# Evaluation of greenhouse gas emissions and carbon sink capacity of amended soils under laboratory conditions

C. Mondini[a], M.L. Cayuela[a,b], T. Sinicco[a], F. Cordaro[a] , A. Roig[b], M.A. Sánchez-Monedero[b]

[a] *CRA - Istituto Sperimentale per la Nutrizione delle Piante, Sezione di Gorizia, via Trieste 23, I-34170 Gorizia, Italy*

[b] *CEBAS-CSIC, Campus Universitario de Espinardo, Apartado de Correos 164, E-30100 Espinardo, Murcia, Spain*

## Abstract

Soil sequestration of atmospheric $CO_2$ through land application of organic residues may have a worthwhile impact as a strategy to offset the increase in the concentration of greenhouse gases (GHG) in the atmosphere. The significance of different variables on GHG production and soil C sink capacity was investigated by monitoring $CO_2$ and $N_2O$ fluxes from amended soils under laboratory conditions. In the first experiment, the effects of the chemical composition and complexity of three N-rich organic fertilisers (blood meal, hydrolysed leather, and hoof and horn meal) on the $CO_2$ and $N_2O$ productions were studied. A second experiment was aimed at evaluating the effects of the degree of transformation of composts prepared from two-phase olive mill waste on soil C sink capacity. The three N-rich organic fertilisers caused different $CO_2$ and $N_2O$ evolution patterns in the amended soils, despite their similar elemental composition. The total amount of added C that was mineralised in the soil ranged from 10.4 to 15.5%, while $N_2O$-N originated from horn and hoof meal was 6 and 13 times higher than that coming from hydrolysed leather and blood meal, respectively. Mineralisation of the C added to the soil was inversely correlated to the degree of stabilisation

of the composting mixtures. Soils amended with the initial composting mixture evolved from 2 to 7.3 times more $CO_2$-C than the soil amended with the more stabilised compost. However, the C conservation efficiency of organic residues, calculated by the combined losses during composting, and after land application, was higher for the less transformed organic materials. Both studies showed the key importance of the variables studied on the GHG emissions and C sink efficiency of amended soils under controlled conditions. Laboratory experiments could represent a useful tool to assist in the designing of field scale experiments for an effective quantification and monitoring of the overall changes in soil C and N pools.

*Key words:* C sink capacity; GHG emission; Organic residues; Composting; Degree of stabilisation; Chemical complexity

## 1. Introduction

The concentration of greenhouse gases (GHG) in the atmosphere has been constantly increasing during the last decades due to anthropogenic activities (Smith et al., 2001). This is giving rise to growing concern about the consequences of such increases on global warming and related climate changes. As a consequence, measures are being implemented at different levels (from regional to supranational) in order to reduce GHG emissions. Some of the measures internationally agreed under the Kyoto Protocol (1997) involve the reduction of the GHG emissions associated with agricultural production, as well as the enhancement of the C sink potential of agricultural soils.

The release of $N_2O$ is one of the major sources of GHG emissions associated with agriculture. The mechanisms involved in $N_2O$ evolution have traditionally been investigated in relation to the economic implication of N losses from soils. However, in recent years, the

54 interest in $N_2O$ research has focussed on the environmental implications regarding global
55 warming and on the depletion of the stratospheric ozone layer (IPCC, 1995). The
56 concentration of $N_2O$ in the atmosphere is increasing at an annual rate of 0.2-0.3% (Conrad,
57 1996) and globally agriculture is responsible of about 70% of the anthropogenic emissions.
58
59 There is increasing interest in understanding the mechanisms involved in $N_2O$ production
60 in terrestrial ecosystems. The microbial processes leading to $N_2O$ formation are nitrification
61 (both autotrophic and heterotrophic), denitrification, coupled nitrification-denitrification and
62 nitrifier denitrification (Wrage et al., 2001). The relative contribution of single mechanisms or
63 their combined effect depends on soil environmental conditions, such as soil pH, moisture,
64 texture, cation exchange capacity (CEC), temperature, the degradation rate of the added
65 organic matter, and the temporary lack of oxygen in soil (Bolan et al., 2004).
66
67 Apart from controlling agricultural $N_2O$ emissions, the use of the C sink potential of
68 agricultural soils is an effective and widely accepted strategy to offset the increase in the
69 concentration of GHG in the atmosphere (IPCC, 2003). Among the activities that could affect
70 the soil C sink capacity, the sequestration of atmospheric $CO_2$ through land application of
71 organic residues may have a significant impact in meeting the emission reduction targets
72 agreed under the Kyoto Protocol (Lal, 1999; Smith et al., 2000). For example, it has been
73 estimated that agricultural soils in the EU have a biological potential for C sequestration
74 (when consideration is taken of socio economic constraints and limitations to land-use, land
75 suitability and amounts of organic residues) in the range between 16 and 29 Mt $CO_2$ per year
76 (Freibauer et al., 2004; Smith, 2004). To understand the importance of this strategy, the
77 amount of C that could be potentially sequestered in the soil roughly corresponds to one third
78 of the EU emission reduction target agreed under the Kyoto Protocol for the period 2008-
79 2012. In Italy, where there is a long established agricultural tradition, an increase of 0.15% in

80 organic C in arable soils would immobilise the same amount of C that is currently released
81 into the atmosphere annually by the use of fossil fuels (Marmo, 2001). However, the
82 temporary nature of immobilised organic C in soil and the practical difficulties of long term
83 monitoring of changes in soil C pools, remain some of the main challenges of soil C
84 sequestration potential (Favoino and Berbel, 2005).
85
86 Besides the contribution to tackling the problems of GHG emissions, soil C sequestration
87 leads to other positive environmental effects, such as an increase in soil fertility and a
88 reduction of C losses, by erosion and/or accelerated organic matter (OM) mineralisation.
89 These effects are associated with the well known beneficial effects of OM on soil properties,
90 and more specifically on soil fertility (Stevenson, 1994). Land application of organic residues
91 could represent a useful tool in maintaining and increasing levels of soil OM (Nortcliff and
92 Amlinger, 2001). Applications of soil amendments have also been effective in significantly
93 reducing soil losses and total run off due to soil erosion (Strauss, 2001). Lal (1999) estimated
94 that of the 78 ± 12 Pg of the historic soil organic C loss, 26 ± 9 Pg (33% of total) was caused
95 by mineralisation of OM accelerated by erosion, which has the most severe impact on the soil
96 organic C (SOC) pool.
97
98 The amount of organic C sequestered in soil depends not only on the inherent
99 characteristics of the soil (Lal et al., 1998), but also on several other factors including the
100 properties of the organic amendments and their management practices (Lal, 2003). In fact, the
101 contribution of organic residue management to $CH_4$ and $N_2O$ emissions should not be
102 neglected, since the treatment of organic residues could lead itself to GHG generation
103 (Hellebrand and Kalk, 2000; Sommer and Moller, 2000; Hao et al., 2001; Zeman et al., 2002).
104 $CH_4$ and $N_2O$ can also be produced after soil application of organic residues, especially when

105 high rates of N rich materials are used or under anaerobic conditions (Inubushi et al., 2000;
106 Smith et al. 2001).
107
108 Consequently, effective recycling of organic residues in soil requires the optimisation of
109 soil and organic waste management practices in order to minimise GHG emissions and
110 optimise soil C sequestration efficiency, but, to date, limited data are available in the literature
111 on this subject. The main reason for the lack of experimental data on the subject is due to the
112 fact that this kind of study requires long term field experiments addressing a large number of
113 variables.
114
115 In this perspective, laboratory scale experiments can represent a powerful tool in assessing
116 the effect of different variables on C and N cycles during the transformation of organic
117 residues and following their application to soil. Laboratory scale experiments allow the study
118 of different management strategies on a short term basis and facilitate a fast response to the
119 importance of different factors affecting GHG emission and soil C sink efficiency.
120 Understanding the effect of different variables facilitates the application of this knowledge to
121 field scale experiments for an effective quantification and assessment of the overall changes
122 in the soil C and N pools.
123
124 In this regard, the aim of this work was a preliminary laboratory study to evaluate the
125 significance of different organic amendments on GHG production and C sink potential of soil
126 under controlled conditions. In particular we investigated, by continuous monitoring of gas
127 fluxes, the effects of the chemical composition and complexity of organic residues and the
128 effects of degree of transformation of OM.
129

## 2. Materials and methods

### *2.1. Soil incubation experiments*

Two incubation experiments involving soil amended with different organic materials were carried out under controlled laboratory conditions in order to study their effect on GHG evolution and soil C sink potential.

The soil used for the incubation experiments was a calcareous sandy clay loam soil sampled (5-20 cm depth) from a olive orchard in a semiarid area in Southeast Spain. Soil was sieved (< 2 mm), adjusted to ca. 40% of water holding capacity and pre-conditioned by incubation at 25ºC under aerobic conditions for 7 d prior to use. Selected characteristics of the soil are reported in Table 1.

#### *2.1.1. Soil incubation with N-rich organic fertilisers*

Pre-conditioned moist soil samples (50 g oven dry basis) were incubated at 25ºC in sealed 130 ml plastic bottles after addition (0.5% w/w) of 3 different commercial organic fertilisers with high N contents (blood meal: obtained by spray drying at low temperatures the fresh whole blood from animal processing plants, $C_{ORG}$ 44.5%, $N_{TOT}$ 12.6%, C/N 3.5; hydrolysed leather: a slow release N fertiliser derived from hydrolysed animal proteins, $C_{ORG}$ 45.4%, $N_{TOT}$ 11.5%, C/N 3.9; horn and hoof meal: organic fertiliser produced by the drying of horns and hoofs from animal processing plants, $C_{ORG}$ 44.6% $N_{TOT}$ 13.5%, C/N 3.3). The three organic fertilisers were ground and sieved (< 0.5 mm) before application. Soil $CO_2$ and $N_2O$ emissions were measured every hour for 28 d (Section 2.2).

155

156 *2.1.2. Soil incubation with organic amendments having different degrees of transformation*

157

158 In the second experiment, two different two-phase olive mill wastes (TPOMWs) and two
159 composts prepared from the same TPOMWs were used. Compost 1 was a mixture of
160 TPOMW 1 and sheep manure (w-w: 67%-33% fresh weight; 55%-45% dry weight), whereas
161 compost 2 was prepared by mixing TPOMW 2, sheep manure and grape stalks (w-w-w: 63%-
162 27%-10% fresh weight; 42%-46%-12% dry weight).

163

164 Both mixtures were composted in a pilot plant forming trapezoidal piles (1.0 m high
165 with a 2×3m base) with forced aerations and occasional turnings (Cayuela et al., 2006). The
166 composting process was considered to be finished, when the temperature of the mixture
167 remained stable and near ambient. Sampling was performed at four different stages of the
168 composting process (Table 2):

169 - I: initial mixture of raw wastes after one week of composting

170 - M: during the mesophilic phase, when compost temperatures were around 40 ºC.

171 - T: during the thermophilic phase, when compost temperatures were over 60 ºC.

172 - F: final product obtained at the end of the composting process (mature compost).

173

174 Composting time and chemical properties of composting mixtures and TPOMWs are
175 shown in Table 2. The composting process was monitored by conventional stability
176 parameters (total organic C divided by total organic N: $C_{ORG}/N_{TOT}$; humic-like acid carbon
177 divided by total alkali extracted organic carbon: $C_{HA}$/TEC) to check the typical performance
178 of the process (Bernal et al., 1998a).

179

The olive mill wastes and compost samples were air dried, ground to 0.5 mm and added (2% w/w) to pre-conditioned moist soil samples (50 g oven dry basis). Amended soils were incubated at 25ºC in sealed 130 ml plastic bottles and $CO_2$ evolution was measured daily for 35 d (Section 2.2).

*2.2. GHG analyses*

GHG evolution was determined by means of an automated system for continuous gas sampling and analysis. The system operates as an "open chamber" system in which the plastic bottles containing the soil sample are continuously aerated at constant flow rate by means of an air pump. At regular time intervals, a single bottle is made a "close chamber" for a selected period (usually in the range 10-60 min) by means of two appropriate valves. The gas concentration in the chamber is automatically measured at the beginning and the end of this period by a gas chromatograph specifically fitted for gas measurements (Varian, CP2003) and the difference between the final and initial measurements provides the rate of gas production for the selected time interval. The system can facilitate up to 16 samples and allows the measurement of GHG evolution rate over regular periods of time (usually every 1-4 h).

All results are expressed on an oven-dry basis (105ºC, 24 h) and represent the mean of three replicates.

**3. Results**

*3.1. Soil incubation with N-rich organic fertilisers*

205 The dynamics of $CO_2$ and $N_2O$ evolution of the amended soils are shown in Fig. 1. In the
206 case of the soil amended with blood meal, $CO_2$ and $N_2O$ evolution occurred within 3 d of
207 incubation. The evolution rate of both GHG peaked on the first day of the incubation
208 (maximum evolution rate of 105 μg $g^{-1}$ $h^{-1}$ for $CO_2$-C and 7.9 μg $kg^{-1}$ $h^{-1}$ for $N_2O$-N),
209 followed by a sharp decrease, approaching values of the control after 1 week of incubation in
210 the cases of the blood meal and the hydrolysed leather, and two weeks in the case of the horn
211 and hoof meal.
212
213 FIGURE 1
214
215 The addition of hydrolysed leather gave a lower initial peak of $CO_2$-C evolution (17 μg $g^{-1}$
216 $h^{-1}$ $CO_2$-C), followed by a gradual decrease during the incubation. The dynamics of $N_2O$ for
217 the hydrolysed leather was different from that of $CO_2$, with a low and nearly constant
218 production rate throughout the whole incubation period (Fig. 1).
219
220 Horn and hoof meal showed a characteristic pattern for both GHG. Its addition gave rise
221 to two GHG peaks for both $CO_2$ and $N_2O$. That could possibly indicate the presence of pools
222 of different degradability. However, dynamics of $CO_2$ and $N_2O$ were not superimposable,
223 with peak rate of $CO_2$ evolution preceding that of $N_2O$. Also the rate of $N_2O$ evolution
224 measured after the addition of horn and hoof meal was noticeably higher than the rates
225 observed for the other materials tested.
226
227 The different patterns shown by each organic fertiliser was also evident when considering
228 the cumulative curves of extra $CO_2$-C and $N_2O$-N ($CO_2$-C and $N_2O$-N evolved from the
229 treatment minus the $CO_2$-C and $N_2O$-N produced by the control) (Fig. 2). The percentage of
230 added C mineralised in soil at the end of the incubation varied from 10.4 to 15.5% for blood

231 meal and horn and hoof meal, respectively. The most remarkable fact was the significant
232 difference between the amount of $N_2O$ evolved from the soil amended with horn and hoof
233 meal and that released from the soil amended with the others two organic fertilisers. $N_2O$-N
234 originated from horn and hoof meal was 6 and 13 times higher than that coming from
235 hydrolysed leather and blood meal, respectively.
236
237 FIGURE 2
238
239 *3.2. Soil incubation with organic amendments with different degrees of transformation*
240
241 Dynamics of the $CO_2$-C evolution rate from soil amended with TPOMWs and compost
242 samples are shown in Fig. 3. Soil addition of both initial composting mixtures (I) caused a
243 sharp increase in the respiration rate that reached the maximum within the first 24 hours of
244 incubation. After this initial increase there was a decline approaching the control levels. Soil
245 addition of more stabilised compost samples (T and F) caused a slight increase in the rate of
246 soil respiration that was significantly higher than control only during the first 10-15 d of the
247 incubation, showing no significant differences respect to the control after this.
248
249 FIGURE 3
250
251 A different behaviour was recorded in the case of TPOMW 1 and TPOMW 2 amended
252 soils, that achieved the maximum respiration rate about 2 d after the addition of the organic
253 residue. After the maximum rate of respiration, $CO_2$ evolution in TPOMW 1 and TPOMW 2
254 amended soils decreased, but more slowly than in soils amended with any of the compost
255 samples prepared from olive mill wastes, regardless of the degree of stabilisation.
256 Differences among treatments tended to decrease with time, but it is remarkable to note that,

for both TPOMWs, the respiration rate of soil amended with either the raw TPOMW or the initial composting mixture (I), was significantly different from the control even after 30 d of incubation (Fig. 3).

The differences in $CO_2$ production among the treatments become more evident when the cumulative respiration of extra $CO_2$-C (i.e. cumulative respiration of the treatment minus cumulative respiration of the control) (Fig. 4) is considered. Cumulative respiration curves clearly show an inverse relationship between $CO_2$ evolution and degree of stabilisation of the materials. The total amounts of extra $CO_2$-C evolved from the soils amended with TPOMW and the initial composting mixture samples (I) indicate that these soils evolved from 2 to 7.3 times more $CO_2$-C than the soil amended with the more stabilised compost samples (F) (Fig. 4). The total amount of extra $CO_2$-C released after 5 weeks of incubation, in the case of mature composts (F), was very low (between 1-2% of added C). This range was considerably lower than the amount of extra C evolved by mature composts of diverse origin (between 15-20% of added C) for similar incubation periods (Bernal et al., 1998b).

FIGURE 4

## 4. Discussion

### *4.1. Effect of chemical composition and complexity on GHG evolution*

The first experiment was aimed at estimating the significance of the chemical composition and complexity of three N-rich organic fertilisers on the dynamics and amount of GHG evolution from amended soils under laboratory conditions. Despite the similar chemical composition and particle size (<0.5 mm) of the 3 materials (Table 1), their $CO_2$ and $N_2O$ evolution dynamics were very different. Therefore, the characteristic behaviour shown by

283 each material should be attributed to the diverse complexity of their structure rather than their
284 chemical composition.
285
286 All three N-rich organic materials mainly consisted of proteins, or protein derived
287 materials, of diverse origin and are extensively used in organic farming as a source of N.
288 Apart form the high N concentrations, the different origin of the N source would lead to
289 different N release rates when applied to the soil, having important implications for crops
290 fertilisation management. Blood meal is mainly composed of non-complex fibrous and
291 globular proteins (secondary structure formed by α-helix), which are easily hydrolysable
292 (Petsko and Ringe, 2003) and, consequently, quickly mineralised in the soil. The hydrolysed
293 leather, which is derived from the partial hydrolysis of shavings of tanned cattle hides, is
294 mainly formed by connective tissue, the proteins of which are ascribable to the structure of
295 collagen (Petsko and Ringe, 2003). The tertiary structure of this protein is characterised by
296 triple-helix of 3 polypeptide chains (secondary structure formed by a mixture of α-helix and
297 β-sheet). As a consequence, the products obtained by partial hydrolysis of collagen are likely
298 to be more slowly degraded than blood meal in the soil. Finally, hoof and horn meal is
299 predominantly composed of an insoluble protein called keratin, characterised by β-sheet
300 secondary structure (Petsko and Ringe, 2003). One characteristic of the amino acid
301 composition of the horn and hoof meal is the presence of sulphur-containing amino acids
302 (methionine, cystine and cysteine), which are responsible for cross-linking in proteins, and
303 cause a reduction in their degradability.
304
305 The diverse chemical complexity of the protein structures of the organic fertilisers would
306 induce different C and N mineralisation rates when applied to the soil. Consequently, $N_2O$
307 production would be also affected, since the pathways leading to its formation are controlled
308 by among other soil environmental conditions, the amount of $NH_4^+$ available in the soil.

309

310 A possible mechanism for $N_2O$ production in blood meal amended soil is autotrophic
311 nitrification as proposed by Inubushi et al. (2000). These authors, in a experiment involving
312 the addition of sewage sludge compost to soil, found that the maximum $N_2O$ production rate
313 corresponded with maximum $NH_4^+$ accumulation in soil, indicating that N mineralisation
314 from the applied compost and successive nitrification were the main processes contributing to
315 $N_2O$ production. In our experiment the fast mineralisation of the easily available organic
316 matter (blood meal), as evidenced by the $CO_2$ respiration, could have created conditions
317 conducive to a considerable release of $N_2O$ through nitrification.

318

319 The rate of $N_2O$ production in the case of hydrolysed leather and horn and hoof meal
320 differed from that of blood meal. The maximum rate of $N_2O$ production was delayed with
321 respect to that of $CO_2$, when most of the easily available C was already depleted. Therefore,
322 the formation of $N_2O$ in this case could be attributed to a different mechanism, namely
323 nitrifier denitrification (Wrage et al., 2001), a pathway of nitrification that is carried out
324 exclusively by one group of autotrophic $NH_3$-oxidisers microorganisms. This mechanism
325 could occur at low levels of available C (Wrage et al., 2001). For instance, He et al., (2001)
326 found significant production of $N_2O$ in an aerated composting pile at the late stages of the
327 process after the readily available C source was depleted and suggested nitrifier denitrification
328 as the possible mechanism for $N_2O$ production. In the case of the addition of more complex
329 organic fertilisers, $N_2O$ production could have been controlled by the limited amount of
330 available $NH_4^+$, that was slowly released during the incubation, as evidenced by the $CO_2$
331 evolution rate.

332

333 Another possible mechanism that could have contributed to the $N_2O$ production, in all the
334 cases studied, is denitrification under aerobic conditions, as recently reported by Muller et al.,

335 (2004). Under the experimental conditions of their work (temperate grassland soil), aerobic
336 nitrate reduction was the predominant $N_2O$ producing mechanism. On the other hand,
337 anaerobic denitrification was unlikely to play a key role in the case of present study, since the
338 soils were kept continuously aerated (Section 2.2).
339
340 Understanding the mechanisms responsible for $N_2O$ production is difficult due to the well
341 known spatial and temporal variability in the physical, chemical and biological properties of
342 the soil. Besides, the continuous changes in soil environmental conditions recorded in our
343 experiment could have favoured the concurrent or subsequent occurrence of several $N_2O$
344 pathways. Therefore it is most likely that $N_2O$ emitted from the soil in our experiment derived
345 from a range of different microbial processes rather than from a single pathway. The different
346 response recorded for each of the three materials studied and the large number of variables
347 affecting the $N_2O$ production suggest that soil application of N-rich organic fertilisers should
348 be carefully investigated. In this perspective, laboratory experiments represent a valuable tool
349 to quickly evaluate the contribution of a large number of variables. Results obtained under
350 controlled conditions could assist in the optimisation of field scale experiments aimed to
351 quantify changes in the global pools of N cycles and to establish agricultural practices that
352 minimise the release of GHG and N losses after land application of N-rich fertilisers.
353
354 *4.2. Effect of the degree of stabilisation of organic matter on soil C sink efficiency*
355
356 The second experiment was designed to evaluate the effects of the degree of stabilisation
357 of organic residues on soil C sink potential, by measuring the amount of $CO_2$-C evolved from
358 the amended soil under laboratory conditions.
359

360 The degree of stabilisation of the added organic matter determined the amount of $CO_2$
361 evolved from the soil (Fig. 4). The inverse relationship between $CO_2$ evolution and degree of
362 stabilisation of the organic materials is in agreement with the findings reported by Bernal et
363 al. (1998b), who found a lower $CO_2$-C evolution from soils amended with mature composts.
364 Similarly, Sánchez-Monedero et al. (2003) found that prior sludge stabilisation through
365 composting improves the conservation of organic carbon from sludge (i.e. for unit of organic
366 carbon supplied to the soil the more the added organic matter is stabilised the more organic
367 carbon is retained in the soil). Finally, Nortcliff and Amlinger (2001) showed that the
368 potential of soil C sequestration could be enhanced by increasing the degree of stability of
369 OM applied to soil.
370
371 However, a proper evaluation of the efficiency of an organic residue stabilisation practice
372 as a way to maximise C conservation capacity, should take into account the C losses during
373 the whole life cycle of the organic materials. This evaluation should include C dynamics not
374 only after addition to the soil, but also during the stabilisation process. These are usually
375 characterised by considerable C losses. Results from previous research, which have
376 considered dynamic C losses of a range of organic materials during composting and
377 subsequent land application, showed that an increased degree of stabilisation of the OM led to
378 an enhanced efficiency in C conservation of organic residues, and consequently the amount of
379 C remaining in the soil after the application of the organic materials (Bernal et al., 1998b;
380 Kirchman and Bernal, 1997). Kirchmann and Bernal (1997) calculated the total amount of C
381 losses from organic residues during the stabilisation process and the incubation of soil
382 amended with material having different degrees of stabilisation. They found that total C losses
383 of the untreated material were 26% higher than those of end products.
384

385 The composting mixtures prepared from TPOMW showed a distinctive behaviour,
386 compared to the organic materials studied by Kirchman and Bernal (1997) and Bernal et al.
387 (1998b). In the case of TPOMW compost, the use of composting mixtures with a higher
388 degree of stabilisation led to higher overall C losses from the organic residues, considering
389 the C losses during composting and subsequent mineralisation in the soil during laboratory
390 incubation. In fact, C losses during composting of TPOMW increased, as expected, with
391 composting time up to 40% of the initial C content of the organic materials (data not shown),
392 whereas the amount of added C evolved after 5 weeks of soil incubation were always lower
393 than 7% (Fig. 4). Even considering an incubation period similar to that of composting (about
394 40 weeks), C losses would not exceed 12% of added C, as estimated by fitting experimental
395 data to kinetic models. Consequently, since the amount of C mineralised during composting
396 would be always higher than the amount of extra $CO_2$-C evolved from the amended soils, the
397 total C losses from the composting materials would be minimised when amending the soil
398 with the initial mixture of compost (I), as the high C losses occurring during composting
399 would be avoided.
400
401 The high lignocellulosic content of TPOMWs has been reported to significantly slow
402 down the degradation rate of these materials during composting (Cayuela et al., 2006). This
403 peculiar characteristic is likely to affect the degradation of these materials in soil, as suggested
404 by the results obtained in the present experiment. The low degradation rate of TPOMW
405 composts in soil is a property that could have relevant implications from the point of view of
406 organic C storage in soils. These preliminary findings, obtained under laboratory conditions,
407 encourage the evaluation of the actual soil C sequestration efficiency of TPOMW compost
408 under field scale. Furthermore, laboratory data suggest that to optimise the efficiency of soil C
409 sequestration, it would be advisable to use composting mixtures that have only undergone a

short composting period, long enough to achieve an adequate sanitisation of the material and the degradation of the phytotoxic substances characteristic of fresh materials.

### *4.3. Conclusions*

On the whole, both laboratory experiments showed the key importance of the variables studied, chemical complexity and degree of stabilisation of the organic residues, on the GHG emissions and C sink efficiency of amended soils. In particular, $N_2O$ emissions were shown to be highly influenced by the chemical complexity of the organic fertiliser used as soil amendment. There is a large number of variables affecting $N_2O$ production from amended soils such as soil environmental conditions and the properties of soil and organic residues, that need to be tested under controlled conditions before performing the research at full scale. Similarly, the mineralisation of TPOMW composting mixtures in amended soils was inversely correlated to the stabilisation degree of the OM. However, the low degradation rate of TPOMW during composting and after its mineralisation in the soil suggest that this material should be tested under field scale conditions to quantify the actual amount of C remaining in the soil after soil incubation. Since field experiments are time and cost demanding, laboratory experiments could represent an effective tool for obtaining in a short time useful information in order to optimise field scale experiments for an effective quantification and monitoring of the overall changes in C and N pools.

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438

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521 greenhouse gas emission and global warming. Compost Science & Utilization 10, 72-86.
522

523 **Table 1.** Selected characteristics of the soil

| Management | Sand | Silt | Clay | pH | CEC[a] | $CaCO_3$ | $N_{TOT}$ | $C_{ORG}$ | $B_C$[b] |
|---|---|---|---|---|---|---|---|---|---|
| | | (%) | | ($H_2O$) | ($c_{mol}^{(+)}$ $kg^{-1}$) | | (%) | | (µg $g^{-1}$) |
| Olive orchard | 52 | 21 | 27 | 8.0 | 8.6 | 41.5 | 0.10 | 1.04 | 119 |

524

525 [a] CEC: cation exchange capacity; [b] $B_C$: soil microbial biomass C.

526

527 **Table 2.** Composting phase, composting time and selected chemical characteristics of two-
528 phase olive mill wastes (TPOMWs) and composting mixtures

| Sample | Phase of composting | | Weeks of composting | $C_{ORG}$[a] (%) | $N_{TOT}$[b] (%) | $C_{ORG}/N_{TOT}$ | $C_{AH}/TEC$[c] |
|---|---|---|---|---|---|---|---|
| **TPOMW 1** | | | | 53.0 | 1.3 | 40.8 | -- |
| **TPOMW 2** | | | | 53.1 | 1.1 | 48.3 | -- |
| **Compost 1** | Initial | 1-I | 1 | 37.8 | 1.5 | 25.2 | 26.5 |
| | Mesophilic | 1-M | 5 | 36.4 | 1.5 | 24.3 | 33.5 |
| | Thermophilic | 1-T | 19 | 32.4 | 1.7 | 19.1 | 68.8 |
| | Final | 1-F | 40 | 30.1 | 1.9 | 15.8 | 73.1 |
| **Compost 2** | Initial | 2-I | 1 | 30.0 | 1.3 | 23.1 | 33.8 |
| | Thermophilic | 2-T | 18 | 27.3 | 1.7 | 16.1 | 73.5 |
| | Final | 3-F | 34 | 23.9 | 1.9 | 12.6 | 76.8 |

529

530 [a] $C_{ORG}$: total organic carbon; [b] $N_{TOT}$: total nitrogen; [c] $C_{HA}$: humic-like acid carbon, TEC: total
531 alkali extracted organic carbon.

532

533 **Figure captions**

534

535 Fig. 1. Rate of $CO_2$-C (a) and $N_2O$-N (b) evolution from soil amended with N-rich organic
536 fertilisers.

537

538 Fig. 2. Cumulative extra $CO_2$-C (a) and $N_2O$-N (b) evolution from soil amended with N-rich
539 organic fertilisers. Bars represent standard deviation *(n = 3)*.

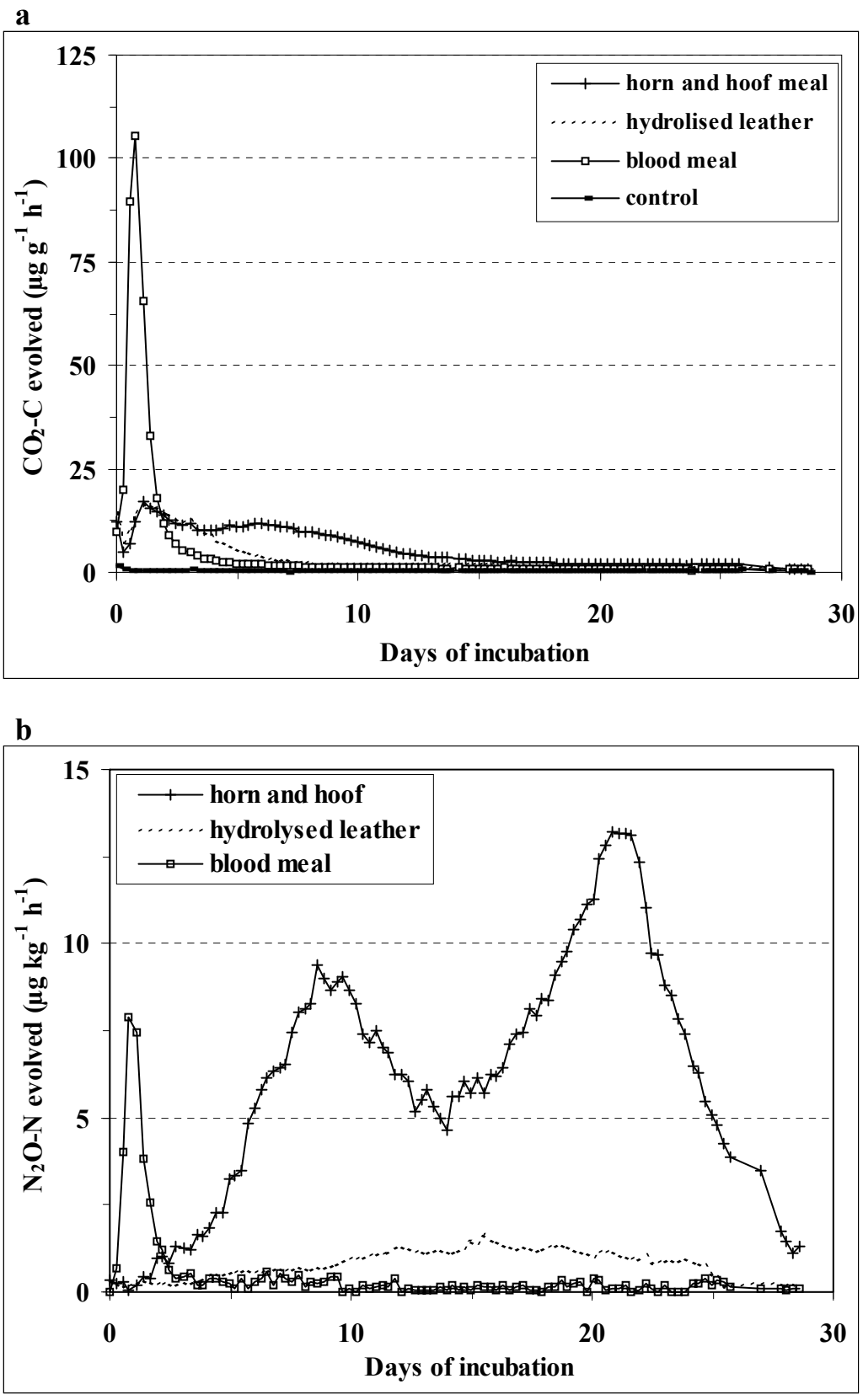
540

541 Fig. 3. Rate of $CO_2$-C evolution from soil amended with two-phase olive mill waste 1
542 (TPOMW 1) and compost 1 (a) and two-phase olive mill waste 2 (TPOMW 2) and
543 compost 2 (b). I, M, T and F indicate the phase of composting (I = initial, M =
544 mesophilic, T = thermophilic, F = final). Bars represent standard deviation *(n = 3)*.

545

546 Fig. 4. Cumulative extra $CO_2$-C evolution from soil amended with two-phase olive mill waste
547 1 (TPOMW 1) and compost 1 (a) and two-phase olive mill waste 2 (TPOMW 2) and
548 compost 2 (b). I, M, T and F indicate the phase of composting (I = initial, M =
549 mesophilic, T = thermophilic, F = final). Bars represent standard deviation *(n = 3)*.

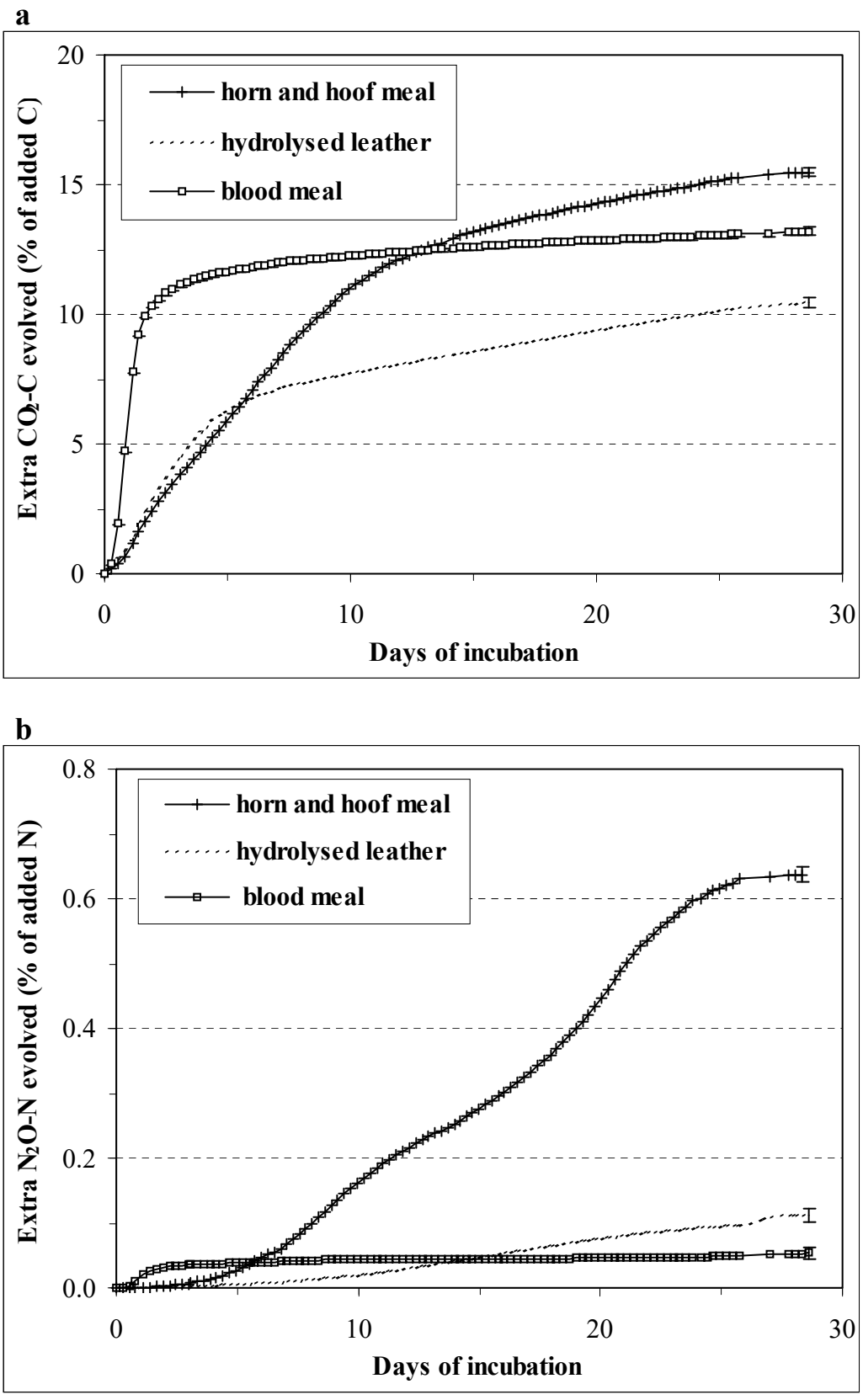
550




551

552 Fig. 1

553



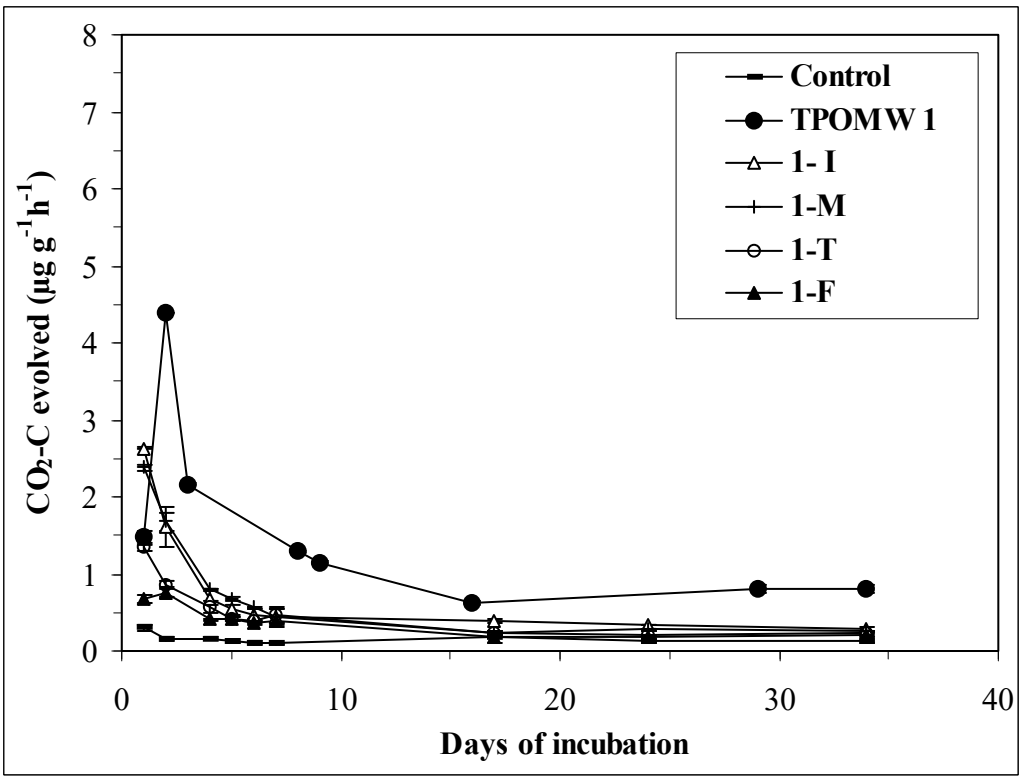

554

555

556 Fig. 2

557

**a**

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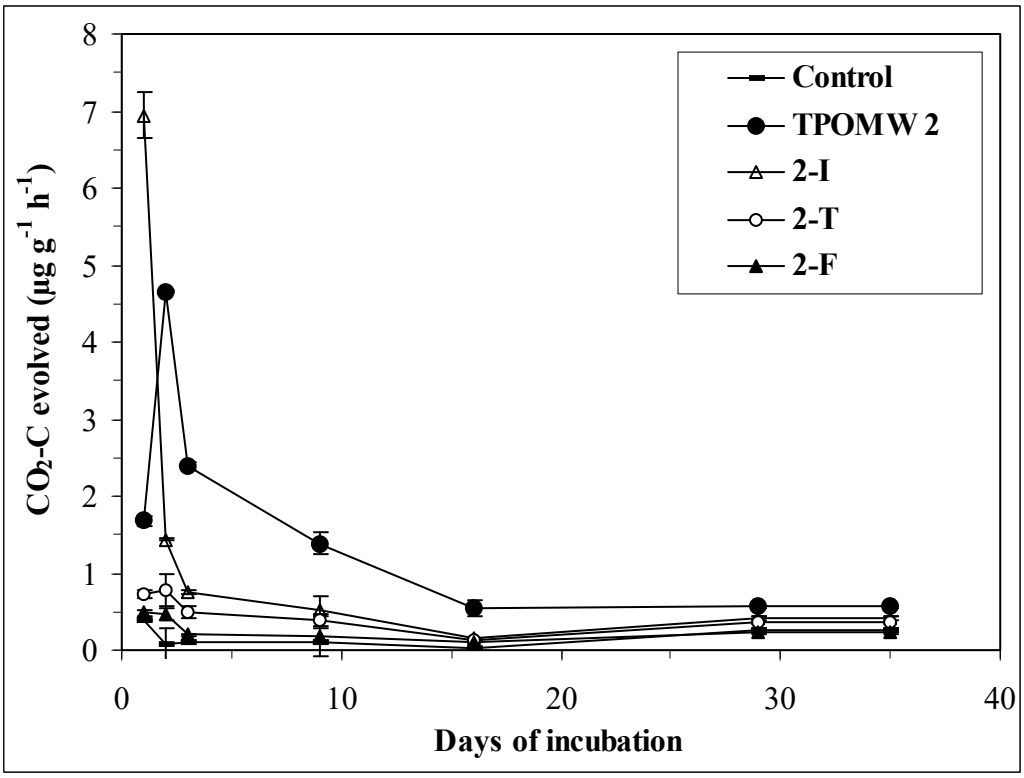
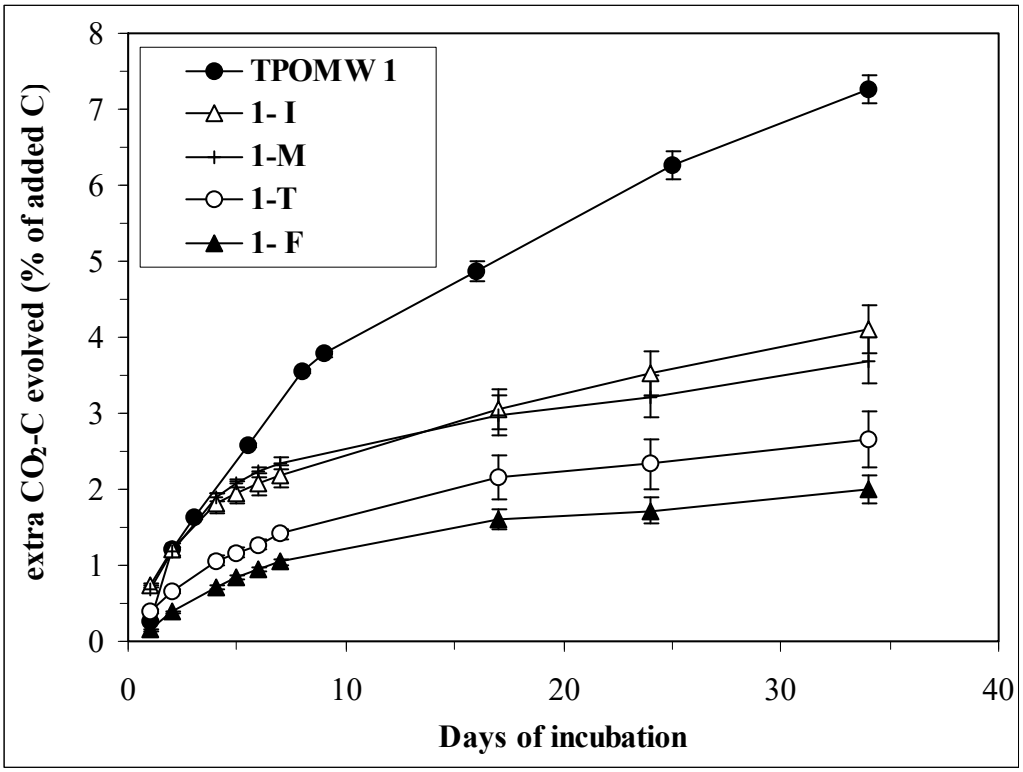



558

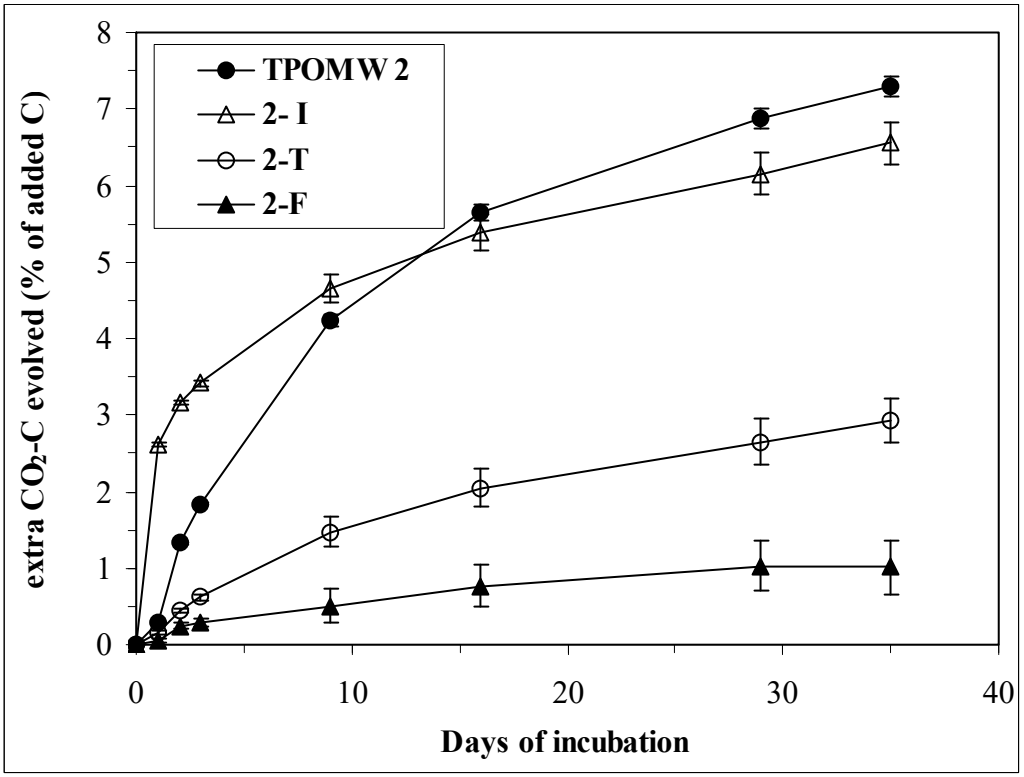
559 Fig. 3

560

**a**




**b**




561

562 Fig. 4

563