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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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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 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 25 26 (163 kg ha<sup>-1</sup> yr<sup>-1</sup>). CA and ORG received high amounts of N derived from biological fixation from alfalfa. The annual SON storage rates markedly differed between CA (55 kg ha<sup>-1</sup> yr<sup>-1</sup>) and 27 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 28 leaching, calculated using soil mineral N measurements, reached an average of 21 kg ha<sup>-1</sup> yr<sup>-1</sup> 29 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, 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 32 monitored with automatic chambers during 40 months. They varied from 1.20 kg ha<sup>-1</sup> yr<sup>-1</sup> in LI 33 to 4.09 kg ha<sup>-1</sup> yr<sup>-1</sup> in CA system and were highly correlated with calculated gaseous N 34 35 emissions. The GHG balance, calculated using SOC and N<sub>2</sub>O measurements, varied widely 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> 36 respectively. In CA, the GHG balance was much more favourable (306 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>), 37 38 despite important N<sub>2</sub>O losses which partly offset the benefit of SOC storage. ORG was the 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 39 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.

### 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 CO2 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 to the groundwater, or the "4 per 1000" initiative launched by the COP21 in 2015 62 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.

Most studies focused on evaluating separately the environmental impacts of each cropping
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 fertilization or increased by no-till (Constantin et al., 2010). These improved practices are often 73 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).

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

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

order to assess the agronomic, economic and environmental performances of three alternative
systems compared to a prevalent conventional cropping system of Northern France. Before the
experimental establishment, the site was evenly maintained under a conventional management.
The climate is oceanic temperate with mean annual temperature of 11.3°C and mean annual
precipitation of 673 mm. The soil is an artificially drained deep Luvisol (IUSS Working Group
WRB, 2006). Its clay content measured in 1998 is 167 g kg<sup>-1</sup> over the whole field and the pH
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 used in the CON, LI and CA systems for weed, pest and disease control; their application rate 144

145 was lower in LI and CA compared to CON system. The ORG system did not receive any pesticide and received authorized P and K fertilizers, according to the European specifications 146 147 for organic farming. Phosphate fertilizer was applied at a mean rate of 14, 14, 9 and 3 kg P ha<sup>-</sup> <sup>1</sup> 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 148 149 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 is159 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.

#### 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 $N_2O$ emissions

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  $N_2$ 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.

212

#### 2.4 Calculations

213

### 2.4.1 Biological N fixation

The biological nitrogen fixation (BNF) of legume crops was estimated, either for the main crops, cover crops undersown in the main crop, or catch crops preceding the main crop. The total amount of fixed N was calculated as the sum of the contributions of each category of grown legumes. BNF was calculated using the empirical relation established by Anglade *et al.*  (2015) between the amount of N derived from atmosphere (*Ndfa*) and the N yield of the legumecrop:

(1)

220 
$$Ndfa = \alpha . Ny + \beta$$

where  $\alpha$  and  $\beta$  are crop specific parameters (Appendix B.2) and *Ny* is the N yield, defined as the total N accumulated in the aboveground biomass (kg N ha<sup>-1</sup>), calculated as follows:

$$223 Ny = Y. Nc/NHI (2)$$

224 where Y is the harvested crop yield (t DM  $ha^{-1}$ ), Nc is the nitrogen content of the harvested material (g kg<sup>-1</sup>), and NHI is the nitrogen harvest index (ratio of N in the harvested material to 225 226 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

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

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

where  $N_{fert}$  is the annual N fertilization,  $N_{fix}$  the N derived from symbiotic fixation,  $N_{atm}$  the atmospheric N deposition and  $N_{exp}$  the N exported from the field by harvests, all values expressed in kg ha<sup>-1</sup> yr<sup>-1</sup>. The values of  $N_{fert}$  and  $N_{exp}$  were recorded each year whereas  $N_{fix}$  was calculated as indicated previously.  $N_{atm}$  was estimated based on the European Monitoring and Evaluation Program (<u>http://www.emep.int/</u>), providing an annual deposition of 12.9 kg ha<sup>-1</sup> yr<sup>-</sup> 1 at the regional scale in France over the 2000-2015 period.

246

#### 2.4.3 N leaching

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

Drainage and leaching were estimated differently according to the time period. First, they were calculated for the five drainage seasons (2012 to 2017) during which water and mineral N contents were measured both in autumn and winter. During this period, the amounts of drained 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 measurements together with LIXIM model. This calculation was not possible during the other
period (1998-2011), because SWC and SMN contents were measured only in winter. Therefore,
drainage was calculated using a simulator based on evapotranspiration, precipitation and soil
water capacity (Leenhardt, 1991) and leaching was determined using a statistical relationship
between N leached, drained water and SMN measured in February, as follows:

273 
$$L = D^{2} \left( a_{1} S_{1} + a_{2} S_{2} + a_{3} S_{3} \right)$$
(4)

where  $S_1$ ,  $S_2$  and  $S_3$  are the amounts of soil nitrate-N measured in mid-February in layers 0-30, 30-60 and 60-90 cm, respectively. This relationship, established with the data of the second period (2012-2017), explained satisfactorily the leaching simulated by LIXIM:  $r^2 = 0.85$ , n=20. 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

Based on N surplus, N leaching and SON storage estimated between 1998 and 2016, we calculated the total gaseous N losses (G, in kg ha<sup>-1</sup> yr<sup>-1</sup>) with the following equation (Mary *et al.*, 2002; Constantin *et al.*, 2010):

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

where  $N_{stored}$  is the SON sequestration rate which was measured between 1998 and 2014. This equation assumes that the variation in soil mineral N between the beginning and end of the period is negligible, which is true. *G* includes all N emissions through denitrification (N<sub>2</sub>+N<sub>2</sub>O), nitrification (N<sub>2</sub>O+NOx) and volatilization (NH<sub>3</sub>), without detail of their respective proportions.

288

#### 2.4.5 GHG balance

The total emissions of greenhouse gases at La Cage experiment result from total equivalent CO<sub>2</sub> losses, deriving from the soil and crop management of each cropping. We calculated the annual GHG balance (*GHG*<sub>b</sub>, in kg CO<sub>2</sub>eq ha<sup>-1</sup> yr<sup>-1</sup>) as follows:

292 
$$GHG_b = F + M + 296.\frac{44}{28} (direct N_2 Oe + indirect N_2 Oe) - \frac{44}{12} SOC_{storage}$$
 (6)

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  $N_2Oe$  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 297 298 fertilizer applied per hectare and the corresponding emission factors which were 6.17 kg CO<sub>2</sub>eq 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> 299 300 of potassium (K) for binary P-K fertilizers (Gac et al., 2010). M was obtained by multiplying 301 the amount of fuel consumed per hectare for soil and crop management (Appendix D) by the 302 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 303 measured for the 2014-2017 period. They were used to calculate the impact factors relative to 304 mineral fertilizers and organic N residues for each system during three years. These factors 305 were then used to estimate the *direct*  $N_2O$  emissions from 1998 to 2013. The *indirect*  $N_2Oe$ 306 emissions were estimated with the emission factors defined in the IPCC Guidelines for National 307 GHG Inventories (De Klein et al., 2006), namely 1% of the N volatilized derived from synthetic 308 fertilizer being transformed into N<sub>2</sub>O and 0.75% of the leached N being transformed into N<sub>2</sub>O 309 all along the N cascade in groundwater, rivers and estuaries. Finally, the SOCstorage was taken 310 from by Autret et al. (2016) who measured it during the 1998-2014 period, with 78, 22, 625 311 and 277 kg C ha<sup>-1</sup> yr<sup>-1</sup> for CON, LI, CA and ORG respectively.

312

#### 2.5 Statistical analysis

Statistical analyses were performed using the R software (R Core Team, 2017). The weakness
of the experiment was its design with only two randomized blocks, previously underlined by
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>-</sup> <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 333 334 cropping systems, respectively (Table 1). It was much lower in the ORG system, with an 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 335 336 fertilizer. The N input derived from symbiotic fixation, related to the legume crop N vield, 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 337 338 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 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 CON, LI, CA and ORG systems, respectively. After 2008, organic N fertilizers were no longer applied to the ORG system, which then relied exclusively on legume crops to inject reactive N. The part of BNF in total N inputs also increased with time in the CA system, due to the more frequent use of legumes as cover crop and green manure, from 30% until 2008 to an average of 77% since 2009.

N outputs (export) are directly linked to the grain yields and aerial biomass of alfalfa. Average wheat yields varied between cropping systems, respectively 8.1, 7.4, 6.2 and 4.6 t DM ha<sup>-1</sup> in CON, LI, CA and ORG. They were correlated to the N fertilizer rate (r=0.51, n=152, p<0.001). The yields of spring pea and winter rapeseed were also lower in the ORG than in the other cropping systems. The mean N export through wheat grains was 149, 137, 123 and 76 kg N ha<sup>-1</sup>  $^{1}$  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 (Table 2). It varied from 43 to 163 kg N ha<sup>-1</sup> yr<sup>-1</sup> and ranked as follows: LI  $\leq$  CON = ORG  $\leq$ 354 355 CA. The smaller N surplus observed in LI compared to CON results from lower N inputs (169 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 356 357 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, especially from alfalfa which accounts for 107 kg N ha<sup>-1</sup> yr<sup>-1</sup>, corresponding to 65% of the total 360 361 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.

#### **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 change in SON stocks during the 16 years varied between 0.10 and 0.89 t N ha<sup>-1</sup>. It was 370 371 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 years were lower, respectively 29, 33, 35 and 42 kg N ha<sup>-1</sup>. The winter SMN during the rest of 378 379 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>.

The calculated drainage did not vary significantly between cropping systems but varied markedly between years, from 11 to 334 mm yr<sup>-1</sup>. The average drainage over the whole period (1998-2017) was 145 mm yr<sup>-1</sup>. Similarly, the amounts of N leached did not differ significantly between systems but widely among years. The mean amounts of leached N over the whole 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 concentration in drained water did not differ significantly between systems with an average 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 maximum content of 50 mg NO<sub>3</sub> L<sup>-1</sup> set by the Nitrate Directive (91/676/CEE). The concentrations calculated during the last five years differed more between systems (37 mg NO<sub>3</sub> 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 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. (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 403 43 to 95 kg N ha<sup>-1</sup> yr<sup>-1</sup> in ORG system. The average values were close to those previously 404 405 calculated,  $161 \pm 20$  kg N ha<sup>-1</sup> yr<sup>-1</sup> for CA and  $65 \pm 17$  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,  $95 \pm 3 \text{ kg N} \text{ ha}^{-1}$ yr<sup>-1</sup>. This indicates that despite the rather large uncertainty in the estimates of N surplus, the 407 408 CA system is characterised by a much higher surplus than the ORG system.

The unrecovered N, *i.e.* the difference between the N surplus and the sum of N stored in soil and N leached, corresponds to the gaseous N losses (denitrification + volatilization). Figure 2 displays the N surplus and its partitioning into its three components over the 1998-2017 period. N surplus was mainly correlated with gaseous losses (r=0.97, p<0.001), moderately with SON storage (r=0.93, p<0.01) and not correlated with N leaching (r=0.60, ns). The gaseous losses differed widely among cropping systems: they were much greater in the CA ( $83 \pm 22 \text{ kg N ha}^{-1}$ yr<sup>-1</sup>) than in the CON system ( $32 \pm 11 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ) and smallest in the LI ( $15 \pm 24 \text{ kg N ha}^{-1}$ yr<sup>-1</sup>) and ORG system ( $12 \pm 14 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ). Similar differences were obtained when 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 (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 421 422 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.

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

### 3.7 Global GHG balance

The GHG balance calculated for each cropping system is presented in Table 7. The upper part
of the table shows the GHG balance during the 2014-2017 period, during which N<sub>2</sub>O fluxes
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 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 439 440 organic fertilizers in the early years of the trial. The CO<sub>2</sub> emissions related to agricultural 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 441 ha<sup>-1</sup> yr<sup>-1</sup> in CON. The differences were mainly due to the absence of soil tillage in CA, ploughing 442 443 being particularly fuel consuming. The indirect N<sub>2</sub>O emissions occurring during the N cascade 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 444 445 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  $(+2198 \text{ kg CO}_2\text{eq ha}^{-1} \text{ yr}^{-1}) > \text{LI} (+1763) > \text{CA} (+306) > \text{ORG} (-65)$ . A similar ranking and 448 449 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**

We have studied the long-term impacts of alternative cropping systems on the N fate, including N uptake by crops, SON storage, N leaching and gaseous N emissions, and the GHG balance, including direct and indirect emissions. The results are summarized in Figure 3: each variable of each cropping system was scored between 0 (adverse impact) and 1 (beneficial impact), 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 in481 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 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 486 487 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 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 497 498 by Poisvert et al. (2017) in the same region over the period 2000-2010. The ORG system is characterized by an unusual high N surplus (63 kg N ha<sup>-1</sup> yr<sup>-1</sup>) compared to other published 499 500 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.

### 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 55 kg N ha<sup>-1</sup> yr<sup>-1</sup>. This result is consistent with Autret *et al.* (2016) who found significant SOC 507 508 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" 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 511 512 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) and/or attributed to higher application of organic fertilizer in organic farming experiments 518 519 (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.

#### **3.11** N leaching

527 The N leaching mitigation (Figure 3) was assessed by comparing the NO<sub>3</sub> concentrations in drained water to a reference threshold of 50 mg L<sup>-1</sup> defined by the Nitrate Directive. The average 528 leaching loss over the 1998-2017 period was 16 kg N ha<sup>-1</sup> yr<sup>-1</sup>, without any difference between 529 530 cropping systems. This value was lower than the average N leaching reported by Benoit et al. (2014) in the same region for conventional cropping systems (32-77 kg N ha<sup>-1</sup> yr<sup>-1</sup>) but closer 531 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 532 533 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 decreased to 40 instead of 84 mg NO<sub>3</sub> L<sup>-1</sup>, respectively. In our study, the favourable effect of 550

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

555

### 3.12 Gaseous N emissions

556 The  $N_2O$  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  $N_2O$ -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 N2O 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 NOx 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 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>). 582 583 If we assume that most gaseous losses are due to denitrification, then the  $N_2O/(N_2+N_2O)$  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 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 590 591 system and 20% smaller in the LI system. Due to its very high C sequestration rate, the CA 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 592 593 atmosphere. The best situation was found in the ORG system which was a sink for the 594 atmosphere (-65 kg  $CO_{2eq}$  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 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 603 systems (4030 kg CO<sub>2eq</sub> ha<sup>-1</sup> yr<sup>-1</sup>). Aguilera et al. (2015) estimated the carbon footprint of 604 605 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 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>). 607 608 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).

### 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.

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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-1; N2O 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 fei	tilizer	B	NF	N of	ftake	Grai	Grain yield		
						kg N ł	na <sup>-1</sup> yr <sup>-1</sup>			t DI	∕I ha⁻¹		
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	a	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			
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 19	98	SON stock 202	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)	а	4.34 (0.31)	b A	13 (11)	b	
LI	4.47 (0.74)	а	4.57 (0.43)	b A	6 (24)	b	
CA	4.44 (0.64)	a	5.33 (0.58)	a B	55 (21)	а	
ORG	3.95 (0.42)	a	4.44 (0.27)	b A	30 (14)	ab	

# 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.

	CON							LI			(	CA		ORG			
Drainage season	Drained water	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> ]
	mm yr <sup>-1</sup>	kg l	ha <sup>-1</sup>	kg ha <sup>-1</sup> yr <sup>-1</sup>	mg L <sup>-1</sup>	kg l	na <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 ł	na⁻¹	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 (kg N ha<sup>-1</sup> yr<sup>-1</sup>) 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	DCN factor	Relative variation of AG biomass									
system	BOIN factor	90%	100%	110%	mean	sd					
CA	2.1	176	190	204							
	1.7	152	163	173	167	(21)					
	1.4	139	148	157							
ORG	2.1	76	86	97							
	1.7	55	63	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-1) 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 peri	od Duration	Crop		Cropping system											
	days			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  $N_2O$  monitoring) and the whole experimental period (1998-2017).

Period	Cropping system	F	М		direct N2Oe	i	ndirect N2Oe	SOC storage rate		GHG balance				
			k	(g (	CO <sub>2eq</sub> ha	₁ <sup>-1</sup> y	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 a	281	а	1088	b	94	286 *	**	2039 a	10.1 a	14.7 a		
	LI	644 b	251	а	558 0	С	85	81 *	**	1457 b	8.6 <i>b</i>	11.5 b		
	CA	606 b	131	b	1900 a	а	79	2292 *	**	425 c	1.6 c	4.0 c		
	ORG	0 c	243	а	698 d	С	41	1016 *	**	-33 d	-0.2 d	-0.4 d		
1998-2017	CON	927 a	272	а	1153 *	*	131	286 <i>c</i>	ı	2198 a	10.9 <i>a</i>	15.8 <i>a</i>		
	LI	728 b	249	b	736 *	*	132	81 <i>c</i>	ı	1763 b	10.4 <i>b</i>	14.0 b		
	CA	659 b	161	С	1645 *	*	132	2292 c	2	306 c	1.1 c	2.9 c		
	ORG	44 c	255	b	576 *	*	76	1016 <i>k</i>	6	-65 d	-0.5 d	-0.8 d		