxes	CO_2 fluxes	N_2O fluxes
Tillage (T)	*	n.s.	n.s.
CT	0.073 a¶	1333.67	0.754
NT	-0.207 b	1406.19	0.919
N fertilization (N)	n.s.	*	n.s.
Control	-0.175	1217.45 b	0.556
Mineral	0.074	1199.36 b	0.866
Organic	-0.096	1669.39 a	1.042
Sampling time (t)	**	***	***
T x N	n.s.	*	n.s.
CT-Control	0.046	1021.40 d	0.458
CT-Mineral	0.214	1155.13 dc	0.676
CT-Organic	-0.040	1824.48 a	1.127
NT-Control	-0.470	1478.84 ab	0.686
NT-Mineral	-0.066	1243.58 bcd	1.056
NT-Organic	-0.153	1514.30 abc	0.956
T x t	***	n.s.	n.s.
N x t	n.s.	*	n.s.
N x T x t	n.s.	n.s.	n.s.

824

825 n.s.: not significant; *P<0.05; **P<0.01; *** P<0.001

826 ¶ For each gas and treatment, different letters indicate significant differences between
827 treatments at P<0.05.

Table 3 Organic C concentration (OC, g kg^{-1}) and mineral N content (nitrate, NO$_3^-$, and ammonium, NH$_4^+$, in mg kg^{-1}) of dry-sieved macroaggregates (0.250-8 mm) before (0 hours) and after (840 hours) incubation, microbial biomass C and N (MBC and MBN, respectively; mg C or N kg^{-1}) and water-soluble C (WSC; mg C kg^{-1}) after the incubation (840 hours), and % of variation of C, nitrate and ammonium during the incubation as affected by tillage (CT, conventional tillage; NT, no-tillage) and N fertilization treatments (0, control; mineral N at 150 kg N ha^{-1}; and organic N with pig slurry at 150 kg N ha^{-1}), and their interaction.

Treatment	0 hours			840 hours						% variation 840-0 hours		
	OC	NO$_3^-$	NH$_4^+$	OC	NO$_3^-$	NH$_4^+$	MBC	MBN	WSC	OC	NO$_3^-$	NH$_4^+$
CT	6.07	110.4	20.1	5.78	147.9	1.7	865.8	228.2	197.2	-6.41	59.0	-79.0
	(0.8)	(83.1) a¶	(15.1)	(0.8)	(72.7)	(0.7)	(232.4)	(115.6)	(43.1) b	(3.7)	(68.9) b	(28.9) b
NT	6.16	92.6	13.3	5.72	141.9	2.0	954.4	236.5	234.9	-7.78	100.8	-62.3
	(0.9)	(56.0) b	(12.4)	(0.8)	(58.3)	(0.4)	(236.9)	(142.5)	(71.9) a	(5.6)	(92.5) a	(35.9) a
Control	5.54	24.1	3.0	5.21	87.3	2.0	893.0	177.0	199.5	-6.07	182.2	-32.5
	(0.4) b	(4.5) c	(0.7) c	(0.6) b	(56.9) b	(0.8)	(278.4)	(70.98) b	(16.7) b	(5.1)	(58.8) a	(30.6) a
Mineral	5.94	180.0	19.0	5.46	183.6	1.8	977.9	182.6	174.3	-7.61	7.1	-86.8
	(0.9) b	(43.4) a	(11.5) b	(0.5) b	(66.3) a	(0.5)	(213.1)	(96.5) b	(33.3) b	(5.3)	(37.9) c	(10.4) b
Organic	6.87	100.4	28.1	6.60	163.6	1.7	859.4	337.5	274.3	-8.05	65.9	-92.7
	(0.5) a	(16.4) b	(11.7) a	(0.6) a	(12.3) a	(0.4)	(219.9)	(140.1) a	(68.5) a	(3.8)	(22.9) b	(4.4) b
CT-Control	5.51	26.0	3.4	5.19	104.3	1.9	688.3	123.2	191.7	-5.87	132.2	-49.2
	(0.2)	(5.5) d	(0.8)	(0.2)	(82.2)	(1.3)	(81.3)	(44.7)	(19.0)	(3.3)	(30.2) b	(35.7)
CT-Mineral	5.62	214.4	19.5	5.29	179.5	1.3	1023.2	208.9	156.5	-5.61	-13.8	-93.0
	(0.3)	(28.7) a	(4.2)	(0.1)	(92.4)	(0.2)	(272.6)	(50.9)	(18.4)	(4.7)	(45.8) e	(1.1)
CT-Organic	7.08	90.9	37.4	6.86	159.9	1.9	885.9	352.6	243.4	-9.08	77.0	-94.8
	(0.4)	(8.3) c	(6.4)	(0.5)	(6.1)	(0.2)	(203.6)	(92.6)	(31.7)	(2.2)	(16.6) c	(0.8)
NT-Control	5.56	22.2	2.5	5.23	70.4	2.1	1097.8	230.8	207.3	-6.27	219.7	-15.7
	(0.6)	(2.6) d	(0.3)	(0.8)	(5.8)	(0.1)	(249.9)	(45.1)	(11.3)	(7.0)	(43.9) a	(13.0)
NT-Mineral	6.27	145.7	18.6	5.62	187.8	2.3	932.5	156.2	192.1	-9.61	28.1	-80.5
	(1.2)	(20.7) b	(17.0)	(0.8)	(40.7)	(0.1)	(161.6)	(131.5)	(37.5)	(5.6)	(10.1) de	(12.1)
NT-Organic	6.66	109.9	18.7	6.34	167.4	1.6	832.9	322.5	305.2	-7.36	54.7	-90.5
	(0.6)	(17.8) c	(6.5)	(0.6)	(16.7)	(0.5)	(263.6)	(191.4)	(86.0)	(5.0)	(24.8) cd	(5.6)

¶ For each variable, different letters indicate significant differences between treatments at P<0.05. Values between parentheses are the standard deviations of the mean.

835 **Table 4** R^2 coefficients of the linear relationships between carbon dioxide (CO_2) and nitrous oxide (N_2O) production in conventional tillage (CT)

836 and no-tillage (NT) macroaggregates at different times of the incubation period.

Tillage system	Sampling (hours)										
	0	4	12	24	48	72	192	384	504	672	840
CT	n.s.	0.71***	0.56**	0.61**	0.76***	0.33*	0.40*	n.s.	n.s.	n.s.	n.s.
NT	n.s.	0.72***	n.s.	n.s.	n.s.	n.s.	0.62**	n.s.	n.s.	n.s.	0.78***

837

838 n.s.: not significant; *P<0.05; **P<0.01; ***P<0.001

839 **Table 5** Mineral N content (nitrate, NO_3^-, and ammonium, NH_4^+, in mg kg^{-1}) and water-

840 soluble C (WSC; mg C kg^{-1}) of dry-sieved macroaggregates (0.250-8 mm) before

841 incubation, as affected by N fertilization treatments (0, control; mineral N at 150 kg N

842 ha^{-1}, and organic N with pig slurry at 150 kg N ha^{-1}).

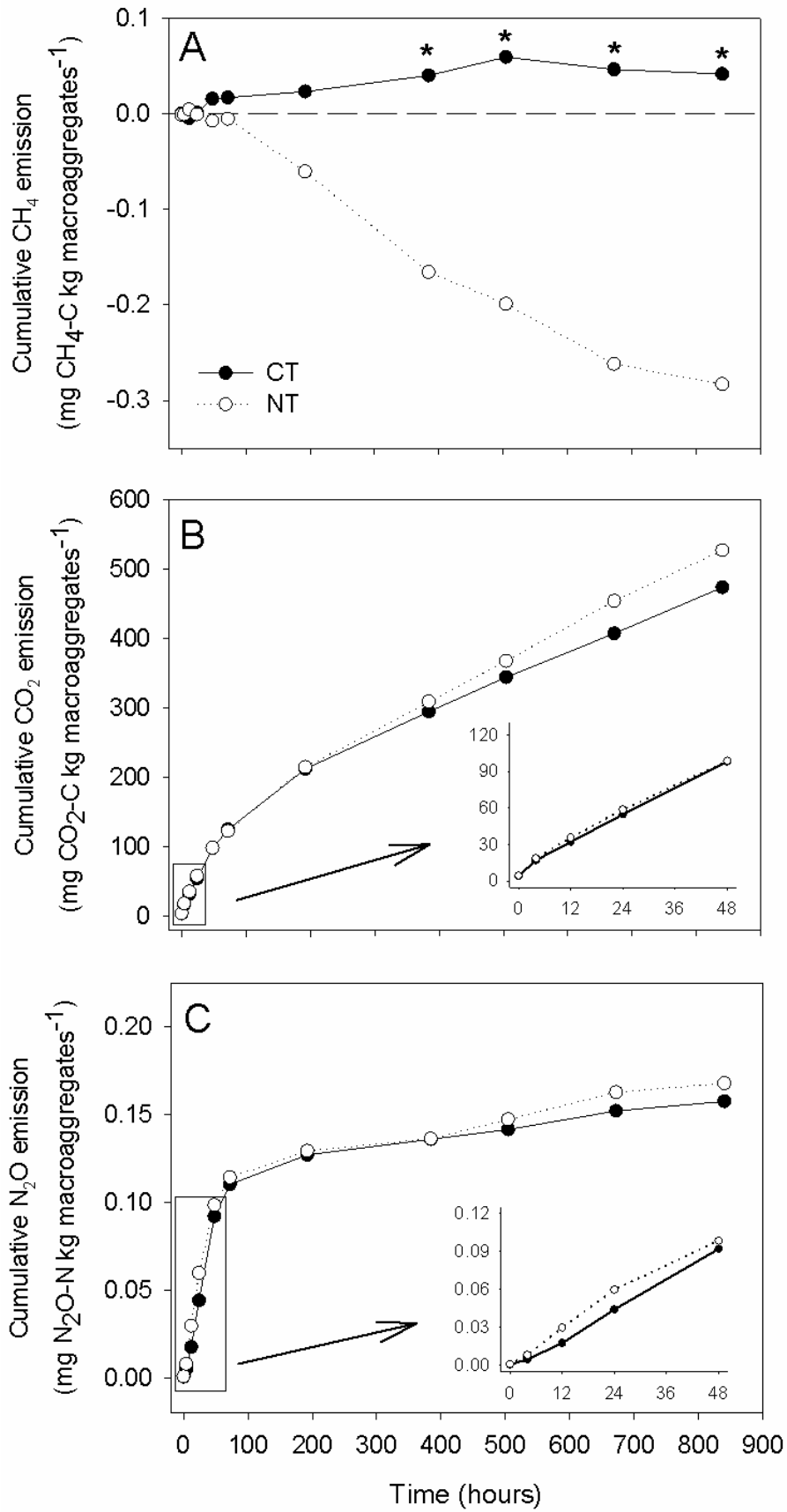| Treatments	NH_4^+	NO_3^-	WSC
Control	1.88	20.19 b¶	90.08 b
Mineral	2.13	85.22 a	92.45 b
Organic	2.58	88.96 a	114.47 a

843

844 ¶ For each variable, different letters indicate significant differences between N fertilization

845 treatments at $P<0.05$.

846   **Table 6** Analysis of variance of the production of N$_2$O (mg N$_2$O-N kg^{-1}

847   macroaggregates h^{-1}) from nitrification and denitrification processes and N$_2$ from

848   denitrification process of dry-sieved macroaggregates as affected by N fertilization

849   treatments (0, control; mineral N at 150 kg N ha^{-1}, and organic N with pig slurry at 150

850   kg N ha^{-1}), soil moisture (15 and 30% gravimetric water content), and their interactions.

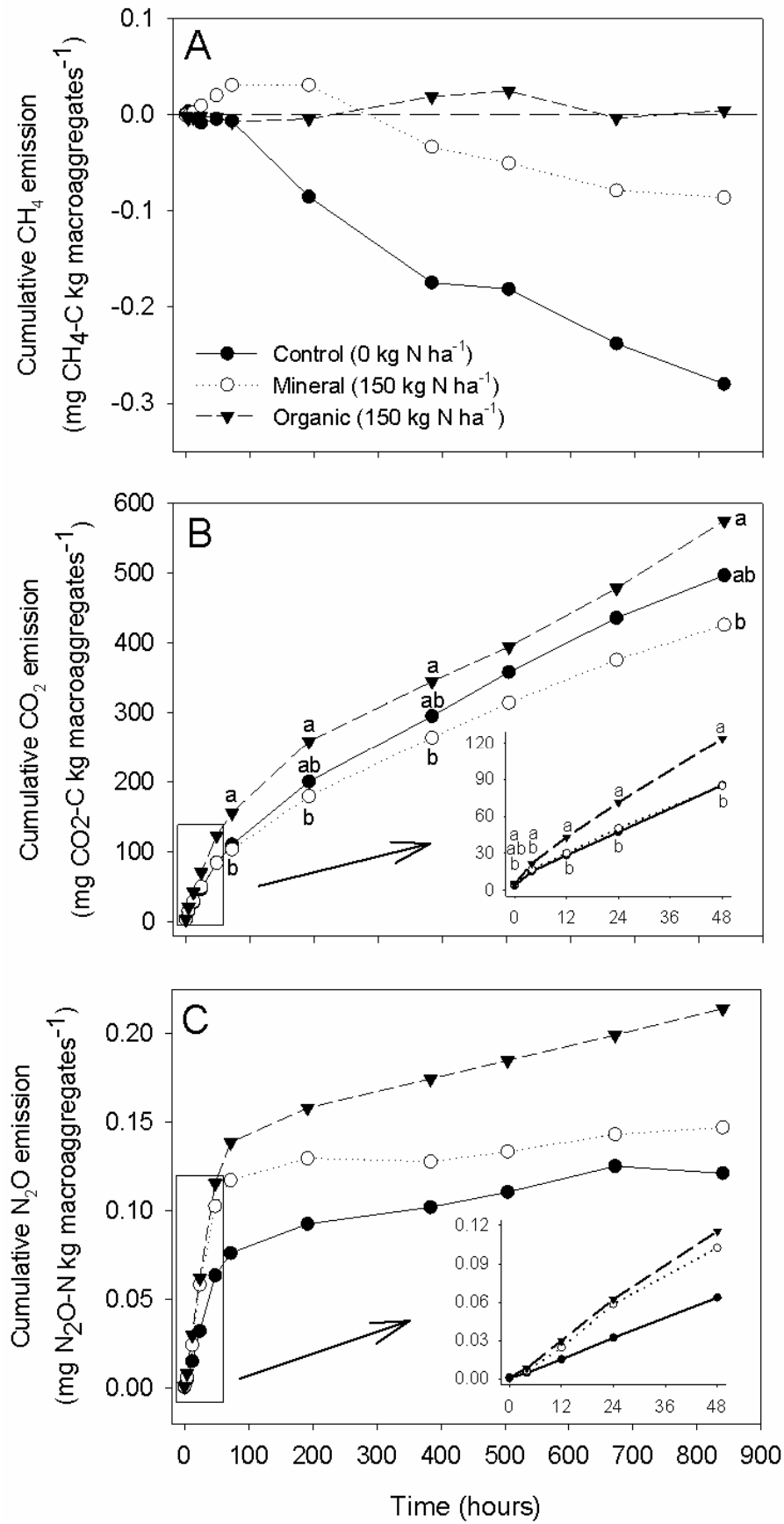| Effects	Nitrification-N$_2$O	Denitrification-N$_2$O	Denitrification-N$_2$
N fertilization (N)	*	*	n.s.
Control	122.51 b¶	180.06b	1949.91
Mineral	455.25 a	303.12 ab	1436.76
Organic	247.90 ab	381.07 a	1752.38
Soil moisture (SM)	***	***	***
15%	115.06 b	69.68 b	5.32 b
30%	500.84 a	506.49 a	4120.01 a
NxSM	n.s.	n.s.	n.s.
Control – 15%	87.15	56.94	29.33
Control – 30%	193.22	303.18	4254.60
Mineral – 15%	159.37	73.39	21.0
Mineral – 30%	751.14	532.85	4268.27
Organic – 15%	60.13	78.70	0
Organic – 30 %	341.78	683.44	3896.46

851   n.s.: not significant; *$P<0.1$; **$P<0.01$; *** $P<0.001$

852   ¶ For each process, different letters indicate significant differences between N fertilization or
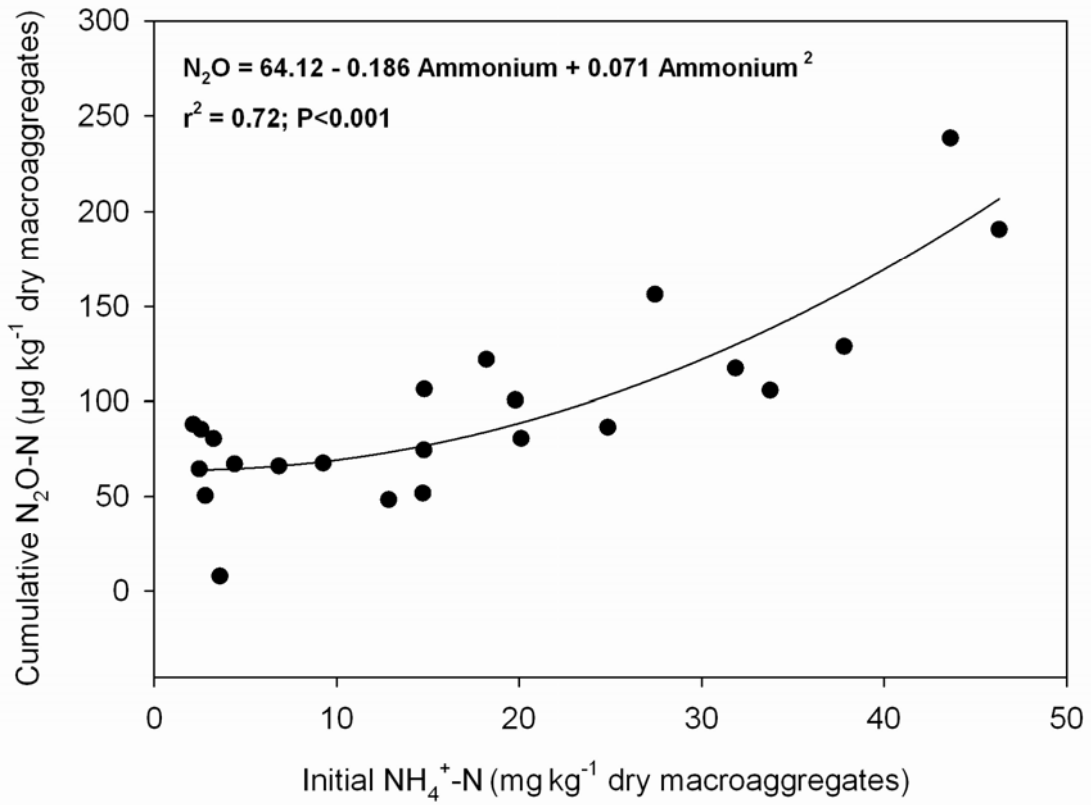
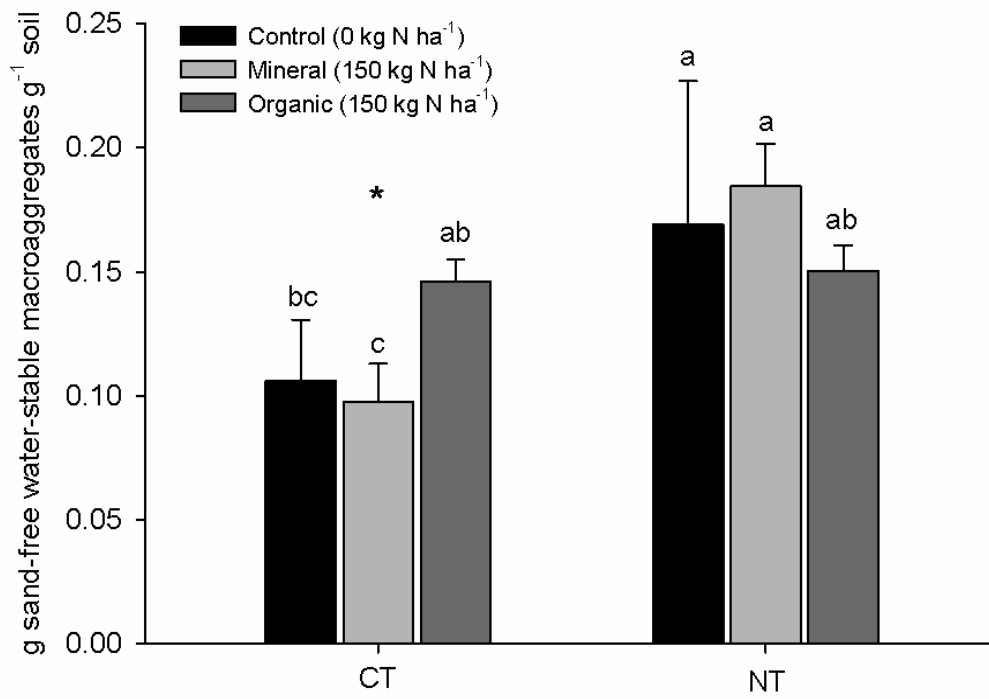853   moisture treatments at P<0.1.

854

855 **Fig 1.**

Fig. 2

858

N₂O = 64.12 - 0.186 Ammonium + 0.071 Ammonium^2

$r^2 = 0.72$; P<0.001

859

Fig. 3

861

862

863 **Fig. 4**