

42 **Highlights**

- 43 • N balance from an urban soilless lettuce crop was performed.
- 44 • The N₂O emission factor for an urban soilless lettuce crop was determined.
- 45 • Soilless systems could reduce GHG emissions from urban agriculture crops.
- 46 • Further research is needed to generate precise, agricultural N₂O emissions factors.

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48 **1. Introduction**

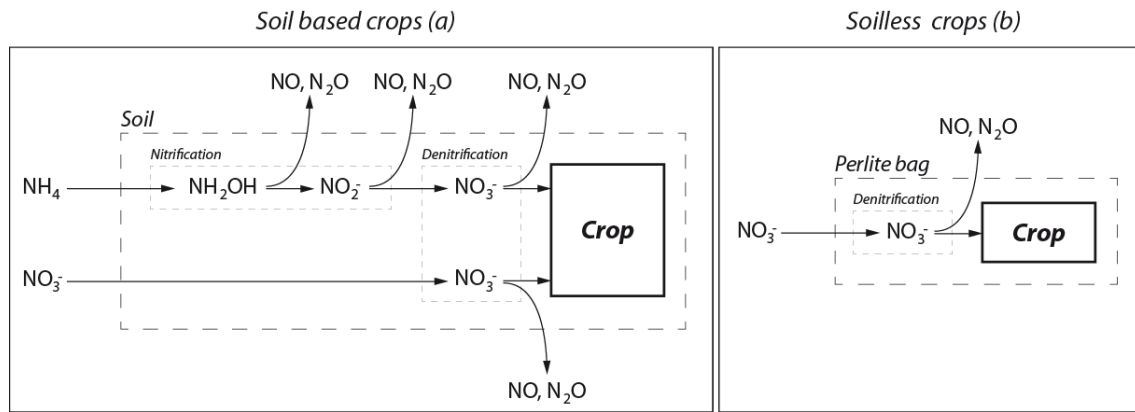
49 High nitrogen (N) demand for food production due to an ever-growing population has resulted
50 in an alteration of the natural N cycle in the air, on land and in the water on both regional and
51 global levels (Galloway et al., 2004). Direct N₂O emissions from the agricultural sector
52 represented 6% of total European greenhouse gas (GHG) emissions in 2012. The atmospheric
53 concentration of N₂O is of great concern because N₂O is a GHG with a global warming
54 potential (GWP) 298 times that of CO₂ for a 100-year time span (IPCC, 2013) and is also
55 responsible for stratospheric ozone depletion (IPCC, 2014a, 2014b). By 2050, global food
56 production is estimated to rise by 30% (Alexandratos and Bruinsma, 2012), as a consequence of
57 an increase in the global population to 9.6 billion humans by the same year (UN, 2012).
58 Moreover, 70% of the global population will be concentrated in cities (UN, 2012), requiring
59 more energy for food transportation and packaging and resulting in further GHG emissions.
60 Finding more sustainable ways of producing food is imperative, especially for urban areas. One
61 way to do this is to look for agricultural systems that produce fewer N₂O emissions to reduce
62 GHG emissions from worldwide feeding systems (Maraseni and Qu, 2016).

63 Urban agriculture (UA) is a potential solution to reducing the carbon footprint of urban areas
64 (Mok et al., 2013) because it reduces food transportation distances, packaging and food loss.
65 Rooftop greenhouses (RTGs) are a specific type of UA consisting of a greenhouse on the roof
66 of buildings. RTGs are of great interest due to their potential to reduce the environmental
67 impact and costs of food production, while creating jobs in in cities (Cerón-Palma et al., 2012).
68 Sanyé-Mengual et al. (2015) have analyzed the environmental performance of RTGs in
69 comparison with conventional crop production using a cradle-to-grave, cradle-to-gate and
70 cradle-to-consumer approach. The study's results suggest that producing tomatoes in a RTG in a
71 *soilless system* can result in fewer GHG emissions than conventional tomato production, if the
72 annual production rate reaches at least 24 kg m⁻². For lack of a more appropriate N₂O emission
73 factor (EF) for soilless crops, the Sanyé-Mengual et al. (2015) study used the IPCC EF to
74 quantify N₂O emissions from the N fertilizers supplied. However, the IPCC method used (IPCC,
75 2006) is based on data generated from *soil-based crops* and does not take into consideration the
76 different substrate and fertilizer reduction of soilless crops, as well as solar radiation,

77 temperature, humidity and other factors that could significantly influence the EF. Consequently,
78 the GHG emissions may not be correctly calculated.

79 Similar to the Sanyé-Mengual et al. (2015) study, other life cycle assessment (LCA) studies that
80 attempt to evaluate the environmental performance of soilless crops have the same handicap due
81 to a lack of more appropriate EFs. For example, three soilless tomato crops grown in Spain,
82 Hungary and The Netherlands were evaluated with LCA methodology in the European project
83 EUPHOROS (Montero et al., 2011) using the same IPCC soil-based EF for all locations,
84 without taking into account how the soil-less substrate and the differing climate conditions
85 could affect the GHG emissions during the growth phase. Similarly, Torrellas et al. (2012)
86 assessed how various degrees of fertilization affect the LCA of a soilless tomato crop grown in
87 Southern Spain. Even though they used the IPCC EF, the study concluded that one of the most
88 important factors in curtailing the carbon footprint of the fruit is the reduction of direct
89 emissions to air caused by N fertilizers, further evidencing the need for a more appropriate N₂O
90 EF for soilless crops. Payen et al. (2015) reached comparable conclusions in their cradle-to-
91 market analysis of local versus imported tomatoes grown with soilless systems in France. They
92 concluded that the environmental assessment is especially sensitive to both the EF used to
93 determine the direct N₂O emissions and the transportation requirements of the fruit.

94 In soil-based crops, emissions of N₂O result from nitrification and denitrification reactions as
95 shown in Fig. 1a. The inert conditions of substrates used in soilless crops and their low capacity
96 to retain water can inhibit the microbial population growth that produces N₂O emissions through
97 nitrification, making denitrification the main mechanism (Ruser and Schulz, 2015).
98 Consequently, applying the IPCC EF, even as a rough approximation, could significantly
99 overestimate the N₂O emissions from soilless crops. Furthermore, in soilless crops, N fertilizers
100 can be exclusively provided through nitrate (NO₃⁻) doses via the irrigation system, as shown in
101 Fig. 1b, thereby limiting the amount of ammonium (NH₄) present in the substrate, which could
102 lead to N₂O via nitrification (Gianquinto et al., 2013). In addition, an optimal NO₃⁻ dose, as well
103 as favorable pH conditions (between 5.5 and 6.5) to facilitate optimal plant N assimilation,
104 should prevent the accumulation of NO₃⁻ in the substrate, which would otherwise lead to N₂O
105 via denitrification (Gianquinto et al., 2013; Savvas et al., 2013). The denitrification route shown
106 in Fig. 1b is further limited by the fact that the high porosity of the substrate results in an
107 aerobic environment (Ruser and Schulz, 2015). In an aerobic environment, microorganisms
108 have enough available oxygen to continue with their activity, so the denitrification process is
109 not necessary for obtaining the required oxygen.



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111 **Figure 1. (a) N_2O emissions through the nitrification – coupled – denitrification process in**
 112 **soil based crops, adapted from Reiner Ruser and Rudolf Schulz (Ruser and Schulz, 2015).**

113 **(b) Only the denitrification process produces N_2O emissions for soilless crops.**

114 There are several studies that analyze direct N_2O emission from fertilized crops, but very few
 115 that determine the EF for soilless crops (table 1). Yoshihara et al. (2014) experimentally
 116 measured an emissions factor of 0.01-0.046 $\text{kg N}_2\text{O}^{-1}$ per kg N^{-1} for a tomato crop grown in
 117 rockwool substrate. The study examined the change in N_2O emissions response after
 118 fertilization with different concentrations of fertilizer and found that 90% of N_2O emissions
 119 occurred within the first two hours of fertilization, with a main emissions peak at the 90th
 120 minute. Furthermore, they realized that halving the concentration of fertilization reduced N_2O
 121 emissions by more than half. A second study by Daum and Schenk (2013) estimated an
 122 emission of 0.004-0.016 $\text{kg N}_2\text{O}^{-1}$ per kg N for a cucumber, closed-loop crop cultivated with
 123 rockwool substrate. The study found that the highest emissions rates are produced during fruit
 124 stem growth. In addition, root density increases N_2O emissions because high root respiration
 125 favors the growth of microorganisms in the substrate. Both studies provide a range for the N_2O
 126 EF that could be between 20% and 68% less than the EF published for soil crops (Bouwman,
 127 1996) and accepted by the IPCC (2006), if efficient fertilization methods are used.

128 The two studies mentioned serve to note that the EFs can vary depending on the crop, climate
 129 conditions, soil, and the type of fertilization used and that further study is needed in order to
 130 establish a more generalized method to account for the emissions to evaluate food production
 131 systems. Moreover, Daum and Schenk (1998) describe how a variation between pH 4 and pH 7
 132 of the nutrient solution influences N_2O emissions in cucumbers grown on a rockwool substrate.
 133 Emissions varied between 0.016 and 0.21 $\text{kg N}_2\text{O}^{-1}$ per kg N . Other studies, based on soil
 134 culture crops, made evident the direct relation between N_2O emissions and soil temperature
 135 (Hosono et al., 2006), the type of soil used (Rochette et al., 2008), the water-filled pore space
 136 (Bateman and Baggs, 2005; Weier et al., 1993), the available carbon in soils (Weier et al.,
 137 1993), the crop season (Daum and Schenk, 2013; Dobbie et al., 1999) or the amount of rainfall
 138 (Dobbie et al., 1999).

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140 With this study, we aim to add to this pool of knowledge by estimating the N₂O EF for a soilless
 141 crop cultivated in an RTG using perlite substrate. To meet the objective, N₂O was continuously
 142 monitored during the cultivation cycle of *Lactuca sativa* (lettuce) in an RTG located in the
 143 metropolitan area of Barcelona, Spain. A calculation of N balance was also performed for two
 144 experiments for further analysis. Additionally, we discuss how the N₂O EF can alter LCA
 145 results and the importance of standardizing an EF specifically for N₂O generation associated
 146 with soilless crops.

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148 **Table 1. Selection of papers that analyze experimentally the N₂O emissions of crops.**

<i>Main author</i>	<i>Reference</i>	<i>Region</i>	<i>Soil typology</i>	<i>Crop variety</i>	<i>Protected crop</i>	<i>Emission factor</i> (kg N ₂ O ⁻¹ per kg N ⁻¹)
Yoshihara et al.	(Yoshihara et al., 2014)	Japan	Rockwool	Tomato	Yes	0.01 – 0.046
D. Daum et al.	(Daum and Schenk, 1998)	Denmark	Rockwool	Cucumber	Yes	0.016 and 0.21
D. Daum et al.	(Daum and Schenk, 2013)	Denmark	Rockwool	Cucumber	Yes	0.004-0.016
Xiong et al.	(Xiong et al., 2006)	China	Soil	2 years consecutives crops: radish, baby bok choy, lettuce, second planting of baby bok choy, and finally celery	Yes	0.0039 -0.0224
Pfad et al.	(Pfab et al., 2011)	Germany	Soil	Rotation crop: Lettuce (summer) and cauliflower (winter) rotation crop	No	0.013-0.016
Rochette et al.;	(Rochette et al., 2008)	Canada	Soil	-	No	Arid zones: 0.0016 Humid zones: 0.017
Henault et al.	(Henault et al., 1998)	Northeastern of France	Soil	-	No	Châlons: 0.0014-0.0018 Messigny: 0.0066-0.0076 Longchamp: 0.0248-0.0249
Hiroko Akiyama et al.	(Akiyama et al., 2006)	Japan	Soil	Tea and rice	No	Tea: 0.0282 ± 1.80% Rice: 0.0031 ± 0.31%
Qiaohui Liu et al.	(Liu et al., 2013)	China	Soil	Vegetables	Protected and not protected	Avg.: 0.0063±0.09%
H. Flessa et al.	(Flessa et al., 1995)	Germany	Soil	Sunflower	No	0.018
A. F. Bouwman	(Bouwman, 1996)	-	Soil	-	No	0.0125±1%*

*Emission factor from the literature used for the IPCC method

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150 **2. Materials and Methods**151 **2.1. Location and crop description**

152 The experiment was carried out in an open chamber in an integrated RTG (i-RTG) with a
 153 southeast orientation, named the Agrouurban Lab 1 (AU-Lab1) located in the Environmental
 154 Science and Technology Institute (ICTA) and Catalan Institute of Paleontology (ICP) building
 155 at the Universitat Autònoma de Barcelona campus near Barcelona (41°29'51.6"N; 2°06'31.9"E).
 156 This RTG is considered an i-RTG because it is built to exchange water, energy and air flow
 157 with the rest of the building to increase its efficiency and optimize resource use. A scheme of

158 the symbiosis between the building and the greenhouse can be found within the supporting
159 information.

160 The experiment to determine N₂O emissions was developed with a *Lactuca sativa* (lettuce)
161 soilless crop in a perlite substrate located on a concrete floor. The fertilizer composition and
162 concentration supplied to the crop through a drip irrigation system is provided in table 2. N was
163 exclusively provided in terms of NO₃⁻ to reduce the nitrification process from NH₄ as shown in
164 Fig. 1b. Once mixed with fertilizers, the irrigation water used had a pH of 6.4 and an electric
165 conductivity of 2.2 mS cm⁻¹, which provides favorable fertilizer assimilation conditions (Savvas
166 et al., 2013). The i-RTG provided the 2015 crop with an average relative humidity of 45% and a
167 temperature of 19°C during the winter and 23°C during the summer. The atmospheric N₂O
168 concentration inside the i-RTG oscillated between 200 and 500 ppb_v.

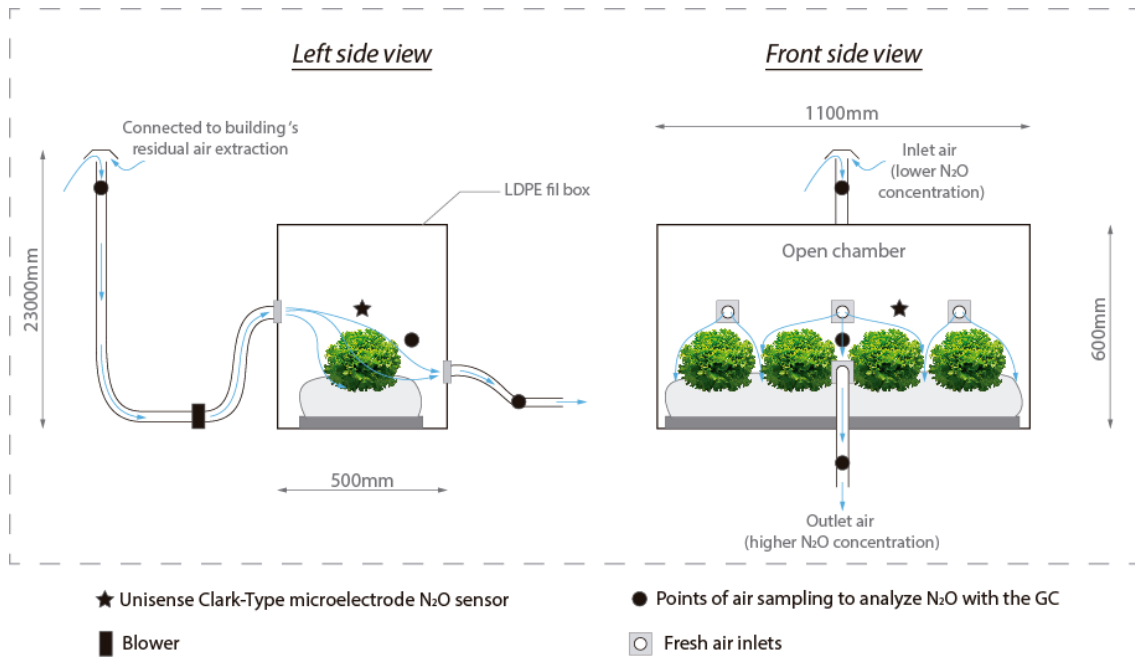
169 **Table 2. Fertilizer concentrations used for irrigation.**

	KPO ₄ H ₂	KNO ₃	K ₂ SO ₄	Ca(NO ₃) ₂	CaCl ₂	CaCl ₂ *2H ₂ O	Mg(NO ₃) ₂	Hortrilon	Sequestrene
mg/l	0.136	0.203	0.348	0.451	0.139	0.184	0.223	0.01	0.01

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171 2.2. Open chamber design & description

172 Open chambers are small systems, based on a validated methodology (Garcia et al., 1990),
173 which allow for the measurement of canopy gas exchange with a certain precision. For the
174 present study, an open chamber was used to measure the potential N₂O emissions from the
175 substrate of soilless lettuce crops. The open chamber was built with LDPE (a film with low
176 reflection and high transmissivity to solar radiation) to reduce the effect of the chamber
177 structure on the temperature and humidity inside, as recommended by a previous study (Burkart
178 et al., 2007). The chamber was built with a volume of 0.33 m³ to fit four lettuces (Fig. 2). The
179 inlet flow was forced by a blower that collected air from the building's cooling and heating air
180 extraction system and injected it into the chamber through a PVC tube with a 50 mm diameter.
181 The inlet air was between 20 and 24°C and had a CO₂ concentration of more than 400 ppm due
182 to human respiration inside the building. A potentiometer was used to adjust the power of the
183 blower and ensure a flow rate that could maintain inner chamber temperatures at the desired
184 levels.



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Figure 2. Open chamber design and measurement points located inside the UA-LAB1 in the ICTA-ICP building (Bellaterra, Spain).

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2.3. Open chamber validation

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The relative humidity and temperature of the i-RTG and the open chamber followed a similar evolution during day and night as shown in section 2.1 of the supporting information. However, from 08/05/2015 to 12/05/2015, daily temperatures and relative humidity were 5°C and 10% higher, respectively, in the open chamber than in the greenhouse. Consequently, the blower was regulated to 330 m³ h⁻¹ (ensuring a renewal of the open chamber air 100 times per hour) to increase air circulation and reduce these differences with respect to the i-RTG. This was achieved after 13/05/2015, when differences were low enough to be considered negligible. This assumption was confirmed by the similar growth rates for lettuces grown inside (10.86 ± 1.87 g day⁻¹) and outside the chamber (10.51 ± 1.57 g day⁻¹). Temperature and humidity were monitored inside and outside the open chamber in the proceeding experiments and never presented higher differences than the ones established as insignificant (supporting information, sections 2.2 and 2.3 for experiments 2 and 3, respectively).

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2.4. Measurements & data acquisition

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To achieve the objectives of the study, air samples at specific measurement points (Fig. 2), inside the chamber and from the inlet and outlet air, were collected with 1-liter air bags (SKC¹ - model 1.252-01) to measure N₂O concentrations. Moreover, one air sample from the greenhouse was collected at the beginning of experiments 2 and 3 (table 3). Air samples were analyzed with gas chromatography using an Agilent chromatograph 6890N and the Agilent HP-PLOT-Q 30 m,

¹ <http://www.skinc.com/catalog/index.php>

208 0.53 mm, 40 μm column (hereafter referred to as GC). These samples were collected to
209 calculate the differences in N_2O concentration between the inlet and the outlet air. The
210 calibration line used to analyze N_2O air samples by GC had a precision of 96.8%. Therefore, an
211 error of $\pm 3.2\%$ may be assumed. Three air samples were analyzed three times to quantify the
212 human error that can be introduced by the manual injection of the samples into the GC, which
213 was 16 ppb_v; therefore, the maximum deviation produced by manual injection could produce an
214 error of $\pm 5.3\%$, adding to a total potential error of $\pm 8.5\%$. To completely minimize the error,
215 each air sample was analyzed 3 times.

216 Additionally, a Unisense Clark-Type microelectrode N_2O sensor was placed inside the chamber.
217 Data from this sensor was collected with a Unisense 4 channel microsensors amplifier
218 multimeter connected to a computer. Calibration and measurements were realized according to
219 the method described by Marques et al. (2014). This method requires knowing the temperature
220 of the Clark-type microelectrode, which was measured with a Campbell SCI 107 thermistor. Air
221 samples from inside the chamber were collected and analyzed by GC to correct possible
222 deviations of the N_2O sensor for the continuous monitoring of N_2O inside the chamber (Fig. 2).
223 Moreover, two Campbell SCI CS215-L sensors were used to record the temperature and relative
224 humidity inside the open chamber and inside the greenhouse (outside the chamber). To collect
225 data from Campbell devices, a CR3000 data-logger was used. A Testo² hot-wire anemometer
226 was used to quantify both inlet and outlet rate flows.

227 To determine leachate N concentration, samples of the leachate from the perlite bags were
228 collected and analyzed in a chromatograph DIONEX-ICS 1000 Ion System with a Dionex Ion
229 Pac AS9-HC RFIC Analytical 4 x 250 mm column. In addition, the volume of leachates was
230 measured. The total N content of lettuces at the end of the crop was determined by an external
231 laboratory. N concentrations within the perlite bag were measured according to the two-step
232 process for analyzing different forms of N in soils as described by Bremner and Keeney (1966).

233 **2.5. N balance**

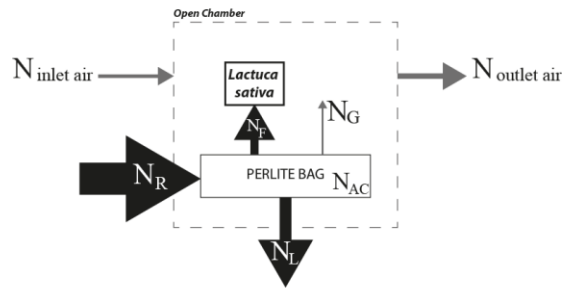
234 The N balance of the crop was calculated according to the following equations:

$$235 \quad [1] \quad N_R = N_L + N_F + N_E + N_{Ac}$$

$$236 \quad [2] \quad N_E = N_{\text{outlet air}} - N_{\text{inlet air}}$$

237 N_R represented the amount of N provided through the irrigation system (inputs); the remaining
238 elements of the equation were related to the different outputs generated (also represented in Fig.
239 3): N_L was the amount of N contained within the leachates; N_F was the amount of N fixed by
240 the crop (lettuces in this case); N_E was the emissions generated by the perlite substrate; and N_{Ac}
241 was the amount of N that accumulated within the perlite bag.

² <https://www.testo.com/en-US/>



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243 **Figure 3. N flows under study. The thickness of the arrow is correlated to the amount of N**
 244 **within each flow.**

245 **2.6. Description of experiments**

246 Three experiments were conducted between May 2015 and October 2015 (table 3). The first
 247 experiment served to understand and validate the open chamber as a suitable representation of
 248 the i-RTG. The N_2O emissions were measured during the second and third experiments. The
 249 latter served to validate the results of the previous experiment, and more data were gathered to
 250 be able to close the N balance. A new perlite bag was used for each experiment so as not to
 251 confound the analysis with N accumulated from a previous experiment.

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Table 3. Description of experiments

Measurement	Units	Experiment 1	Experiment 2	Experiment 3
Date	-	04/05/2015 - 03/06/201	07/07/2015 - 14/08/2015	16/09/2015 - 15/10/2015
Objective	-	<ul style="list-style-type: none"> Compare greenhouse and open chamber climate conditions. Ensure that open chamber has no effect on lettuces growth rate. 	<ul style="list-style-type: none"> Measure the N input and output flows from a lettuce crop to realize the N balance. Analyze N₂O emissions of an over fertilized crop. 	<ul style="list-style-type: none"> Improve the quality and repetitiveness of results from experiments 1 & 2 to realize the N balance. Analyze N₂O emissions from a crop with a more efficient fertilization.
Greenhouse N₂O concentrations	ppmv	-	1 sample at the beginning of the experiment	1 sample at the beginning of the experiment
Greenhouse temperature & humidity	°C; %	Yes	Yes	Yes
Open Chamber temperature & humidity	°C; %	Yes	Yes	yes
N content from irrigation (chromatography)	g	-	At the beginning and end of the crop	1 sample every 4 days
N content from leachates (chromatography)	g	-	4 samples during the whole experiment	3 samples per week
Total irrigation	L	-	Yes	Yes
Open chamber inlet, inner and outlet air N₂O concentration (chromatography)	ppmv	-	3 samples per day, during 5 days at 10:00; 13:00 and 16:00	3 samples twice a week at 10:00, 13:00 and 16:00
Continuous measurements of open chamber inner N₂O concentration (N₂O microsensor)	ppmv	-	Yes	Yes
N content of lettuces cropped	g	-	Yes	Yes
N content of the perlite bag at the end of the crop	g	-	yes	Yes

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257 3. Results and Discussion

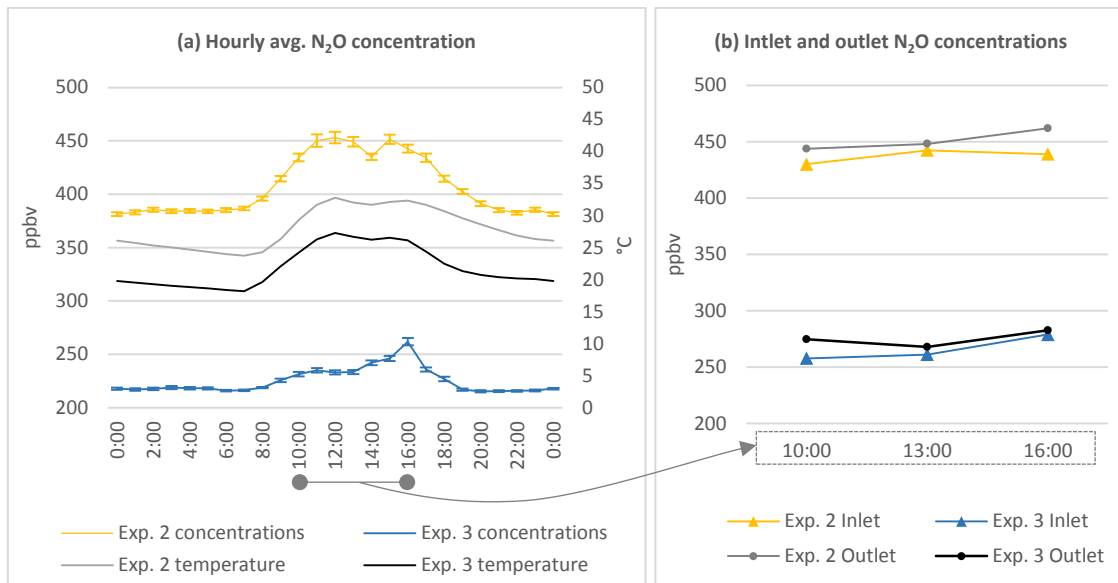
258 3.1. Determining N₂O emissions

259 Fig. 4 shows both the average daily evolution of N₂O concentration inside the open chamber,
260 measured continuously with the Unisense microsensor for the duration of both entire
261 experiments (Fig. 4-a), and the chamber inlet and outlet air N₂O concentrations analyzed by GC
262 (Fig. 4-b) for experiments 2 and 3 from 04/05/2015 to 03/06/2016 and 07/07/2015 to
263 14/08/2015, respectively.

264 It seems that N₂O generation was mostly affected by the increase in chamber temperature during
265 the day (Fig. 4-a). Higher temperatures, which enhance the activity of microorganisms such as
266 the thermophilic *Bacillus* sp, increase the denitrification process (Keeney et al., 1979) and
267 consequently N₂O emissions resulting from this process (Maag and Vinther, 1996). This is best
268 reflected by the results from experiment 2, where temperature and N₂O concentration,
269 respectively, began to rise from 24°C and 380 ppb_v at 8:00 to 32°C and 450 ppb at noon, when
270 they remained stable until 16:00, at which point both temperature and concentration started
271 decreasing back to their initial values. The dip in concentration at 14:00 further correlated the
272 generation of emissions with temperature because during that time, the open chamber received
273 direct shade from the greenhouse structure for approximately one hour. The correlation between
274 temperature and N₂O emissions was not as evident for experiment 3, where the concentration
275 peak at 12:00 did not coincide with the highest temperature. Upon closer inspection, we found
276 that the low average concentration was a result of eight days during experiment 3 that had
277 temperatures over 30°C with low N₂O concentrations ranging between 205 and 212 ppb_v. The
278 relative humidity (RH) during those days (Fig. 7 in supporting information) was lower than on
279 any other day of the experiment (less than 50%, see Fig. 8 in supporting information). We
280 believe that the low RH and the increased evaporation rate due to the high temperatures most
281 likely caused the water-filled pore space (WFPS) of the perlite substrate to be low enough to
282 inhibit nitrification (Merino et al., 2004; Pihlatie et al., 2004). Furthermore, the lettuces in
283 experiment 3 were watered for 4 minutes every two hours instead of for 8 minutes every two
284 hours as were the lettuces in experiment 2, thereby further reducing the amount of water in the
285 substrate pores. For the rest of the days where RH is higher and temperatures are lower, the
286 positive correlation between temperature and N₂O generation holds true (Fig. 8, supporting
287 information). To summarize, it seems that temperature plays an important role in N₂O
288 generation, but watering frequency and RH can also affect the substrate significantly and even
289 inhibit N₂O formation.

290 Ambient N₂O concentrations in the i-RTG during experiments 2 and 3 were approximately 380
291 and 250 ppb_v, respectively, which explains the difference of 130 ppb_v between daily
292 concentrations measured for the two experiments (Fig. 4-a). The higher ambient concentration

293 for experiment 2, which took place during July, could be explained by the fact that N₂O
 294 concentrations are usually higher during the warmer months in the northern hemisphere (Khalil
 295 and Rasmussen, 1983).



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 297 **Figure 4. The average hourly N₂O concentrations in the open chamber as measured with a**
 298 **Unisense Microsensor (Fig. 4-a) and the N₂O concentration difference between inlet and**
 299 **outlet air as measured by gas chromatography (Fig. 4-b) for experiments 2 and 3.**

300 An increase in N₂O emissions 30-120 minutes after fertilization via irrigation has been reported
 301 (Daum and Schenk, 2013). Nevertheless, we did not find a correlation between the fertilization
 302 periods and N₂O generation.

303 Whereas Fig. 4-a shows the hourly average N₂O concentration inside the open chamber during
 304 the entire experiment, Fig. 4-b shows the hourly average N₂O concentration, punctually
 305 measured 3 times a week during the for the duration of the experiments (table 3), of the air
 306 going into and out of the open chamber (as depicted by the circles in Fig. 2). In other words,
 307 Fig. 4-b allows an estimation of the N₂O generation inside the open chamber by calculating the
 308 difference between outlet and inlet N₂O concentrations. Total N₂O emissions were calculated
 309 considering a constant flow rate of 330 m³ h⁻¹ and the ideal gas law, resulting in 8.53·10⁻¹¹ and
 310 6.28·10⁻¹¹ kg N₂O s⁻¹ (equivalent to a total of 0.29 g and 0.15 g of N as shown in table 4) for
 311 experiments 2 and 3, respectively.

312 Grab air samples were taken at 10:00, 13:00 and 16:00 (Fig. 4-b) because it was considered a
 313 time frame during the day when N₂O emissions were the most significant due to more intense
 314 solar radiation and consequently higher temperatures. As can be expected, there are higher N₂O
 315 concentrations in the outlet air, reflecting N₂O generation during these hours. N₂O generation
 316 ranges from 0.014 ppmv to 0.023 ppmv and from 0.004 ppmv to 0.017 ppmv for experiments 2
 317 and 3, respectively.

318 Table 4 presents the N balance for experiments 2 and 3 as calculated according to equation 1
 319 (Fig. 3). In experiment 2, 25% of the input is unaccounted for in the output. A possible reason
 320 for this is the fact that only four samples from the leachates were collected during the entire 5-
 321 week experiment to establish an average N concentration, which was used to determine
 322 leachates N (N_L). Differences in daily volume and N content amongst the 4 samples were
 323 significant, ranging between 4.0 and 6.7 liters, with N contents between 0.09 and 0.13 g liter⁻¹.
 324 Therefore, to determine N_L based on an average of the four samples was inappropriate. Thus the
 325 N_L value of 23.59 g of N is most likely inaccurate. Consequently, for experiment 3, leachates
 326 were measured three times a week for 4 weeks to improve the N balance calculation. As a
 327 result, 95% of the input N is accounted for in the output N (table 4). The remaining 5% can be
 328 other forms of N output that are unaccounted for in this study, such as NO (Hosono et al.,
 329 2006), as well as the error introduced by the flowmeter (5%), which could overestimate the total
 330 N input.

331 **Table 4. N balance for experiments 2 and 3. All values are given in g of N.**

	<i>2nd experiment</i>				<i>3rd experiment</i>			
	Input		Output		Input		Output	
	Total	Per 1 kg of lettuces	Total	Per 1 kg of lettuces	Total	Per 1 kg of lettuces	Total	Per 1 kg of lettuces
Irrigation	40.08	42.07	-	-	17.88	22.38	-	-
Emissions measured	-	-	0.29 (+/- 8.5%)	0.30 (+/- 8.5%)	-	-	0.15 (+/- 8.5%)	0.19 (+/- 8.5%)
Leachate	-	-	23.59	24.76	-	-	11.80	14.77
Perlite bag	-	-	3.93	4.12	-	-	3.34	4.18
Lettuces	-	-	2.27	2.39	-	-	1.50	1.88
Total	40.08	42.07	30.08	31.57	17.88	22.38	16.79	21.02

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 333 More than half of the N supplied in experiment 2 was lost in the leachate. Thus, for experiment
 334 3, 17.9 g of N was administered via irrigation instead of the 40.1 g of N that was used in
 335 experiment 2 (table 4). This significantly reduced N emissions from 0.29g ($\pm 8.5\%$) to 0.15 g
 336 ($\pm 8.5\%$), as well as N in the leach. In addition, the percentage of N absorbed by each kg of
 337 lettuce for experiment 3 (8.4%) was higher than for lettuces from experiment 2 (5.6%). This
 338 means that for experiment 3, the N in the fertilizers was used more efficiently. However, N per
 339 kg of lettuces is lower for experiment 3 (1.88 g, table 4) than for experiment 2 (2.39 g, table 4).
 340 A lower concentration of N fertilization reduces the final N content in lettuces, but it contributes
 341 to the absorption of other substances such as chloride, glucose or sucrose (Mccall and
 342 Willumsen, 2015), so that some nutritional compounds are substituted by others. (It is out of the
 343 scope of this research to determine if the nutritional function of these compounds is more or less
 344 important than the nutritional value of N.)

345 Compared with experiment 2, more N was emitted than supplied during experiment 3 (0.72%
 346 vs. 0.83%, respectively). The difference is insignificant when we consider the error introduced

347 by GC analysis, which had a $\pm 8.5\%$ of 250-450 N_2O ppmv. When we included the errors from
348 the measurements, the ratio of N emitted to N supplied varied between 0.6% and -0.79% for
349 experiment 2 and 0.76% and 0.91% for experiment 3.

350 The N accumulated in the perlite can potentially be emitted to the atmosphere in the form of
351 N_2O . The emissions from the substrate at the end of its life should be accounted and attributed
352 proportionally to each crop cultivated with the substrate. This calculation is beyond the scope of
353 this study.

354

355 **3.2. Determining the emission factor**

356 The N_2O EFs for the soilless lettuce crop determined for experiments 2 and 3 were very similar:
357 0.0072 and 0.0085 $\text{kg N}_2\text{O}^{-1}$ per kg N^{-1} , respectively. These values are roughly half the EF
358 provided by the IPCC (0.0125 $\text{kg N}_2\text{O}^{-1}$ per kg N^{-1}) and, on average, are lower than the EFs
359 shown in Table 1 for rockwool based crops (0.004-0.21 $\text{kg N}_2\text{O}^{-1}$ per kg N^{-1}). The lower EF we
360 obtained could be explained by (1) the high porosity and low water retention potential of the
361 perlite substrate, which provided an aerobic environment that limited denitrification reactions
362 (Ruser and Schulz, 2015), (2) the fact that nitrification reactions are limited by supplying N in
363 the form of NO_3^- and not in the form of NH_4 , as shown in Fig. 1, and (3) the use of an
364 appropriate concentration of NO_3^- to optimize the assimilation of fertilizer as was demonstrated
365 by experiment 3.

366 The results obtained are specific to perlite substrate under certain temperature, humidity and
367 irrigation conditions. As explained by Yoshihara et al. (2014) and Daum and Schenk (2013),
368 N_2O emissions can oscillate significantly depending on the season (affecting temperature),
369 fertilization frequency, efficiency of fertilizer use, irrigation timing (day or night), and type of
370 substrate. We cannot assume that the EF we obtained can be used for all soilless crops. What
371 has been determined through this study is that further research is needed to determine an EF that
372 can be used by LCA practitioners to evaluate the advantages of such crops. In our experiments,
373 a more efficient use of fertilizers (using 55% less N fertilizers) reduced total N_2O emissions by
374 48.3%.

375 **4. Applicability in LCA studies**

376 To determine how the EF of soilless crops can influence LCA studies, we applied the EF
377 determined by our analysis to an LCA study evaluating tomato crop production conducted by
378 Sanyé-Mengual et al. (2015) in the same i-RTG where the experiments realized in this study
379 took place. Although the Sanyé-Mengual et al. (2015) study evaluated tomato production
380 instead of lettuce production, the EF determined for lettuce production could be applied to the

381 study of tomato production because both crops had the same fertilization system, climate
382 conditions and substrate.

383 The system boundaries of Sanyé-Mengual et al. (2015)'s study include the assessment of the i-
384 RTG structure, the production of tomatoes, and the distribution for commercialization of
385 tomatoes. For the assessment of the greenhouse structure, the study accounted for the
386 environmental impact of raw material extraction, processing and transportation, as well as the
387 structure's maintenance and end of life. The assessment of the production of tomatoes included
388 the fabrication, use, and end of life of the auxiliary equipment and resources required for
389 growing the tomatoes, such as the perlite substrate, the irrigation system, water, energy,
390 fertilizers and pesticides as well as waste management. The assessment of crop distribution
391 encompassed the packaging production and tomato distribution up to 25 km from the
392 greenhouse located in the city of Barcelona, Spain.

393 Sanyé-Mengual et al. (2015) used the IPCC EF ($0.0125 \text{ kg N}_2\text{O}^{-1} \text{ per kg N}^{-1}$) to estimate N_2O
394 emissions from N fertilizers and concluded that direct N_2O from the substrate was responsible
395 for 18.05% of the total GHG emissions ($0.8 \text{ kg CO}_2 \text{ eq. kg tomato}^{-1}$ - see table 5). Consequently,
396 N_2O emissions from N fertilizers represented an environmental impact higher than that of the i-
397 RTG structure (12.0% of total GHG emissions) and more than half of the environmental impact
398 of the production stage (26.8%, see table 5). This reduced transportation avoids 0.44 kg CO_2
399 eq./kg tomato in comparison with tomatoes that are produced 900 km away in southern Spain
400 and transported to and consumed in Barcelona.

401 If the environmental analysis from Sanyé-Mengual et al. (2015) is calculated with the average
402 EF determined by the present study ($0.0079 \text{ kg N}_2\text{O}^{-1} \text{ per kg N}^{-1}$), the total GWP is reduced
403 from 0.80 to $0.75 \text{ kg CO}_2 \text{ eq. kg tomato}^{-1}$ (table 5). That represents a 7.5% reduction of total
404 GWP ($1.5 \text{ kg CO}_2 \text{ eq. m}^{-2} \text{ year}^{-1}$ or $112.5 \text{ kg CO}_2 \text{ eq. year}^{-1}$ for the entire i-RTG). It should be
405 taken into account that in the i-RTG, N was provided in the form of NO_3^- . For crops where N is
406 also provided in the form of NH_4 , the measured EF is not applicable. Further studies are needed
407 to quantify EF from soilless conditions when N is applied in the form of NH_4 .

408 Between 1990 and 2012 European agricultural GHG emissions were reduce by 23%, from 600
409 million tons $\text{CO}_2 \text{ eq.}$ to 460 million tons (Benjamin et al., 2015). This was achieved by reducing
410 the surplus of N fertilizers and by decreasing N losses to the hydrosphere and the lithosphere
411 from the leachates of crops. However, the European Union still needs to look for new
412 approaches, measures and technologies to mitigate agricultural GHG emissions (Dalgaard et al.,
413 2014). Sanyé-Mengual et al. (2015) achieved a 33% GWP reduction in relation to conventional
414 tomato production due to the reduction of transport distances. We show here that the
415 environmental burden in terms of GWP is actually less when we also consider an appropriate

416 EF for soilless crops. In the case of the Sanyé-Mengual et al. (2015) study, the GWP could be
 417 further reduced to 40.5% of that of conventional tomato production.

418 Our results highlight the necessity of developing more specific EFs that can be used in LCA
 419 studies to ensure more precise analysis. For the case of urban agriculture, using an erroneous EF
 420 could significantly overestimate GHG emissions and consequently hinder proper policy making.

421

422 **Table 5. GWP contributions for 1 kg of tomato production in an i-RTG according to a**
 423 **cradle-to-consumer study by Sanyé-Mengual et al. (2015): a comparison of results between**
 424 **GWP when using the IPCC and our measured EFs.**

Emission factor applied			Total	i-RTG Structure	Tomato production *	Packaging	Distribution	N fertilizer direct emissions
IPCC (0.0125 kg N ₂ O ⁻¹ per kg N)	GWP	kg CO ₂ eq.	0.80	0.10	0.22	0.49	4.74E-05	0.15
	Contribution to total GWP	%	100%	12.0%	26.84%	61.13%	0.01%	18.1%
Measured (0.0079 kg N ₂ O ⁻¹ per kg N)	GWP	kg CO ₂ eq.	0.75	0.10	0.16	0.49	4.74E-05	0.09
	Contribution to total GWP	%	100%	12.9%	21.6%	65.5%	0.01%	12.2%

*Includes direct N₂O emissions from N fertilizers

425

426 5. Conclusions

427 The experiments performed have helped determine that urban agriculture using soilless
 428 substrates has the potential to reduce GHG from feeding urban areas than what is presently
 429 estimated. This study found that by using a perlite substrate and controlled fertilization, the
 430 direct N₂O emissions from fertilization was reduced in average by up to 0.00785 kg N₂O⁻¹ per
 431 kg N⁻¹, which is a 38% reduction in emissions compared with the IPCC EF (0.0125 kg N₂O⁻¹
 432 per kg N⁻¹). This result demonstrated that using the IPCC EF in soilless urban agriculture
 433 would overestimate the GWP of such food systems. In the LCA case study analyzed, there
 434 were 7.5% fewer GHG emissions (0.06 kg CO₂ eq. per kilogram of tomatoes produced) if a
 435 more appropriate EF was used.

436 This study does not serve to provide an EF applicable to all soilless crops, but it does show that
 437 the IPCC method presently used for LCA studies can be a significant overestimation of direct
 438 N₂O emissions and that a new method should be determined. We hope the results here help in
 439 this endeavor and shed some light on some of the parameters that influence the generation of
 440 N₂O by providing irrigation and substrate details, temperature and emission profiles, and
 441 nitrogen balance measurements and calculations.

442

443

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456

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