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### ► To cite this version:

Bénédicte Autret, Nicolas Beaudoin, Lucia Rakotovololona, Michel M. Bertrand, Gilles Grandeau, et al.. Can alternative cropping systems mitigate nitrogen losses and improve ghg balance? Results from a 19-yr experiment in northern France. *Geoderma*, 2019, 342, pp.20-33. 10.1016/j.geoderma.2019.01.039 . hal-02624613

**HAL Id: hal-02624613**

**<https://hal.inrae.fr/hal-02624613>**

Submitted on 22 Oct 2021

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1 **Can alternative cropping systems mitigate nitrogen losses and improve GHG**  
2 **balance? Results from a 19-yr experiment in Northern France**

3

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14 Keywords: nitrogen surplus, nitrogen storage, nitrate leaching, N<sub>2</sub>O emission, greenhouse gas,  
15 organic farming, conservation agriculture, low input

16

## 17 **Abstract**

18 Alternative cropping systems are promoted to reduce nitrogen (N) losses in the environment  
19 and mitigate greenhouse gas (GHG) emissions. However, these supposed benefits are not fully  
20 known, rarely studied together and on the long-term. Here, we studied the N inputs, N exports,  
21 soil organic N (SON) storage, N leaching, gaseous N emissions and GHG balance in a 19-yr  
22 field experiment comparing four arable cropping systems without manure fertilization, under  
23 conventional (CON), low-input (LI), conservation agriculture (CA) and organic (ORG)  
24 managements. The N surplus, *i.e.* the difference between total N inputs and exports, was lowest  
25 in LI (43 kg ha<sup>-1</sup> yr<sup>-1</sup>), intermediary for CON and ORG with 63 kg ha<sup>-1</sup> yr<sup>-1</sup> and highest in CA  
26 (163 kg ha<sup>-1</sup> yr<sup>-1</sup>). CA and ORG received high amounts of N derived from biological fixation  
27 from alfalfa. The annual SON storage rates markedly differed between CA (55 kg ha<sup>-1</sup> yr<sup>-1</sup>) and  
28 both CON and LI (13 and 6 kg ha<sup>-1</sup> yr<sup>-1</sup>), with intermediary value in ORG (30 kg ha<sup>-1</sup> yr<sup>-1</sup>). N  
29 leaching, calculated using soil mineral N measurements, reached an average of 21 kg ha<sup>-1</sup> yr<sup>-1</sup>  
30 and did not significantly differ between treatments. The gaseous N emissions (volatilization +  
31 denitrification), calculated as the difference between N surplus, SON storage and N leaching,  
32 ranged from 12 kg ha<sup>-1</sup> yr<sup>-1</sup> in ORG to 83 kg ha<sup>-1</sup> yr<sup>-1</sup> in CA. N<sub>2</sub>O emissions were continuously  
33 monitored with automatic chambers during 40 months. They varied from 1.20 kg ha<sup>-1</sup> yr<sup>-1</sup> in LI  
34 to 4.09 kg ha<sup>-1</sup> yr<sup>-1</sup> in CA system and were highly correlated with calculated gaseous N  
35 emissions. The GHG balance, calculated using SOC and N<sub>2</sub>O measurements, varied widely  
36 between systems: it was highest in CON and LI, with 2198 and 1763 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>  
37 respectively. In CA, the GHG balance was much more favourable (306 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>),  
38 despite important N<sub>2</sub>O losses which partly offset the benefit of SOC storage. ORG was the  
39 system with the smallest GHG balance (-65 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>), acting as a CO<sub>2</sub> sink in the  
40 long-term. Similar trends were observed when GHG was expressed per unit of N input or N  
41 exported. The N surplus alone was not a good indicator of the N fate in the four agricultural

42 systems. Complementary predictors of N losses and GHG balance are required to obtain a true  
43 overview of the C and N environmental impacts of cropping systems. On an operational point  
44 of view, these results should lead to investigate the variability of the GHG emissions within  
45 each cropping system.

## 46        **1. Introduction**

47        The objective of increasing crop yields to meet the worldwide increasing food demand has been  
48        putting pressure on the use of nitrogen (N) fertilizers for sixty years, the amount of synthetic N  
49        fertilizer applied being multiplied by 7 between 1960 and 1995 (Tilman et al., 2002). However,  
50        the N use efficiency (NUE), *i.e.* the ratio between crop N export and N inputs, is often less than  
51        50% (Tilman et al., 2002). The unrecovered N in the crop can be stored in the soil or released  
52        in the environment throughout the “nitrogen cascade” (Galloway et al., 2003) as dinitrogen  
53        (N<sub>2</sub>), nitrogen oxides (NO<sub>x</sub>), nitrous oxide (N<sub>2</sub>O), ammonia (NH<sub>3</sub>) and nitrate (NO<sub>3</sub>). These N  
54        losses cause environmental damages, with eutrophication of rivers, algal blooms in estuaries or  
55        slimes under forest (Sutton et al., 2011) and for human health (Habermeyer et al., 2015; WHO,  
56        2013). The other important environmental challenge of sustainable agriculture is to improve the  
57        greenhouse gas (GHG) balance, with two aspects: reduce the CO<sub>2</sub> emissions or increase the  
58        soil organic C (SOC) stocks and decrease the emissions of N<sub>2</sub>O, with regard to its high global  
59        warming potential (296 times greater than CO<sub>2</sub> over a 100-year time span).

60        The awareness of this situation led to the implementation of regulations or initiatives such as  
61        the Nitrate Directive in Europe (91/676/CEE), which aims at reducing N losses from agriculture  
62        to the groundwater, or the “4 per 1000” initiative launched by the COP21 in 2015  
63        (<http://4p1000.org>) in order to increase SOC stocks by 4‰ every year. To meet these objectives,  
64        alternative farming practices have been promoted, such as the reduction of mineral N  
65        fertilization, the establishment of catch crops, the cultivation of legume crops as organic N  
66        fertilizer, the introduction of perennial crops in arable systems, the reduction of tillage. An ideal  
67        cropping system combining these practices would result in high N exportations, high NUE, low  
68        N surplus (Eurostat, 2016) and high C and N storage in soil.

69        Most studies focused on evaluating separately the environmental impacts of each cropping  
70        practice with regard to N losses. For example, straw incorporation, catch crops and reduced N

71 fertilization practices were shown to reduce N leaching (e.g. Beaudoin *et al.*, 2005; Constantin  
72 *et al.*, 2010; Hansen *et al.*, 2015), while gaseous N emissions can be decreased by reduced  
73 fertilization or increased by no-till (Constantin *et al.*, 2010). These improved practices are often  
74 clustered in alternative farming systems, such as low-input farming, organic farming or  
75 conservation agriculture. These systems are generally assumed to be more environmental-  
76 friendly than conventional farming systems, because of their smaller nutrient inputs, improved  
77 biodiversity and/or lower soil disturbance. However, the combination of various practices may  
78 complicate the effects of alternative cropping systems on the N flows, and the biochemical  
79 processes involved in N fate still need to be clarified. While the positive effect of long-term  
80 reduced fertilization on N leaching is commonly accepted, the effect of organic farming and  
81 conservation agriculture is still a matter of controversy. Conservation agriculture is  
82 characterized by no-tillage and permanent living mulch growing under the main crop (Soane *et*  
83 *al.*, 2012) and its impact on N flows is unclear. For example, it may either result in a reduced  
84 N leaching by immobilization of N in the topsoil layer during crop residues decomposition or  
85 increase it because of preferential water flows occurring under no-till (Kay *et al.*, 2009). Palm  
86 *et al.* (2014) report inconsistent results concerning N<sub>2</sub>O emissions which could be increased  
87 with a higher moisture at soil surface in CA or decreased in the long-term. In organic farming  
88 systems, N input to soil is mainly ensured by organic fertilizers and crop residues derived from  
89 N<sub>2</sub> fixing legumes, but this N needs to be mineralized before becoming available (Thorup-  
90 Kristensen and Dresbøll, 2010). The N inputs in stockless organic systems mainly derive from  
91 the second source and are usually lower than in the corresponding conventional systems. A  
92 greater N sequestration seems to occur with these inputs compared to mineral N inputs (Kramer  
93 *et al.*, 2002). Gaseous N emissions from organic cropping systems are expected to be smaller  
94 since soil mineral N is scarcer than in highly fertilized conventional systems (Skinner *et al.*,  
95 2014).

96 At the field scale, the N surplus of a cropping system is calculated as the difference between N  
97 inputs and N exported by crop harvests. N surplus is often considered as an indicator of the  
98 negative impact of agricultural practices on the environment (OECD, 2001). N surplus must be  
99 calculated over long enough period, in order to characterize properly the long-term effect of a  
100 cropping system on the N fate in the soil-plant system.

101 Few studies have simultaneously estimated the components of the N balance (N surplus, N  
102 leaching, SON storage, gaseous N emissions), the SOC storage and the GHG balance of  
103 alternative cropping systems in the long-term. This was the general objective of this paper,  
104 which analyses the long-term experiment of “La Cage”, comparing conventional (CON), low  
105 input (LI), conservation agriculture (CA) and organic (ORG) cropping systems. Autret *et al.*  
106 (2016) previously determined the changes in soil organic carbon (SOC) stocks in these four  
107 cropping systems. In this paper, we i) compare changes in SON stocks between systems from  
108 1998 to 2014; ii) quantify the N losses through leaching and N<sub>2</sub>O emission, iii) determine the  
109 N surplus and iv) calculate the GHG balance of each cropping system on the long-term (1998-  
110 2017). The SON stocks measured in 1998 and 2014 were used to estimate the SON  
111 sequestration rates. N leaching was calculated using measurements of soil mineral N and  
112 LIXIM model (Mary et al., 1999). The direct N<sub>2</sub>O emissions were continuously measured  
113 during 40 months (2014-2017). The N surplus was calculated using records of N inputs and  
114 exportations. With regard to the previous observations concerning SOC changes, we  
115 hypothesized that the GHG balance could vary widely among the four cropping systems.

## 116 **2. Material and methods**

### 117 **2.1 Experimental site**

118 The study was conducted at the experimental site of “La Cage” at Versailles, France (48°48’  
119 N, 2°08’ E) as described by Autret *et al.* (2016). The experiment was established in 1998 in

120 order to assess the agronomic, economic and environmental performances of three alternative  
121 systems compared to a prevalent conventional cropping system of Northern France. Before the  
122 experimental establishment, the site was evenly maintained under a conventional management.  
123 The climate is oceanic temperate with mean annual temperature of 11.3°C and mean annual  
124 precipitation of 673 mm. The soil is an artificially drained deep Luvisol (IUSS Working Group  
125 WRB, 2006). Its clay content measured in 1998 is 167 g kg<sup>-1</sup> over the whole field and the pH  
126 in water is 7.38 (Appendix A).

## 127 **2.2 Cropping systems and management**

128 Four cropping systems are compared: a conventional (CON), low input (LI), conservation  
129 agriculture (CA) and organic farming (ORG) system. They differ in soil tillage, crop succession  
130 and protection, fertilization and crop residues management. Soil was ploughed every year in  
131 CON and ORG, and only one out of two years in LI. The CA system, consisting in direct seeding  
132 with a permanent plant cover, is conducted in no-till since 1998. The crop rotation of CON and  
133 LI was the following: rapeseed (*Brassica napus* L.), winter wheat (*Triticum aestivum* L.), spring  
134 pea (*Pisum sativum* L.) and winter wheat. It slightly differed in CA where maize (*Zea mays* L.)  
135 was grown two years instead of rapeseed. The main difference occurred in the ORG system in  
136 which alfalfa (*Medicago sativa* L.) was introduced thereafter to replace pea and rapeseed. All  
137 crop residues were left on the soil surface at harvest. Some of the alfalfa cuts were exported  
138 while the others were returned to soil: the proportion of cuts return was 50% and 67% in CA  
139 and ORG systems respectively. Catch crops were grown only in the CA system, being  
140 composed of oat (*Avena sativa* L.), vetch (*Vicia sativa* L.), white mustard (*Sinapis alba* L.) or  
141 fodder radish (*Raphanus sativus* L.). A permanent cover crop, composed of festuca (*Festuca*  
142 *rubra* L.) in the first years and alfalfa thereafter, was maintained under the main crop in CA. It  
143 was chemically destroyed at least once every 4 years, before seeding pea crops. Pesticides were  
144 used in the CON, LI and CA systems for weed, pest and disease control; their application rate



145 was lower in LI and CA compared to CON system. The ORG system did not receive any  
146 pesticide and received authorized P and K fertilizers, according to the European specifications  
147 for organic farming. Phosphate fertilizer was applied at a mean rate of 14, 14, 9 and 3 kg P ha<sup>-1</sup>  
148 yr<sup>-1</sup> and potassium at a rate of 26, 26, 18 and 6 kg K ha<sup>-1</sup> yr<sup>-1</sup> in CON, LI, CA and ORG  
149 systems respectively. The rate was proportional to the crop yield. Mineral N fertilizers were  
150 applied to non-legume crops of CON, LI and CA systems. The N fertilizer rate of each crop  
151 was calculated in mid-winter according to a balance-sheet method and split in 2 or 3  
152 applications. The N fertilization rate was highest in CON system and was reduced on average  
153 by 22% in LI and 29% in CA systems. In the ORG system, organic N fertilizers (feather meal  
154 and guano) were applied solely on wheat during the first years and in 1999 and 2005 for  
155 rapeseed, at a low rate (6% of the CON system). No other manure or organic fertilizer was  
156 applied.

## 157 **2.3 Measurements**

158 The timeline of N-related measurements made at La Cage between 1998 and 2017 is  
159 summarized in Figure 1.

### 160 **2.3.1 Crop yields and N uptake**

161 Grain yield was determined based on the quantity of grain collected by the combine harvester  
162 and average values of dry matter content of grain. Alfalfa yield was calculated as the sum of  
163 the biomass content of exported cuts which was measured directly, and the aboveground  
164 biomass returned to soil (not exported) which was estimated using regional references (Autret  
165 *et al.*, 2016). When not available, N content in harvested grains and aboveground biomass was  
166 assumed to be equal to national reference values (Appendix B.1). They were used to estimate  
167 N export by main crops (Table 1). Crop residues, cover crops and catch crops were returned to  
168 the soil and therefore not accounted for in the N outputs.

169 **2.3.2 Soil water and mineral N contents**

170 Soil mineral N (SMN) and soil water content (SWC) were measured three times per year: after  
171 harvest (average date July 30), in autumn (average November 2) and winter (average February  
172 11) between November 2012 and February 2017. Three soil samples were collected in each  
173 subplot from soil surface down to 90 cm depth, corresponding to the maximum rooting depth.  
174 Soil cores were divided into three layers of 30 cm and frozen until mineral N extraction. The  
175 gravimetric water content was determined after 48h drying at 105°C. Mineral N was extracted  
176 from 100 g of soil shaken in 200 mL of a potassium chloride solution (1M). Nitrate (NO<sub>3</sub>) and  
177 ammonium (NH<sub>4</sub>) concentrations of the solution were measured by continuous-flow  
178 colorimetry (Khan et al., 2007). Bulk densities used to calculate SMN stocks were taken from  
179 previous measurements made in 2014 (Autret et al., 2016).

180 **2.3.3 SOC and SON stocks**

181 The SOC and SON concentrations and bulk densities were measured at different dates between  
182 1998 and 2014, in order to calculate SON stocks on equivalent soil mass (ESM) basis (Ellert  
183 and Bettany, 1995) over a depth at least equal to the deepest tillage event. Twenty samples were  
184 taken in each subplot down to 30 cm depth in 1998, whereas six samples were picked up in  
185 each subplot at 60 cm depth in 2014 with a hydraulic gauge of 6 cm diameter (Autret *et al.*,  
186 2016). Soil samples were coarsely crushed, oven dried for 48h at 35°C and sieved (2 mm). Soil  
187 subsamples were finely ground in a ball mill (PM 400, Retsch, Germany) before measurement  
188 of the total N content by Dumas (dry combustion) with an elemental analyser (EURO EA,  
189 Eurovector, Italy). Bulk density was measured in 1998 for the layers 0-10, 10-20 and 20-30 cm  
190 and in 2014 on the top soil layer 0-5 cm, using a steel cylinder of 98 cm<sup>3</sup>. Soil was weighed  
191 after drying during 48 h at 105 °C. A gamma-densitometer (LPC-INRA, Angers, France) was  
192 used in 2014 to measure the bulk density every 5 cm in the layers from 5 to 40 cm depth.

### 2.3.4 *N<sub>2</sub>O emissions*

193  
194 Direct N<sub>2</sub>O emissions were monitored continuously between April 2014 and July 2017, using  
195 automatic chambers as described by Bessou *et al.* (2010) and Peyrard *et al.* (2016, 2017). On  
196 April 8, 2014, twelve automatic, large size chambers (0.70 m x 0.70 m) were installed in four  
197 plots of the block 1, representing the four cropping systems with three replicates. Three  
198 chambers were installed at a mean distance of 10 m each other in each plot of 0.56 ha. They  
199 were maintained in the plot throughout the growing season and removed only for the main  
200 operations (sowing, mechanical weeding, harvest and disk ploughing), receiving the same  
201 management than the rest of the plot. Chambers were connected to two infra-red gas analysers,  
202 one for CO<sub>2</sub> (LiCor 820, LiCor Biosciences, USA) and the other for N<sub>2</sub>O (Thermo 46c, Thermo  
203 Fisher Scientific, USA) installed in an enclosure left *in situ*. The automatic chambers were  
204 closed sequentially four times a day during 20 minutes (starting at 0, 6, 12 and 18 hours GMT).  
205 The CO<sub>2</sub> and N<sub>2</sub>O concentrations were measured every 10 seconds during the closure.  
206 Additional corrections (besides those provided by the manufacturer) were made to account for  
207 the (small) interferences between N<sub>2</sub>O and CO<sub>2</sub> concentrations. N<sub>2</sub>O concentrations were  
208 converted into fluxes by fitting the concentration kinetics to a linear or an exponential model.  
209 The smaller measurable flux using this method is lower than 1 g N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>. Cumulative  
210 N<sub>2</sub>O emissions were obtained by summing up the fluxes measured during 20 minutes every 6  
211 hours.

## 2.4 Calculations

### 2.4.1 *Biological N fixation*

214 The biological nitrogen fixation (BNF) of legume crops was estimated, either for the main  
215 crops, cover crops undersown in the main crop, or catch crops preceding the main crop. The  
216 total amount of fixed N was calculated as the sum of the contributions of each category of  
217 grown legumes. BNF was calculated using the empirical relation established by Anglade *et al.*

218 (2015) between the amount of N derived from atmosphere ( $Ndfa$ ) and the N yield of the legume  
219 crop:

$$220 \quad Ndfa = \alpha \cdot Ny + \beta \quad (1)$$

221 where  $\alpha$  and  $\beta$  are crop specific parameters (Appendix B.2) and  $Ny$  is the N yield, defined as  
222 the total N accumulated in the aboveground biomass ( $\text{kg N ha}^{-1}$ ), calculated as follows:

$$223 \quad Ny = Y \cdot Nc / NHI \quad (2)$$

224 where  $Y$  is the harvested crop yield ( $\text{t DM ha}^{-1}$ ),  $Nc$  is the nitrogen content of the harvested  
225 material ( $\text{g kg}^{-1}$ ), and  $NHI$  is the nitrogen harvest index (ratio of N in the harvested material to  
226 N in total aboveground biomass).  $Ny$  was determined using the following variables: i) the  
227 measured grain yield for pulses (fababean, lupin, pea and soybean), ii) the estimates of  
228 aboveground biomass for the other legumes (alfalfa, vetch and clover), iii) an average value of  
229 measured N content for pea, and iv) standard values of N content for the other species  
230 (CORPEN, 1988; Parr *et al.*, 2011; Anglade *et al.*, 2015). The  $Ndfa$  was finally corrected by a  
231 multiplicative factor (BGN-F) accounting for belowground contributions which varied between  
232 species, in order to estimate the total BNF (Anglade *et al.*, 2015).

### 233 **2.4.2 N surplus**

234 The N surplus ( $N_{sur}$ ) relative to the soil-plant system was calculated for each of the four cropping  
235 systems according to OECD (2001) by subtracting the total N exportation from the total N  
236 inputs. A positive N surplus indicates that N losses occur in the environment and/or that SON  
237 stock increases, whereas a negative surplus means a soil impoverishment. The annual surplus  
238 ( $\text{kg ha}^{-1} \text{ yr}^{-1}$ ) was calculated as:

$$239 \quad N_{sur} = N_{fert} + N_{fix} + N_{atm} - N_{exp} \quad (3)$$

240 where  $N_{fert}$  is the annual N fertilization,  $N_{fix}$  the N derived from symbiotic fixation,  $N_{atm}$  the  
241 atmospheric N deposition and  $N_{exp}$  the N exported from the field by harvests, all values  
242 expressed in  $\text{kg ha}^{-1} \text{ yr}^{-1}$ . The values of  $N_{fert}$  and  $N_{exp}$  were recorded each year whereas  $N_{fix}$  was

243 calculated as indicated previously.  $N_{am}$  was estimated based on the European Monitoring and  
244 Evaluation Program (<http://www.emep.int/>), providing an annual deposition of 12.9 kg ha<sup>-1</sup> yr<sup>-1</sup>  
245 <sup>1</sup> at the regional scale in France over the 2000-2015 period.

### 246 **2.4.3 N leaching**

247 Water drainage, net N mineralization and N leaching were calculated using both measurements  
248 of SWC and SMN contents and the LIXIM model (Mary et al., 1999). LIXIM simulates  
249 simultaneous N mineralization and water and nitrate transfer between soil layers at a daily time-  
250 step, fitting simulated water and mineral N stocks to observed values. We used the adaptation  
251 made by Beaudoin *et al.* (2005) to account for the concomitant crop N uptake which can occur  
252 when soil is not completely bare fallow during winter. Results showed that leaching predictions  
253 were only slightly impacted by crop N uptake.

254 Input data for LIXIM model were the mean daily temperature and precipitation, potential  
255 evapotranspiration, soil bulk density, soil water contents at permanent wilting point and field  
256 capacity of each layer, SWC and SMN contents in autumn and winter. When soil was covered  
257 by a crop (catch crop, cover crop, main crop or rapeseed regrowth), the date of seeding, depth  
258 of rooting, root growth rate, base temperature and N absorption rate were also provided. The  
259 depth of rooting, root growth rate and base temperature of each crop were set to values given  
260 in Appendix C. The actual to potential evapotranspiration ratio (AET / PET) was set at 0.5 for  
261 a bare soil and 0.6 or fitted by the model when a crop was present in winter. The net N  
262 mineralization rate was optimized, as well as the nitrogen absorption rate, by fitting the  
263 simulated values of water and SMN contents to the observed values.

264 Drainage and leaching were estimated differently according to the time period. First, they were  
265 calculated for the five drainage seasons (2012 to 2017) during which water and mineral N  
266 contents were measured both in autumn and winter. During this period, the amounts of drained  
267 water ( $D$ , mm yr<sup>-1</sup>) and N leached ( $L$ , kg N ha<sup>-1</sup> yr<sup>-1</sup>) were estimated using SWC and SMN

268 measurements together with LIXIM model. This calculation was not possible during the other  
269 period (1998-2011), because SWC and SMN contents were measured only in winter. Therefore,  
270 drainage was calculated using a simulator based on evapotranspiration, precipitation and soil  
271 water capacity (Leenhardt, 1991) and leaching was determined using a statistical relationship  
272 between N leached, drained water and SMN measured in February, as follows:

$$273 \quad L = D^2(a_1S_1 + a_2S_2 + a_3S_3) \quad (4)$$

274 where  $S_1$ ,  $S_2$  and  $S_3$  are the amounts of soil nitrate-N measured in mid-February in layers 0-30,  
275 30-60 and 60-90 cm, respectively. This relationship, established with the data of the second  
276 period (2012-2017), explained satisfactorily the leaching simulated by LIXIM:  $r^2 = 0.85$ ,  $n=20$ .  
277 The fitted value of coefficients  $a_1$ ,  $a_2$  and  $a_3$  was 0.98, -0.89 and 0.74 respectively.

#### 278 **2.4.4 Gaseous N losses**

279 Based on N surplus, N leaching and SON storage estimated between 1998 and 2016, we  
280 calculated the total gaseous N losses ( $G$ , in  $\text{kg ha}^{-1} \text{ yr}^{-1}$ ) with the following equation (Mary *et*  
281 *al.*, 2002; Constantin *et al.*, 2010):

$$282 \quad G = N_{sur} - (N_{stored} + L) \quad (5)$$

283 where  $N_{stored}$  is the SON sequestration rate which was measured between 1998 and 2014. This  
284 equation assumes that the variation in soil mineral N between the beginning and end of the  
285 period is negligible, which is true.  $G$  includes all N emissions through denitrification ( $\text{N}_2 + \text{N}_2\text{O}$ ),  
286 nitrification ( $\text{N}_2\text{O} + \text{NO}_x$ ) and volatilization ( $\text{NH}_3$ ), without detail of their respective  
287 proportions.

#### 288 **2.4.5 GHG balance**

289 The total emissions of greenhouse gases at La Cage experiment result from total equivalent  
290  $\text{CO}_2$  losses, deriving from the soil and crop management of each cropping. We calculated the  
291 annual GHG balance ( $\text{GHG}_b$ , in  $\text{kg CO}_2\text{eq ha}^{-1} \text{ yr}^{-1}$ ) as follows:

$$GHG_b = F + M + 296 \cdot \frac{44}{28} (direct\ N_2Oe + indirect\ N_2Oe) - \frac{44}{12} SOC_{storage} \quad (6)$$

292 where  $F$  is the amount of CO<sub>2</sub> emitted during the fertilizer synthesis (in kg CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>),  $M$   
 293 the amount of CO<sub>2</sub> emitted during the plant and soil management (in kg CO<sub>2</sub> ha<sup>-1</sup> yr<sup>-1</sup>), *direct*  
 294 *N<sub>2</sub>Oe* the amount of N<sub>2</sub>O emitted by the soil (kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>), *indirect N<sub>2</sub>Oe* the amount of  
 295 N<sub>2</sub>O emitted throughout the N cascade (kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>) and  $SOC_{storage}$  the amount of carbon  
 296 yearly stored in the soil (kg C ha<sup>-1</sup> yr<sup>-1</sup>).  $F$  was calculated as the product of the amount of  
 297 fertilizer applied per hectare and the corresponding emission factors which were 6.17 kg CO<sub>2</sub>eq  
 298 kg<sup>-1</sup> of N for ammonium nitrate, 1.30 kg CO<sub>2</sub>eq kg<sup>-1</sup> of phosphate (P) and 0.54 kg CO<sub>2</sub>eq kg<sup>-1</sup>  
 299 of potassium (K) for binary P-K fertilizers (Gac et al., 2010).  $M$  was obtained by multiplying  
 300 the amount of fuel consumed per hectare for soil and crop management (Appendix D) by the  
 301 emission factor of 0.81 kg CO<sub>2</sub>eq per liter of fuel consumed. The direct N<sub>2</sub>Oe emissions were  
 302 measured for the 2014-2017 period. They were used to calculate the impact factors relative to  
 303 mineral fertilizers and organic N residues for each system during three years. These factors  
 304 were then used to estimate the *direct N<sub>2</sub>O* emissions from 1998 to 2013. The *indirect N<sub>2</sub>Oe*  
 305 emissions were estimated with the emission factors defined in the IPCC Guidelines for National  
 306 GHG Inventories (De Klein et al., 2006), namely 1% of the N volatilized derived from synthetic  
 307 fertilizer being transformed into N<sub>2</sub>O and 0.75% of the leached N being transformed into N<sub>2</sub>O  
 308 all along the N cascade in groundwater, rivers and estuaries. Finally, the  $SOC_{storage}$  was taken  
 309 from by Autret *et al.* (2016) who measured it during the 1998-2014 period, with 78, 22, 625  
 310 and 277 kg C ha<sup>-1</sup> yr<sup>-1</sup> for CON, LI, CA and ORG respectively.

## 312 **2.5 Statistical analysis**

313 Statistical analyses were performed using the R software (R Core Team, 2017). The weakness  
 314 of the experiment was its design with only two randomized blocks, previously underlined by  
 315 Henneron *et al.* (2014), Pelosi *et al.* (2015) and Autret *et al.* (2016). These authors considered

316 each of the two sided subplots of a given cropping system as replicate, thus producing four  
317 pseudo replicates. Their choice was supported by different arguments and particularly the fact  
318 that the intra-plot variability (between subplots) was as important as the inter-plot variability  
319 (within blocks), as indicated by the comparison of variances ( $F=1.83$ ,  $p<0.05$ ) made by Autret  
320 *et al.* (2016). In the present study, we made a more rigorous analysis as recommended by  
321 Webster and Lark (2018). We did not consider 16 randomized plots (4 treatments x 2 blocks x  
322 2 subplots) such as the previous authors but 8 randomized plots (4 treatments x 2 blocks) with  
323 2 replicates within plots (in the sided subplots), applying a “nested” analysis of variance. This  
324 ANOVA was performed to test the effect of cropping system on 1) SON stocks in 1998 and  
325 2014 and their variation; 2) N inputs, N exportations and N surplus from 1998 to 2016; 3) N  
326 leaching between 1998 and 2017; 4) N<sub>2</sub>O emissions between April 2014 and July 2017 and 5)  
327 GHG balance. The assumptions of ANOVA were checked by using the Shapiro-Wilk and  
328 Levene’s tests. The existence of significant effects ( $p<0.05$ ) was followed by a post-hoc  
329 comparison test of means with the CLD from the *emmeans* package (Lenth, 2018).

### 330 **3. Results**

#### 331 **3.1 N inputs and export by main and cover crops**

332 The mean input of fertilizer-N over the 19 years experiment was 199, 144 and 166 kg N ha<sup>-1</sup> yr<sup>-1</sup>  
333 <sup>1</sup> for wheat crops and 189, 169 and 98 kg N ha<sup>-1</sup> yr<sup>-1</sup> for rapeseed in the CON, LI and CA  
334 cropping systems, respectively (Table 1). It was much lower in the ORG system, with an  
335 average of 12 kg N ha<sup>-1</sup> yr<sup>-1</sup> (wheat) and 44 kg N ha<sup>-1</sup> yr<sup>-1</sup> (rapeseed), both applied as organic  
336 fertilizer. The N input derived from symbiotic fixation, related to the legume crop N yield,  
337 varied between species. It was estimated at 166, 168, 191 and 105 kg N ha<sup>-1</sup> yr<sup>-1</sup> for pea crop in  
338 the CON, LI, CA and ORG systems, respectively. Alfalfa, whether grown as a main crop in CA  
339 and ORG or as a cover crop mixed with a cereal in CA, provided the highest N inputs, ranging



340 from 154 to 350 kg N ha<sup>-1</sup> yr<sup>-1</sup>. BNF represented 20%, 23%, 56% and 84% of total N inputs for  
341 CON, LI, CA and ORG systems, respectively. After 2008, organic N fertilizers were no longer  
342 applied to the ORG system, which then relied exclusively on legume crops to inject reactive N.  
343 The part of BNF in total N inputs also increased with time in the CA system, due to the more  
344 frequent use of legumes as cover crop and green manure, from 30% until 2008 to an average of  
345 77% since 2009.

346 N outputs (export) are directly linked to the grain yields and aerial biomass of alfalfa. Average  
347 wheat yields varied between cropping systems, respectively 8.1, 7.4, 6.2 and 4.6 t DM ha<sup>-1</sup> in  
348 CON, LI, CA and ORG. They were correlated to the N fertilizer rate ( $r=0.51$ ,  $n=152$ ,  $p<0.001$ ).  
349 The yields of spring pea and winter rapeseed were also lower in the ORG than in the other  
350 cropping systems. The mean N export through wheat grains was 149, 137, 123 and 76 kg N ha<sup>-1</sup>  
351 yr<sup>-1</sup> in CON, LI, CA and ORG respectively. It represented 49 to 59% of the total N export.

### 352 **3.2 N surplus**

353 The average N surplus calculated between 1998 and 2016 was clearly positive for all systems  
354 (Table 2). It varied from 43 to 163 kg N ha<sup>-1</sup> yr<sup>-1</sup> and ranked as follows: LI < CON = ORG <  
355 CA. The smaller N surplus observed in LI compared to CON results from lower N inputs (169  
356 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and a similar symbiotic N fixation. The N surplus of the ORG system did not  
357 differ from the CON system, despite its very low fertilizer input but the presence of alfalfa  
358 generated high N input (101 kg N ha<sup>-1</sup> yr<sup>-1</sup>), three times greater than in the CON system. The  
359 very high N surplus observed in CA results from the important amounts of symbiotic N fixation,  
360 especially from alfalfa which accounts for 107 kg N ha<sup>-1</sup> yr<sup>-1</sup>, corresponding to 65% of the total  
361 BNF inputs. The N surplus was positive in all cropping systems, indicating that N had been  
362 stored in the soil and/or lost through leaching and gaseous emissions in all systems.

363 **3.3 SON storage**

364 The SON stocks calculated at ESM in the 0-30 cm layer (old ploughed layer) in 1998 and 2014  
365 are presented in Table 3. Small differences, but not significant, were found between treatments  
366 in SON stocks in 1998 ( $p < 0.05$ ), the average SON value being 4.25 t N ha<sup>-1</sup>. In 2014, SON  
367 stocks were much higher in CA than in the three other systems, which did not differ  
368 significantly each other. SON stocks in the layer 30-60 cm did not differ between systems  
369 (results not shown), indicating that most SON variations occurred in the upper layer. The  
370 change in SON stocks during the 16 years varied between 0.10 and 0.89 t N ha<sup>-1</sup>. It was  
371 significantly different from 0 in the CA system but not in the other systems. The average rates  
372 of N sequestration were 13, 6, 55 and 30 kg N ha<sup>-1</sup> yr<sup>-1</sup> in CON, LI, CA and ORG respectively.

373 **3.4 SMN stocks, drainage and N leaching**

374 The measured SMN stocks and the calculated drainage, N leaching and NO<sub>3</sub> concentrations in  
375 drained water are presented in Table 4. The SMN stocks in autumn, measured from 2012 to  
376 2017, varied little between systems: they were on average 62, 58, 43 and 58 kg N ha<sup>-1</sup> in the  
377 CON, LI, CA and ORG systems respectively. The SMN stocks found in winter during the same  
378 years were lower, respectively 29, 33, 35 and 42 kg N ha<sup>-1</sup>. The winter SMN during the rest of  
379 experiment (1998-2011) were slightly higher but with the same ranking between systems.  
380 Although no significant difference was found, the ORG system which received no mineral N  
381 fertilizer tended to have the highest SMN stocks in February, varying between 18 and 86 kg N  
382 ha<sup>-1</sup>.

383 The calculated drainage did not vary significantly between cropping systems but varied  
384 markedly between years, from 11 to 334 mm yr<sup>-1</sup>. The average drainage over the whole period  
385 (1998-2017) was 145 mm yr<sup>-1</sup>. Similarly, the amounts of N leached did not differ significantly  
386 between systems but widely among years. The mean amounts of leached N over the whole

387 period were 18, 22, 24 and 20 kg N ha<sup>-1</sup> yr<sup>-1</sup> for CON, LI, CA and ORG respectively. The nitrate  
388 concentration in drained water did not differ significantly between systems with an average  
389 value of 56 (period 2012-2017) and 65 mg NO<sub>3</sub> L<sup>-1</sup> (whole period), *i.e.* slightly greater than the  
390 maximum content of 50 mg NO<sub>3</sub> L<sup>-1</sup> set by the Nitrate Directive (91/676/CEE). The  
391 concentrations calculated during the last five years differed more between systems (37 mg NO<sub>3</sub>  
392 L<sup>-1</sup> in CA vs 63 mg NO<sub>3</sub> L<sup>-1</sup> in CON), suggesting that the improvement in the management of  
393 the non-conventional systems might reduce nitrate leaching losses in the near future.

### 394 **3.5 N surplus and gaseous N losses**

395 The greatest source of uncertainty in the calculation of the N surplus lies in the BNF input from  
396 alfalfa in the CA and ORG systems, with two components: 1) the estimate of aboveground  
397 biomass and 2) the belowground N derived from BNF. We conducted a sensitivity analysis to  
398 determine the change in the N surplus in response to a variation in aboveground production of  
399 alfalfa and in the BGN-F factor (ratio of total to aboveground N fixed) (Table 5). Biomass  
400 production varied between 90 and 110% of the nominal value (range determined using the  
401 measurements made in the last years) and three values of the BGN factor were tested: 1.4, 1.7  
402 and 2.1 corresponding to its minimum, average and maximum value reported by Anglade *et al.*  
403 (2015). The N surplus thus calculated varied widely, from 134 to 196 kg N ha<sup>-1</sup> yr<sup>-1</sup> in CA and  
404 43 to 95 kg N ha<sup>-1</sup> yr<sup>-1</sup> in ORG system. The average values were close to those previously  
405 calculated, 161 ± 20 kg N ha<sup>-1</sup> yr<sup>-1</sup> for CA and 65 ± 17 kg N ha<sup>-1</sup> yr<sup>-1</sup> for ORG. It is noticeable  
406 that the difference between the two cropping systems was much more stable, 95 ± 3 kg N ha<sup>-1</sup>  
407 yr<sup>-1</sup>. This indicates that despite the rather large uncertainty in the estimates of N surplus, the  
408 CA system is characterised by a much higher surplus than the ORG system.

409 The unrecovered N, *i.e.* the difference between the N surplus and the sum of N stored in soil  
410 and N leached, corresponds to the gaseous N losses (denitrification + volatilization). Figure 2  
411 displays the N surplus and its partitioning into its three components over the 1998-2017 period.

412 N surplus was mainly correlated with gaseous losses ( $r=0.97$ ,  $p<0.001$ ), moderately with SON  
413 storage ( $r=0.93$ ,  $p<0.01$ ) and not correlated with N leaching ( $r=0.60$ , ns). The gaseous losses  
414 differed widely among cropping systems: they were much greater in the CA ( $83 \pm 22$  kg N ha<sup>-1</sup>  
415 yr<sup>-1</sup>) than in the CON system ( $32 \pm 11$  kg N ha<sup>-1</sup> yr<sup>-1</sup>) and smallest in the LI ( $15 \pm 24$  kg N ha<sup>-1</sup>  
416 yr<sup>-1</sup>) and ORG system ( $12 \pm 14$  kg N ha<sup>-1</sup> yr<sup>-1</sup>). Similar differences were obtained when  
417 calculations were made only during the period 2014-2017 (results not shown).

### 418 **3.6 N<sub>2</sub>O emissions**

419 Emissions varied widely between systems and throughout time (Table 6). Over the whole  
420 monitoring period (April 2014 – July 2017) the N<sub>2</sub>O emissions were highest in the CA system  
421 ( $11.96$  kg N<sub>2</sub>O-N ha<sup>-1</sup>), intermediate in the CON system ( $6.85$  kg N<sub>2</sub>O-N ha<sup>-1</sup>) and lowest in the  
422 LI and ORG system ( $3.51$  and  $4.39$  kg N<sub>2</sub>O-N ha<sup>-1</sup>). Emissions occurred mainly but not  
423 exclusively after fertiliser application, in response to rainfall events, indicating that  
424 denitrification was the main source of N<sub>2</sub>O production. The ORG system produced very small  
425 emissions except during the last period (November 2016 – July 2017) under wheat established  
426 after the destruction of alfalfa. Conversely, the three other systems had small emission rates  
427 during this period; this is likely due to a low denitrification itself linked with a low rainfall  
428 during this period.

429 The N<sub>2</sub>O emission factor relative to the total N inputs (mineral + organic N) during the whole  
430 monitoring period was 1.19%, 0.92%, 1.49% and 1.42% for the CON, LI, CA and ORG  
431 systems, respectively. These values are close to IPCC references. In addition, N<sub>2</sub>O emissions  
432 were highly correlated with total gaseous losses ( $r=0.97$ ,  $p<0.001$ ). This result suggests that a  
433 large part of the gaseous losses originated from denitrification.

### 434 **3.7 Global GHG balance**

435 The GHG balance calculated for each cropping system is presented in Table 7. The upper part  
436 of the table shows the GHG balance during the 2014-2017 period, during which N<sub>2</sub>O fluxes  
437 were continuously measured, whereas the lower part concerns the 19-yr period (1998-2017).  
438 Over the 19 yr-period, emissions deriving from fertilizer synthesis were high in CON (927 kg  
439 CO<sub>2</sub>eq ha<sup>-1</sup> yr<sup>-1</sup>) and low in ORG (44 kg CO<sub>2</sub>eq ha<sup>-1</sup> yr<sup>-1</sup>) which received small amounts of  
440 organic fertilizers in the early years of the trial. The CO<sub>2</sub> emissions related to agricultural  
441 operations varied less between systems, from 161 kg CO<sub>2</sub>eq ha<sup>-1</sup> yr<sup>-1</sup> in CA to 272 kg CO<sub>2</sub>eq  
442 ha<sup>-1</sup> yr<sup>-1</sup> in CON. The differences were mainly due to the absence of soil tillage in CA, ploughing  
443 being particularly fuel consuming. The indirect N<sub>2</sub>O emissions occurring during the N cascade  
444 contributed very little (average 118 kg CO<sub>2</sub>eq ha<sup>-1</sup> yr<sup>-1</sup>) to the GHG balance. The direct N<sub>2</sub>O  
445 emissions and the direct CO<sub>2</sub> emissions (estimated by the SOC variation) were the main sources  
446 of variability. Taking into account the annual SOC sequestration, the net GHG balance was  
447 estimated for each cropping system on the 1998-2017 period and ranked as follows: CON  
448 (+2198 kg CO<sub>2</sub>eq ha<sup>-1</sup> yr<sup>-1</sup>) > LI (+1763) > CA (+306) > ORG (-65). A similar ranking and  
449 amplitude of variation was obtained for the period 2014-2017, showing the robustness of the  
450 calculations. The ranking was conserved when the GHG balance was expressed per unit of N  
451 input or unit of exported N. Intensive mineral N fertilization and mechanization, associated  
452 with a poor SOC sequestration rate lead to high GHG emissions in the CON, as well as in LI  
453 even with reduced farming intensity. The CA system had a much better GHG balance due to its  
454 very high SOC storage rate. However, its high N<sub>2</sub>O emissions offset this beneficial effect,  
455 resulting in a slightly positive GHG balance. Finally, the ORG system was the only cropping  
456 system leading to a negative GHG balance.

## 457 **4. Discussion**

458 We have studied the long-term impacts of alternative cropping systems on the N fate, including  
459 N uptake by crops, SON storage, N leaching and gaseous N emissions, and the GHG balance,  
460 including direct and indirect emissions. The results are summarized in Figure 3: each variable  
461 of each cropping system was scored between 0 (adverse impact) and 1 (beneficial impact),  
462 compared to two reference values (low and high) of each variable, given in the legend.

### 463 **3.8 N use efficiency**

464 The NUE of each cropping system can be compared to the reference value of 0.66 obtained on  
465 average for France during the 2000-2014 period (Eurostat, 2016). A nominal comparison of the  
466 NUE of cropping systems requires precaution because NUE is linked to the N fertilizer rate  
467 (Mary *et al.*, 2002). Thus, the NUE variability within each system would deserve to be  
468 investigated. However, both the N input rate and origin is structurally determined by the nature  
469 of each cropping system. During the 19 years of the experiment, LI system yielded the highest  
470 NUE, 0.84, CON and ORG obtained respectively 0.69 and 0.55, and the lowest was found in  
471 CA system, 0.39. This latter value was explained by the high organic N inputs derived from  
472 legume cover crop residues which were preferentially stored in the soil rather than used for  
473 plant uptake. Given that straw was systematically returned to soil during the experiment in all  
474 cropping systems, the differences in NUE refer mainly to the efficiency of conversion of the  
475 supplied N, either by inorganic fertilizers or through BNF, into crop uptake and grain N.  
476 Aronsson *et al.* (2007) reported similar NUE values in Sweden: 0.54 for an organic system  
477 relying on green manure, 0.68 for a conventional system, but higher efficiency in an organic  
478 system with animal manure (0.70). In Italy, higher NUE were found for an organic system  
479 (average 0.74 over 16 years) than for a conventional (average 0.51) (Migliorini *et al.*, 2014).

480 Lin *et al.* (2016) also reported higher NUE for organic systems in a 20-yr experiment in  
481 Germany.

### 482 **3.9 N surplus, an ambiguous indicator**

483 The N surplus is often presented as an indicator of the N losses in arable fields (OECD, 2001),  
484 useful to compare management practices at annual time step. However, a positive N surplus  
485 may also reflect a SON storage. In our experiment, the N surplus was three times higher in CA  
486 than in the other systems (157 vs 50 kg N ha<sup>-1</sup> yr<sup>-1</sup>), in spite of a smaller addition of mineral N  
487 fertilizer compared to the CON system. The important amounts of symbiotic fixed N combined  
488 with smaller N exportations are responsible of the very high N surplus in CA. The introduction  
489 of legume cover crops in conservation agriculture is used to provide N for the subsequent crop  
490 after crop residues mineralization and allow to reduce mineral N fertilization (Scopel *et al.*,  
491 2012). Blesh and Drinkwater (2013) made contrasted observations in fields from the  
492 Mississippi River Basin, where legumes and complex crop rotations including annual and  
493 perennial species were grown. They found smaller surpluses in these cropping systems  
494 compared to mineral N based systems (<10 and 35 kg N ha<sup>-1</sup> yr<sup>-1</sup> respectively). The difference  
495 between their results and ours can be explained by the large proportion of alfalfa cuts returned  
496 to soil in La Cage experiment: 90% in the CA system and 75% in ORG. The N surplus in the  
497 LI and CON systems is equal or slightly greater than the mean value (47 kg N ha<sup>-1</sup> yr<sup>-1</sup>) reported  
498 by Poisvert *et al.* (2017) in the same region over the period 2000-2010. The ORG system is  
499 characterized by an unusual high N surplus (63 kg N ha<sup>-1</sup> yr<sup>-1</sup>) compared to other published  
500 references. This results from the long duration of legumes in the rotation (42%), particularly  
501 alfalfa (29%) whose cuts were often returned to soil. The asynchrony between the crop N uptake  
502 and the release of mineral N from decomposing residues, described by Crews and Peoples  
503 (2005), may have limited N uptake and increased the N surplus.

504           **3.10 N and C storage**

505   The SON measurements realized in 1998 and 2014 indicated that N storage occurred in all  
506   treatments but was significant only in the CA system which had a mean rate of sequestration of  
507   55 kg N ha<sup>-1</sup> yr<sup>-1</sup>. This result is consistent with Autret *et al.* (2016) who found significant SOC  
508   sequestration in the CA system. The authors attributed the SOC increase to important crop  
509   residues and root inputs, deriving from supplementary catch crops and cover crops. SOC  
510   sequestration rates can be compared to the yearly increase of 4 ‰ targeted by the “4 per 1000”  
511   initiative to mitigate CO<sub>2</sub> emissions. They were 1.8, 0.5, 14.1 and 7.0 ‰ yr<sup>-1</sup> for CON, LI, CA  
512   and ORG systems respectively (Autret *et al.*, 2016). SON sequestration rates were close: 3.2,  
513   1.3, 12.5 and 7.6 ‰ yr<sup>-1</sup> respectively; the conservation and organic systems thus demonstrated  
514   a high potential of C and N storage in soil over almost a two-decade period. If the important  
515   storage could be expected in the CA system (González-Sánchez *et al.*, 2012), the storage in the  
516   ORG system is more surprising. The positive impact of organic systems on SOC/SON storage  
517   claimed by some studies (Gattinger *et al.*, 2012; Lin *et al.*, 2016) is debated (Leifeld *et al.*, 2013)  
518   and/or attributed to higher application of organic fertilizer in organic farming experiments  
519   (Leifeld and Fuhrer, 2010). In our experiment, very low amounts of organic fertilizer were  
520   applied in the ORG system. We hypothesize that the storage in ORG (and also CA) mainly  
521   result from the important amount of legume residues, particularly alfalfa, which provided both  
522   C and N substrate needed for C and N sequestration in soil: large C inputs were reported by  
523   Autret *et al.* (2016) and large N surpluses were shown in this study. This hypothesis is consistent  
524   with Van Groenigen *et al.* (2017) who pointed out the importance of N required for SOC  
525   sequestration.



### 526 3.11 N leaching

527 The N leaching mitigation (Figure 3) was assessed by comparing the NO<sub>3</sub> concentrations in  
528 drained water to a reference threshold of 50 mg L<sup>-1</sup> defined by the Nitrate Directive. The average  
529 leaching loss over the 1998-2017 period was 16 kg N ha<sup>-1</sup> yr<sup>-1</sup>, without any difference between  
530 cropping systems. This value was lower than the average N leaching reported by Benoit *et al.*  
531 (2014) in the same region for conventional cropping systems (32-77 kg N ha<sup>-1</sup> yr<sup>-1</sup>) but closer  
532 to those reported for organic farms (13-37 kg N ha<sup>-1</sup> yr<sup>-1</sup>). N leaching was not related with N  
533 surplus. The correlation between N leaching and N surplus, reported by Billen *et al.* (2013) for  
534 conventional systems, does no longer apply when comparing complex cropping systems  
535 involving diversified cropping practices and/or rotation. Other studies also confirmed this poor  
536 or absence of correlation in arable cropping systems (Sieling and Kage, 2006; Pugesgaard *et*  
537 *al.*, 2017).

538 A moderate reduction in mineral fertilizer N has been shown to have small effects on N leaching  
539 if the reference system is not over-fertilized (*e.g.* Constantin *et al.*, 2010). This was the case of  
540 LI compared to CON. In the case of CA system, opposite effects occurred: the presence of  
541 permanent cover crop favoured catching of mineral N whereas alfalfa destruction and  
542 decomposition during autumn and winter increased SMN. The absence of tillage in this system  
543 did not seem to reduce N leaching, in agreement with previous results (Oorts *et al.*, 2007;  
544 Hansen *et al.*, 2015; Daryanto *et al.*, 2017). The similar N leaching observed in the ORG system  
545 is more surprising with regard to the literature. Organic cropping systems generally lead to a  
546 decrease of N leaching because of a lower level N fertilizer applied, as highlighted by Tuomisto  
547 *et al.* (2012) in their meta-analysis. Syswerda *et al.* (2012) also found smaller nitrate losses in  
548 an organic system using legume catch crops compared to a mineral N fertilizer-based  
549 conventional system; the average nitrate concentration in drained water over 11 years was  
550 decreased to 40 instead of 84 mg NO<sub>3</sub> L<sup>-1</sup>, respectively. In our study, the favourable effect of

551 low fertilizer rate was probably offset by the risky phase of alfalfa destruction. Several authors  
552 have mentioned that the poor synchrony between mineral N availability derived from alfalfa  
553 residues and the subsequent crop uptake could increase N leaching (Crews and Peoples, 2005;  
554 Aronsson *et al.*, 2007).

### 555 **3.12 Gaseous N emissions**

556 The N<sub>2</sub>O emissions monitored continuously during 40 months are assumed to be representative  
557 of the four systems, at least during the last years. The emission factor (ratio of cumulative N<sub>2</sub>O-  
558 N emissions to total N inputs) calculated for this period vary from 0.7% (LI) to 1.5% (CA), *i.e.*  
559 close to the 1% value of IPCC. The CA system was characterized by high rates of N<sub>2</sub>O  
560 emissions after fertilization events, much higher than those observed in the LI system, although  
561 both systems received almost the same amount of fertilizer-N. Such difference may result from  
562 the absence of tillage or the presence of the living mulch or both effects. The influence of no  
563 tillage on N<sub>2</sub>O emissions is not clear yet, since opposite results have been reported. In their  
564 meta-analysis, van Kessel *et al.* (2013) did not point out differences between tilled or no-tilled  
565 systems during the first ten years, and found lower emissions in no-till systems after 10 years  
566 under dry climates. Therefore, tillage does not seem to be the main reason for the difference  
567 between systems at La Cage. The mulch formed by dead crop residues falling on the top soil  
568 layer together with the more humid micro-climate created by the living cover crop may have  
569 stimulated the emissions in the CA system. Shan and Yan (2013) have shown in their meta-  
570 analysis that the presence of mulch of plant residues stimulates N<sub>2</sub>O emissions compared to  
571 their incorporation in soil. Crop residues, left as a mulch on the top soil layer, have been shown  
572 to increase soil moisture content of soil surface and exacerbate the N<sub>2</sub>O emissions under annual  
573 or perennial crops (Peyrard *et al.*, 2017, 2016). Moreover, residues deriving from alfalfa  
574 probably increased N<sub>2</sub>O emissions in the CA system, since legumes residues are known to  
575 induce higher N losses than non-legume (Basche *et al.*, 2014).

576 The total gaseous N emissions, assessed by the N mass balance, correspond to the sum of NH<sub>3</sub>  
577 volatilization, production of N<sub>2</sub> and N<sub>2</sub>O by denitrification and NO<sub>x</sub> and N<sub>2</sub>O by nitrification,  
578 all fluxes being stimulated by mineral N fertilization (De Klein et al., 2006). In our study these  
579 losses were not correlated with the fertilizer rate, but with the N surplus ( $r = 0.97$ ,  $p < 0.001$ ).  
580 They were also well correlated with N<sub>2</sub>O emissions ( $r = 0.98$ ,  $p < 0.001$ ), which suggests that  
581 denitrification was the major source of emission to the atmosphere. The CA system exhibited  
582 the highest N losses (average 79 kg N ha<sup>-1</sup> yr<sup>-1</sup>) and ORG the smallest ones (10 kg N ha<sup>-1</sup> yr<sup>-1</sup>).  
583 If we assume that most gaseous losses are due to denitrification, then the N<sub>2</sub>O/(N<sub>2</sub>+N<sub>2</sub>O) molar  
584 ratio would vary from 5% (CA) to 15% (ORG), *i.e.* in the lower range of reported values (*e.g.*  
585 Wang *et al.*, 2011).

### 586 **3.13 The GHG balance, an ultimate environmental indicator**

587 Our GHG balance accounts for the main sources of CO<sub>2</sub> emissions, including mineral fertilizer  
588 synthesis, fuel combustion due to crop and soil management, direct and indirect N<sub>2</sub>O emissions  
589 from soil, groundwater, rivers and estuaries and net CO<sub>2</sub> emissions from soil (assessed using  
590 SOC change rate). The average GHG balance was 2198 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup> in the conventional  
591 system and 20% smaller in the LI system. Due to its very high C sequestration rate, the CA  
592 system had a much more favourable balance, emitting only 306 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup> to the  
593 atmosphere. The best situation was found in the ORG system which was a sink for the  
594 atmosphere (-65 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>). The relative differences between systems are maintained  
595 when the GHG is expressed per unit of N input or N exported instead of area unit.

596 Very few studies have quantified the GHG balance of such arable alternative cropping systems  
597 without manure application. Most studies focused on gross GHG emissions, without  
598 considering SOC storage and/or N<sub>2</sub>O emissions. Six *et al.* (2004) estimated a negative GHG  
599 balance in no-till systems compared to conventional tillage, but they mentioned the large  
600 uncertainty of their estimation, related to the variability of N<sub>2</sub>O emissions. Mary *et al.* (2014)

601 also compared tilled versus no-tilled systems in a long-term experiment at Boigneville (France)  
602 in which SOC stocks had been monitored for 41 years and N<sub>2</sub>O emissions for three years. They  
603 found high values of the GHG balance both for no-till (3350 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>) and ploughed  
604 systems (4030 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>). Aguilera *et al.* (2015) estimated the carbon footprint of  
605 rainfed crops under conventional and organic management in 8 Spanish farms. They estimated  
606 higher net GHG emissions from conventional than organic systems when expressed per unit of  
607 area (1024 vs 361 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>) and also per unit of production (315 vs 182 g CO<sub>2eq</sub> kg<sup>-1</sup>).  
608 The authors attributed the low carbon footprint of organic management to reduced CO<sub>2</sub>  
609 emissions deriving from synthetic fertilizers use and lower direct N<sub>2</sub>O emissions since SOC  
610 sequestration rates were small and similar in conventional and organic systems.

611 The GHG balance can be presented as an ultimate indicator giving a wider evaluation of  
612 cropping systems, in the context of the global climate change mitigation with alternative  
613 cropping managements. In our study, the ranking of GHG balance among cropping systems  
614 differed completely from the ranking of N surplus. The N<sub>2</sub>O losses accounted for a small share  
615 (5-15%) of the total N losses in the four cropping systems, whereas they represented on average  
616 49% of total GHG emissions. In the CA system, N<sub>2</sub>O emissions offset 66% of the SOC  
617 sequestration, even though SOC change rate reached the top range achievable in arable cropping  
618 systems. The importance of assessing N<sub>2</sub>O fluxes on the global warming potential was  
619 previously pointed out by Six *et al.* (2004). Using a simulation model, Li *et al.* (2005) predicted  
620 that C sequestration often goes along with increased N<sub>2</sub>O emissions in alternative cropping  
621 systems. Our results confirm the idea that no-till management, which is often considered as the  
622 main attribute of conservation agriculture, should not be seen as an ultimate solution to mitigate  
623 global warming (VandenBygaart, 2016). Hence, the need for a complete evaluation of the GHG  
624 balance in designing alternative cropping systems is highly critical, yet being an arduous task  
625 (Skinner *et al.*, 2014).

## 626        **5. Conclusion**

627    The N surplus, N fate and the GHG balance were compared in the conventional and three  
628    alternative arable cropping systems over 19 years in La Cage experiment (Northern France).  
629    Our purpose was not to conclude about the performance of cropping systems in a nominal  
630    approach but to understand how their characteristics could impact their N balance and GHG  
631    emissions. The four systems had contrasted impacts on C and N cycles: they had similar effects  
632    on nitrate leaching, but very different ability to sequester C and N in soil, and contrasted gaseous  
633    N emissions, including denitrification and N<sub>2</sub>O losses. Their GHG mitigation potential was not  
634    reflected by their N surplus. The alternative systems all improved the GHG balance, slightly  
635    for the low input system, markedly for the conservation agriculture system systems and even  
636    more in the organic cropping system, which led to a negative GHG balance. In conservation  
637    agriculture, the high N<sub>2</sub>O emissions partially offset the very high carbon sequestration rate.  
638    Agricultural policies targeting a single environmental objective, such as the “4 per 1000”  
639    initiative (Minasny et al., 2017), are welcome but must be considered cautiously since they may  
640    potentially overestimate the CO<sub>2</sub> sequestration potential (*e.g.* White *et al.*, 2017). Our results  
641    clearly demonstrate that the full GHG balance has to be considered when comparing the  
642    potential of new management practices. Hence, an appropriate assessment of the environmental  
643    impact of a cropping system should be based on a global evaluation, considering both C and N  
644    fluxes modified by the farming practices and not be limited to a patchy indicator. Our study  
645    confirms the interest of long-term monitoring to accurately evaluate the impact of alternative  
646    systems. Moreover, there is a need to investigate other CA or ORG systems varying in the  
647    nature and importance of legume residue returns, which constitute the main alternative to  
648    mineral N fertilizer for injecting reactive nitrogen into soils. This investigation can be done  
649    using experiments, modelling or both.

## 650 **Acknowledgements**

651 This study was funded by the French Ministry of Agriculture, the Water Agency of Seine-  
652 Normandie Basin, INRA Versailles within the ENBIO project and the PIREN-Seine. “La Cage”  
653 experiment is coordinated by INRA Versailles (France). We gratefully acknowledge P. Saulas,  
654 D. Le Floch and C. Montagnier for managing the experiment, J.P. Pétraud, F. Mahu and E.  
655 Venet for their technical assistance in soil samplings, C. Dominiarczyk and A. Teixeira for  
656 processing samples and O. Delfosse for nitrogen analyses.

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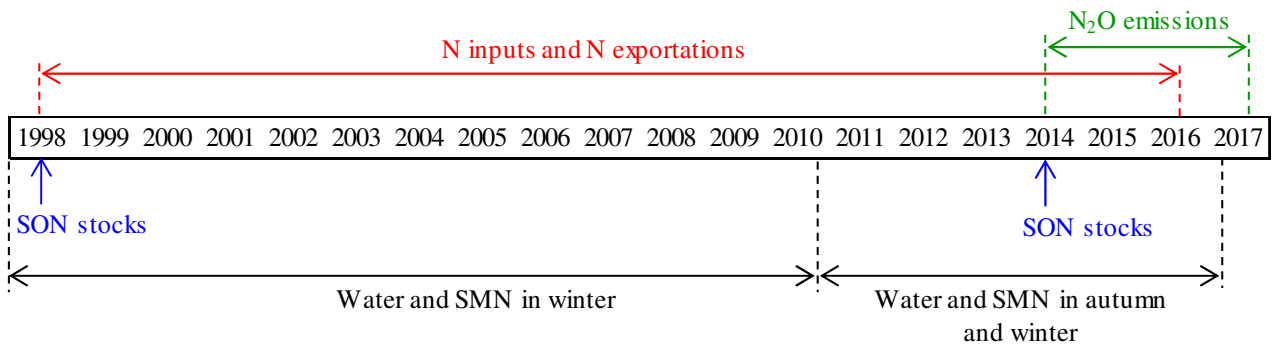
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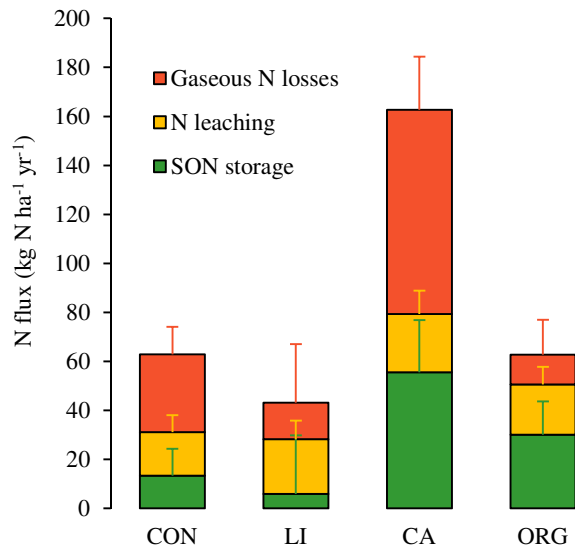
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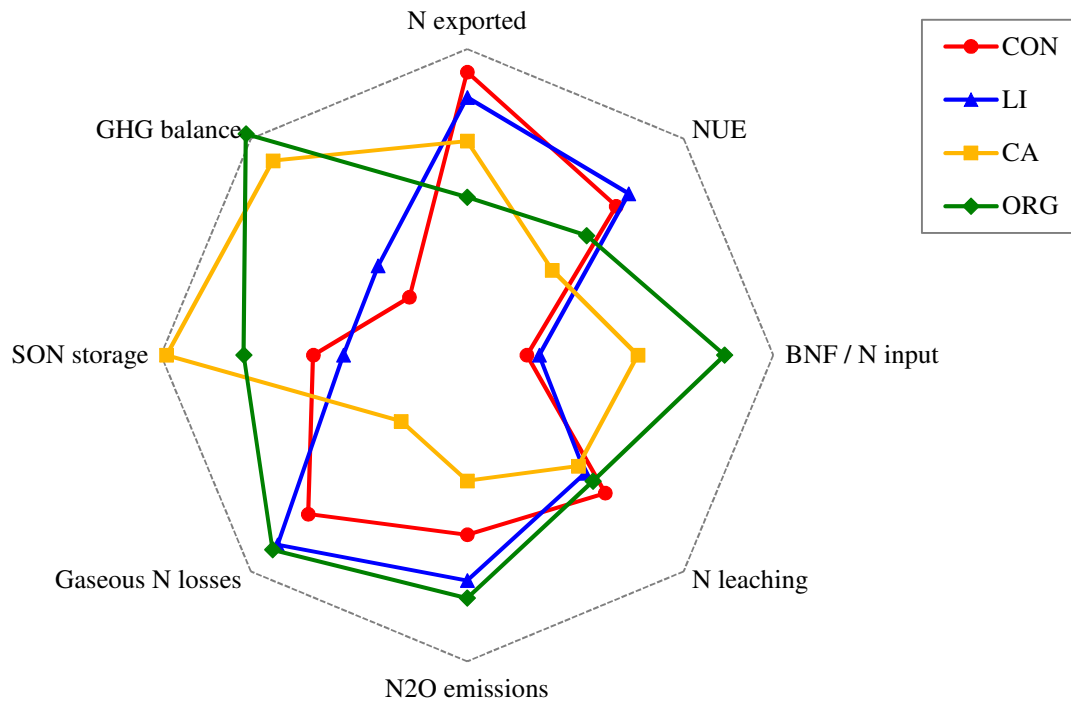
**Figure 1.**

Sequences of N measurements made between 1998 and 2017, used in this paper. SON = soil organic nitrogen; SMN = soil mineral nitrogen



**Figure 2.**

Partitioning of the N surplus between SON storage, N leaching and gaseous N emissions (see eq. 5). Gaseous N losses were calculated as the difference between the N surplus and the sum of SON storage and N leaching. Error bars represent the standard deviations.



**Figure 3.**

Diagram summarizing the environmental C and N impacts of the four cropping systems. Each dimension was scored between 0 (adverse impact, center of the radar) and 1 (beneficial impact, periphery). These scores correspond to the following values (respectively): N exported: 0 and 150 kg N ha<sup>-1</sup>; NUE: 0 and 1; BNF/N input: 0 and 1; N leaching: 150 and 0 mg NO<sub>3</sub> l<sup>-1</sup>; N<sub>2</sub>O emissions: 6 and 0 kg N ha<sup>-1</sup> yr<sup>-1</sup>; gaseous N losses: 120 and 0 kg N ha<sup>-1</sup> yr<sup>-1</sup>; SON storage: -8 and +13 % yr<sup>-1</sup>; GHG balance: 0 and 3000 kg CO<sub>2</sub>eq ha<sup>-1</sup> yr<sup>-1</sup>

**Table 1.**

Crop frequency, mean N inputs from fertilizers and BNF (biological N fixation), mean N offtake by crops and mean grain yield per crop and cropping system during the whole period (1998-2016). Values in brackets are standard deviations between years.

cropping system	main crop	cover crop	crop frequency	N fertilizer	BNF	N offtake	Grain yield	
				kg N ha <sup>-1</sup> yr <sup>-1</sup>			t DM ha <sup>-1</sup>	
CON	wheat		50%	199 (25)		149 (27)	8.1	(1.5)
	rapeseed		26%	189 (31)		123 (24)	3.9	(0.8)
	pea		24%		166 (58)	135 (53)	3.7	(1.3)
LI	wheat		50%	144 (27)		137 (21)	7.4	(1.2)
	rapeseed		26%	169 (15)		99 (26)	3.1	(0.8)
	pea		24%		168 (62)	134 (55)	3.7	(1.4)
CA	wheat	fescue	26%	154 (19)		116 (12)	6.0	(0.9)
	wheat	alfalfa	21%	181 (36)	204 (44)	128 (26)	6.3	(1.5)
	wheat	clover	3%	166	112	149 (0)	7.3	
	rapeseed	alfalfa	5%	98 (4)	154 (7)	91 (57)	2.9	(1.8)
	pea		16%	8	191 (79)	121 (52)	3.3	(1.4)
	oat	alfalfa	3%		237	42 (0)	2.6	
	maize	clover	11%	154 (36)	82 (99)	74 (30)	5.2	(2.1)
	alfalfa		16%		317 (25)	67 (5)	9.8 *	(0.7)
ORG	wheat		50%	12 (23)		76 (27)	4.6	(1.6)
	rapeseed		8%	44 (40)		20 (18)	0.6	(0.6)
	pea		5%		105 (27)	94 (26)	2.6	(0.7)
	barley-pea		3%		112	67 (0)	2.9	
	lupin		3%		190	142 (0)	3.2	
	soyabean		3%		117	84 (0)	1.5	
	alfalfa		29%		350 (30)	87 (37)	10.7 *	(0.9)

\* total aerial biomass produced for alfalfa as a main crop

**Table 2.**

Mean annual values of N input, N exported and N surplus (period 1998-2016). Total N input is the sum of N fertilization, BNF and atmospheric N deposition. Values in brackets are standard deviations between replicates. Different letters indicate significant differences between cropping systems ( $p < 0.05$ ).

Cropping system	N fertilization	BNF	atmospheric deposition	total N input	total N exported	N surplus
	kg N ha <sup>-1</sup> yr <sup>-1</sup>					
CON	149 (9) a	39 (13) b	13	202 (5) b	139 (10) a	63 (6) b
LI	117 (8) b	40 (17) b	13	169 (10) c	126 (11) a	43 (3) c
CA	106 (4) b	149 (9) a	13	268 (7) a	105 (7) b	163 (11) a
ORG	9 (1) c	118 (6) a	13	140 (7) d	77 (6) c	63 (2) b

**Table 3.**

SON stocks at equivalent soil mass in 1998 and 2014 and SON storage rate between 1998 and 2014. Values in brackets are standard deviations. Small letters indicate significant differences between cropping systems, capital letters indicate differences between years ( $p < 0.05$ ).

Cropping system	SON stock 1998		SON stock 2014			SON storage rate (1998-2014)	
	t N ha <sup>-1</sup>		t N ha <sup>-1</sup>			kg N ha <sup>-1</sup> yr <sup>-1</sup>	
CON	4.13	(0.33) <i>a</i>	4.34	(0.31) <i>b</i>	<i>A</i>	13	(11) <i>b</i>
LI	4.47	(0.74) <i>a</i>	4.57	(0.43) <i>b</i>	<i>A</i>	6	(24) <i>b</i>
CA	4.44	(0.64) <i>a</i>	5.33	(0.58) <i>a</i>	<i>B</i>	55	(21) <i>a</i>
ORG	3.95	(0.42) <i>a</i>	4.44	(0.27) <i>b</i>	<i>A</i>	30	(14) <i>ab</i>



**Table 4.**

Mean annual drainage, SMN stocks in autumn and winter, N leached and NO<sub>3</sub> concentration in drained water for each cropping system. NO<sub>3</sub> concentrations are weighted by water drainage. N leached and NO<sub>3</sub> concentrations were estimated with LIXIM model between 2013 and 2017 and calculated during the remaining years using eq. 4 and 5.

Drainage season	Drained water mm yr <sup>-1</sup>	CON				LI				CA				ORG			
		SMN autumn	SMN winter	N leached	[NO <sub>3</sub> <sup>-</sup> ]	SMN autumn	SMN winter	N leached	[NO <sub>3</sub> <sup>-</sup> ]	SMN autumn	SMN winter	N leached	[NO <sub>3</sub> <sup>-</sup> ]	SMN autumn	SMN winter	N leached	[NO <sub>3</sub> <sup>-</sup> ]
		kg ha <sup>-1</sup>	kg ha <sup>-1</sup> yr <sup>-1</sup>	mg L <sup>-1</sup>		kg ha <sup>-1</sup>	kg ha <sup>-1</sup> yr <sup>-1</sup>	mg L <sup>-1</sup>		kg ha <sup>-1</sup>	kg ha <sup>-1</sup> yr <sup>-1</sup>	mg L <sup>-1</sup>		kg ha <sup>-1</sup>	kg ha <sup>-1</sup> yr <sup>-1</sup>	mg L <sup>-1</sup>	
1998-1999	269		9	23	37		21	44	25		24	67	35		18	15	28
1999-2000	286		15	60	93		18	40	29		14	27	23		21	62	27
2000-2001	334		8	39	52		14	64	19		44	142	61		22	68	30
2001-2002	191		32	28	66		46	35	64		20	14	28		41	8	48
2004-2005	99		49	7	31		47	8	64		25	6	35		86	0	94
2005-2006	37		49	0	5		80	0	120		32	1	49		25	1	37
2006-2007	171		12	20	53		42	50	59		44	45	64		63	62	67
2007-2008	60		19	2	16		16	1	16		18	2	25		22	2	24
2008-2009	11		49	0	10		36	0	49		11	0	17		55	0	71
2009-2010	56		25	3	24		29	2	39		17	2	24		33	5	37
2011-2012	150		8	8	24		19	14	10		42	20	61		21	23	29
1998-2012	152		25	17	51		33	23	69		27	30	87		37	22	65
2012-2013	183	113	30	58	140	92	38	56	136	40	28	17	40	63	29	37	89
2013-2014	192	35	19	18	42	45	27	21	48	42	38	24	55	55	43	28	65
2014-2015	139	58	21	10	31	65	29	11	35	38	17	8	26	38	38	7	22
2015-2016	110	29	3	6	23	27	8	6	25	25	9	5	20	58	27	11	45
2016-2017	26	74	70	1	11	60	61	5	78	70	81	1	19	73	71	1	16
2012-2017	130	62	29	18	63	58	33	20	67	43	35	11	37	58	42	17	57
1998-2017	145		26	18	54		33	22	68		29	24	73		38	20	63

**Table 5.**

Sensitivity analysis of N surplus ( $\text{kg N ha}^{-1} \text{ yr}^{-1}$ ) to the aboveground biomass and BGN factor of alfalfa for the conservation agriculture (CA) and organic (ORG) cropping systems. Values in brackets are standard deviations. The N surplus used in the study is shown in bold.

Cropping system	BGN factor	Relative variation of AG biomass				
		90%	100%	110%	mean	sd
CA	2.1	176	190	204		
	1.7	152	<b>163</b>	173	167	(21)
	1.4	139	148	157		
ORG	2.1	76	86	97		
	1.7	55	<b>63</b>	71	67	(17)
	1.4	44	51	58		
Difference	2.1	100	103	107		
CA-ORG	1.7	97	100	103	100	(4)
	1.4	94	97	99		

**Table 6.**

Cumulative N<sub>2</sub>O-N fluxes (kg N ha<sup>-1</sup>) measured continuously during 6 periods (4 crops and 40 months). Values in brackets are standard deviations. Letters indicate significant differences between cropping systems at each period (Student test,  $p < 0.05$ ).

Measurement period	Duration days	Crop	Cropping system				
			CON	LI	CA	ORG	
08/04/2014	20/07/2014	104	wheat	1.50 (0.55) b	0.93 (0.19) b	2.98 (0.90) a	0.22 (0.07) c
02/09/2014	08/07/2015	310	rapeseed *	1.44 (0.14) b	1.30 (0.15) b	2.51 (0.39) a	0.51 (0.04) c
10/07/2015	19/10/2015	102	fallow *	0.17 (0.05) a	0.17 (0.10) a	0.09 (0.05) a	0.23 (0.02) a
10/11/2015	26/07/2016	260	wheat	2.31 (0.54) b	0.86 (0.16) c	5.21 (1.39) a	0.54 (0.13) d
31/08/2016	19/10/2016	50	fallow *	0.38 (0.08) b	0.15 (0.03) c	0.55 (0.09) a	0.57 (0.18) a
16/11/2016	16/07/2017	243	wheat	1.04 (0.10) b	0.11 (0.02) d	0.62 (0.14) c	2.32 (0.56) a
08/04/2014	16/07/2017	1069	all	6.85 (1.48) b	3.51 (0.88) c	11.96 (3.29) a	4.39 (0.95) c

\* *alfalfa for the ORG system*

**Table 7.**

GHG balance and components at La Cage estimated during the 2014-2017 period (with N<sub>2</sub>O monitoring) and the whole experimental period (1998-2017).

Period	Cropping system	<i>F</i>	<i>M</i>	<i>direct</i> N <sub>2</sub> O <sub>e</sub>	<i>indirect</i> N <sub>2</sub> O <sub>e</sub>	SOC storage rate	GHG balance		
							kg CO <sub>2eq</sub> ha <sup>-1</sup> yr <sup>-1</sup>	kg CO <sub>2eq</sub> kg <sup>-1</sup> N input	kg CO <sub>2eq</sub> kg <sup>-1</sup> N exported
2014-2017	CON	861 <i>a</i>	281 <i>a</i>	1088 <i>b</i>	94	286 **	2039 <i>a</i>	10.1 <i>a</i>	14.7 <i>a</i>
	LI	644 <i>b</i>	251 <i>a</i>	558 <i>c</i>	85	81 **	1457 <i>b</i>	8.6 <i>b</i>	11.5 <i>b</i>
	CA	606 <i>b</i>	131 <i>b</i>	1900 <i>a</i>	79	2292 **	425 <i>c</i>	1.6 <i>c</i>	4.0 <i>c</i>
	ORG	0 <i>c</i>	243 <i>a</i>	698 <i>c</i>	41	1016 **	-33 <i>d</i>	-0.2 <i>d</i>	-0.4 <i>d</i>
1998-2017	CON	927 <i>a</i>	272 <i>a</i>	1153 *	131	286 <i>a</i>	2198 <i>a</i>	10.9 <i>a</i>	15.8 <i>a</i>
	LI	728 <i>b</i>	249 <i>b</i>	736 *	132	81 <i>a</i>	1763 <i>b</i>	10.4 <i>b</i>	14.0 <i>b</i>
	CA	659 <i>b</i>	161 <i>c</i>	1645 *	132	2292 <i>c</i>	306 <i>c</i>	1.1 <i>c</i>	2.9 <i>c</i>
	ORG	44 <i>c</i>	255 <i>b</i>	576 *	76	1016 <i>b</i>	-65 <i>d</i>	-0.5 <i>d</i>	-0.8 <i>d</i>