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N_2O Emission Pattern in A Legume-Based Agroecosystem

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Abstract

Legumes provide several ecological services to agroecosystems, but there is a lack of references on services related to N flows for a wide range of legume crops. N_2O emissions were measured in two field experiments using a two-year legume-cereal crop sequence. In the first year (2014 and 2016), different legume crops were grown (lupin, pea, fava bean, common bean, soybean, chickpea) and compared to fertilized cereals (barley and sorghum). Once the seeds were harvested and the residues incorporated in the soil, unfertilized wheat was sown and harvested in the second year (2015 and 2017). N_2O emissions, as well as soil temperature and moisture, were measured continuously using an automated chamber method during the two years of each experiment. Daily N_2O emissions were less than 10 g $N\text{-}N_2O$ ha^{-1} d^{-1}, with higher values (ranging from 10 to 90 g $N\text{-}N_2O$ ha^{-1} d^{-1}) being measured during exceptionally rainy conditions. Daily N_2O emissions were mainly influenced by climatic conditions for field experiments and far less by inorganic N content, except for N-fertilized cereals. For both field experiments, cumulative N_2O emissions during legume and cereal pre-crops + fallow period between pre-crop harvest and wheat sowing (1st year) (mean values 365.4 and 318.1 g $N\text{-}N_2O$ ha^{-1} for experiment I and II, respectively) were higher than during wheat crop cultivation (2nd year) (155.8 and 101.5 g $N\text{-}N_2O$ ha^{-1} for experiment I and II, respectively). For field experiment II, N_2O emissions were slightly higher for the N fertilized cereal pre-crops (529.8 and 523.3 g N-N_2O ha^{-1} for barley and sorghum, respectively) compared to legume pre-crops (mean values 380.6 and 417.2 g N-N_2O ha^{-1} for legumes sown in March and May, respectively), while no significant difference was measured for field experiment I. There was no difference in N_2O emissions during the cultivation of the different legume species. Furthermore, when wheat was grown after legumes or N fertilized cereals, N_2O emissions were comparable for the different experimental treatments with no relation established with the amounts of N present in crop residues or their C: N ratios. Despite the small differences in emissions between N-fertilized cereals and grain legumes, introducing these leguminous species in crop rotation and in these pedoclimatic conditions makes it possible to substitute synthetic N fertilizer and mitigate the greenhouse gases emitted from these cropping systems. However, further research is still needed to clarify and quantify the value of legumes in mitigating and reducing greenhouse gas emissions from cropping systems.

Keywords
Agricultural soil; nitrous oxide; N mineralization; leguminous species; cereal; crop rotation; crop residues

1. Introduction

Global warming is associated with increased greenhouse gas (GHG) atmospheric concentrations, predominantly caused by anthropic activities. The AFOLU (Agriculture, Forestry and Other Land Uses) sector is estimated to produce 21% of total net anthropogenic GHG emissions, including 69% of the potent GHG N_2O emissions [1]. N_2O is emitted from the soil during the N cycle through two microbial processes: nitrification and denitrification. N_2O emissions are related to the inorganic N content of agricultural soils [2] and are greatly impacted by inorganic and organic N fertilization.

Legumes are an alternative source of N for cropping systems. Due to their ability to fix atmospheric N via symbiosis with soil bacteria, legumes provide several ecological services to agroecosystems. Their cultivation results in the harvest of proteins or protein-rich fodder, without any N fertilization. In addition, their N-rich crop residues left in the soil can supply N to the following crop and reduce the amount of N fertilizer required. Lastly, their cultivation reduces fossil fuel energy and the GHG emissions associated with using nitrogen fertilizers, as reported by several authors [3, 4].

Previous studies have shown that N_2O emissions from legume crops without N fertilization are lower than those from non-legume fertilized crops [5-7]. Indeed, the symbiotic fixation of atmospheric nitrogen is recognized as a non-N_2O emitting process [3, 8]. On the other hand, the degradation of nodules at the end of the crop and the mineralization of the organic nitrogen contained in the crop residues can lead to N_2O emissions [7, 9]. Thus, contrasting values of N_2O emissions in cropping systems with or without legumes have been reported in the literature, with emissions being influenced by agricultural practices, particularly tillage, crop rotation and legume cultivation [10-12]. Peaks of N_2O emissions are generally reported after the incorporation of legume crop residues (e.g., [4, 13, 14]). The lower C/N ratio of legume residues is responsible for their rapid mineralization in the soil and mineral nitrogen availability for nitrification and denitrification processes, depending on aerobic/anaerobic soil conditions. However, very few of these studies have compared the N_2O emissions from soil successively cultivated with (1) different species of grain legumes (e.g. [8, 12, 15]) and (2) different species of cereals in the same experimental conditions.

The current study aimed to measure N_2O emissions during a two-year legume-cereal crop sequence: i) the first year with different legume crops compared to N fertilized cereals (named pre-crops in this study), and ii) the second year with unfertilized winter wheat that had taken up mineralized nitrogen from legume or cereal residues. The first main hypothesis was that N_2O emissions are lower for legume crops than N-fertilized cereals. The second main hypothesis was N_2O emissions from a cereal crop are higher when cultivated after incorporating legume residues into the soil than cereal residues. The originality of this study is that it investigated N_2O emissions for a wide range of legumes during their cultivation and the following crop (i.e., wheat).

2. Materials and Methods

2.1 Experimental Site

Two field experiments, each lasting two years, were carried out at the INRAE Dijon experimental site located in F 21110 Breteniere (Eastern France; N 47° 14′ 27.6′′, E 5° 6′ 54′′, altitude = 206 m a.s.l.) in 2014-2015 (Experiment I) and 2016-2017 (Experiment II). The soil is classified as Eutric Cambisol (Anoclayic) [16] with a clay surface layer (depth = 0.65 ± 0.15 m) developed on a coarse alluvial layer (Table S1).

The site is subject to a semi-continental climate but the climatic conditions were contrasted between Experiments I and II. Cumulative rainfall and mean temperature between the beginning of the pre-crop harvest and wheat harvest were 850 mm and 11.8°C in Experiment I and 600 mm and 11.3°C in Experiment II (Figure S1). Rainfall was unusually high between July and November 2014 (541 mm) compared to 2016 (303 mm) and to the 20-year mean rainfall during the same period (362 mm). Rainfall between January and June was similar in 2015 (252 mm) and 2017 (293 mm), but lower than the 20-year mean rainfall during the same period (340 mm).

2.2 Experimental Design

Each experiment consisted of a two-year crop sequence (i.e., legume or cereal pre-crop followed by wheat) as described previously [17]. Legume and cereal pre-crop treatments were applied in 2014, followed by winter wheat harvested in 2015 (Experiment I). In 2016, the same pre-crops were grown near the first experiment, followed by winter wheat harvested in 2017 (Experiment II).

2.2.1 Pre-Crop Treatments

During the first year of both experiments I and II, 8 treatments were performed in the field including six legume pre-crops: fava bean (*Vicia faba*), chickpea (*Cicer arietinum*), common bean (*Phaseolus vulgaris*), lupin (*Lupinus albus*), pea (*Pisium sativum*), and soybean (*Glycine max*) and two N fertilized cereal pre-crops: barley (*Hordeum vulgare*) and sorghum (*Sorghum bicolor*). Pre-crops were sown either in March or in May, depending on the physiology of each species (Table S2), and were called spring pre-crops and summer pre-crops, respectively, after that. Pre-crops were sown on plots 1.5 m wide and 12 m long using four randomly distributed replicates in Experiments I and

II. No N fertilization was applied to any of the legume pre-crops in either experiment. In contrast, N fertilization was applied in the form of NH_4NO_3 at rates of 135 kg N ha^{-1} in Experiment I (60 and 75 kg N on 3rd and 28th April 2014) and 70 kg N ha^{-1} in Experiment II (19th April 2016) for barley. N fertilizer was also applied twice during sorghum growth at rates of 50 kg N ha^{-1} in 2014 (9th May and 13th June) and 2016 (7th June and 7th July). In Experiment I, spring pre-crops was irrigated with 35, 40 and 50 mm on March 26th, May 16th and June 18th 2014, respectively. Summer pre-crops were irrigated with 40, 40 and 50 mm on May 16th, May 27th and June 14th 2014, respectively. In Experiment II, 50 mm was supplied to summer pre-crops on July 11th 2016. Weeds were controlled by hand weeding and one insecticide (Karate Zeon) was applied on April 25th 2014 and April 21th 2016 in Experiments I and II, respectively.

2.2.2 Pre-Crop Harvest and Residue Management

Pre-crop grains were harvested with a harvesting machine. No grains were harvested for chickpeas in 2014, due to rainy conditions coupled with excessively low temperatures unsuitable for grain production. In this case, the whole plant was considered crop residue. Two to seven weeks after the pre-crop grain harvest, crop residues were chopped and incorporated into the soil using a rototiller (see Table S2 for pre-crop incorporation dates).

2.2.3 Following Crop

Winter wheat (*Triticum aestivum*, cv. Rubisko) was sown on the same plots on October 20th 2014 (300 seeds m^{-2}) in Experiment I and October 24th 2016 (350 seeds m^{-2}) in Experiment II. To control weeds and pathogens, a herbicide (February 26th 2015, Arbalète) and a fungicide (April 30th 2015, Adexar + Voxan) were applied on wheat in Experiment I. In Experiment II, irrigation was applied on wheat on April 5th and 19th 2017 (27 mm and 30 mm, respectively) (Figure S1B). In order to better characterize the effect of previous crops on N_2O emissions during wheat cultivation, no N fertilization was applied on wheat.

2.3 N_2O Emissions Measurements

Nitrous oxide (N_2O) emissions were measured continuously using automated chambers (length 70 cm, width 70 cm, height 30 cm) with the method described previously [18]. For each treatment the chambers were randomly placed on two of the four plots (i.e., two chambers on one plot and one chamber on the other plot), because of the limited physical connection between the analyzer and chambers. For an experiment I, the chambers were installed on April 16th 2014 and 3rd June 2014 for pre-crops sown in March and May, respectively. For experiment II, the chambers were installed on 5th April 2016 and 6th June 2016 for pre-crops sown in March and May, respectively. During the measurement period the chambers were removed for very short periods when harvesting, residue incorporation and wheat sowing were performed. Vegetation was present inside the chambers for the early growth stages of the different crops. Nevertheless, the plants were cut when the crop height was higher than the chamber's top to allow chamber closure for measurements. Nitrous oxide (N_2O) concentrations in the headspaces were measured for 20 minutes 4 times a day by a Megatec® IR analyzer 46i (Thermo Scientific), connected to each chamber using an automated screening system. Daily N_2O fluxes were estimated for each experimental treatment and each day by considering the four measurements per day and the three replicates and were calculated as described previously [18]. The absence of data corresponded to periods during which the chambers were out of order or removed for technical operations on the fields (e.g., sowing, harvesting) and for undetectable N_2O emissions. The data in all the remaining systems were excluded to ensure the comparison of cumulative data if measurements were missing. Cumulative N_2O emissions therefore corresponded to the sum of N_2O emissions observed without extrapolation, i.e., during periods for which measurements were available for all the systems simultaneously. On average, the measurements covered 60% of the length of both periods for each experiment.

2.4 Statistics

Data analyses were performed using R software version 4.0.2. [19]. For each period (i.e., pre-crop + fallow and wheat crop) and each kind of crop (i.e., crops sown in March and crops sown in May), a two-way analysis of variance (ANOVA) was performed to test for a date, species and date*species interaction effects on daily N_2O emissions Homoscedasticity and normality of variable residuals were tested with a Bartlett and Shapiro-Wilk test, respectively. A Three-factor ANOVA was performed to analyze the effects of the experiments (I and II), the kind of crops (sown in March or May), the period (pre-crop + fallow vs. wheat crop) and interactions between the three factors on cumulative values. In order to compare the effects of legumes and cereals on cumulative emissions, a one-factor ANOVA was performed separately for each kind of crop and each period. If the effects were significant, a Least significant difference" (LSD) test ($p < 0.05$) was performed to compare multiple means, using the {agricolae} package.

3. Results

3.1 Daily Emissions for Experiment I

For crops sown in March 2014 and during the pre-crop + fallow period, N_2O emissions varied from 0.2 to 36.0 g N ha^{-1} d^{-1} with an average N_2O emission of 3.1 g N ha^{-1} d^{-1} (Figure 1). 73% of N_2O emission values were lower than 2.0 g N ha^{-1} d^{-1}. There was no significant difference for N_2O emission between pre-crops ($P = 0.10$), while the effect of date was highly significant ($P < 0.001$), showing that N_2O emissions can present very high daily variation. Higher N_2O emission peaks were measured in summer-autumn 2014, when the weather conditions were very rainy.

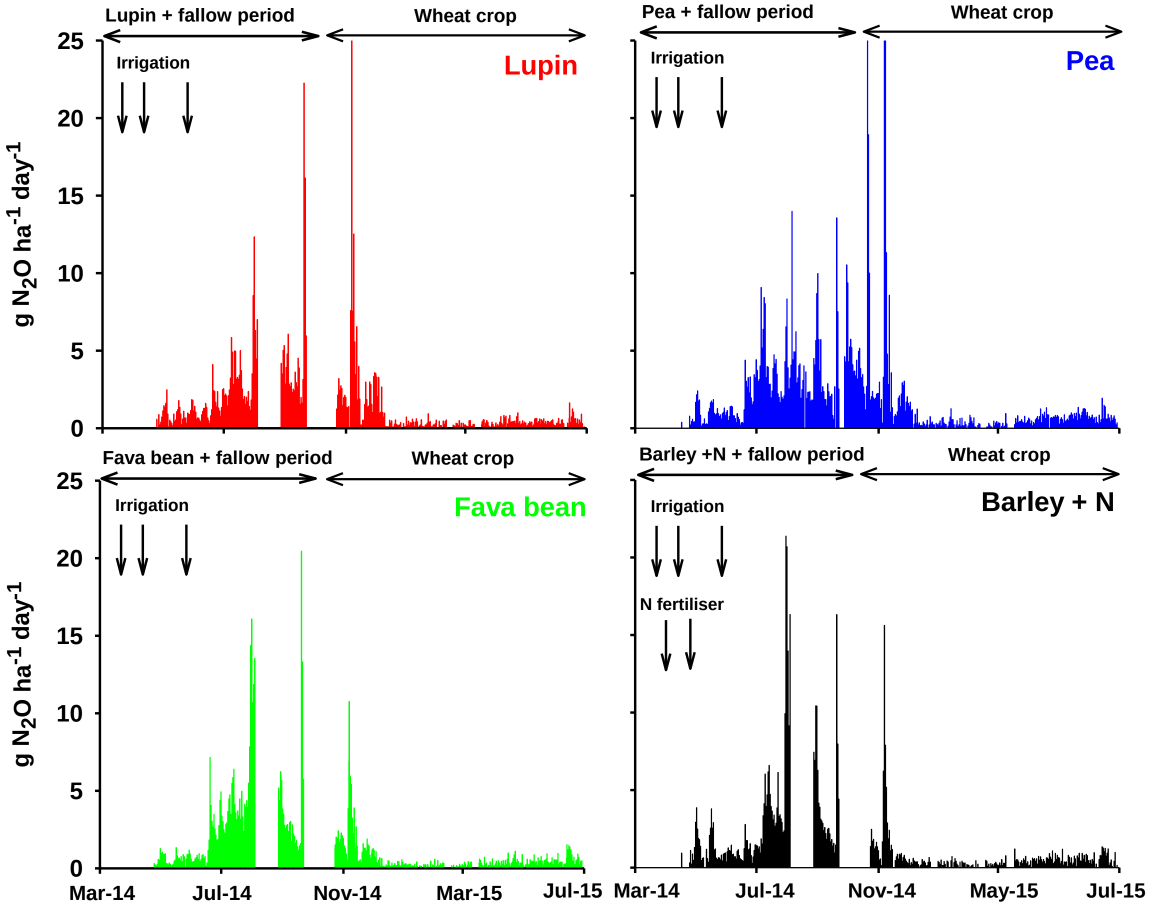

Figure 1 Daily N_2O emissions for crops sown in March and for experiment I (2014-2015). Pre-crops are lupin, fava bean, pea and barley. Measurements were performed 4 times a day. Amounts of N fertilizer (for barley) and irrigated water (for all crops) are given in part 2.2.

N_2O emissions during the pre-crop and fallow period for crops sown in May 2014 are presented in Figure 2. The mean N_2O emission for all treatments and periods was 4.7 g N ha^{-1} d^{-1} with minimal and maximal N_2O emission of 0.2 and, exceptionally, as high as 127.0 g N ha^{-1} d^{-1}, depending on the pre-crop and date. 50% of emission values were lower than 2.0 g N ha^{-1} d^{-1}. The effects of pre-crop ($P < 0.001$), date ($P < 0.001$) and date*pre-crop interaction ($P < 0.001$) were highly significant with the highest daily emissions measured for N fertilized sorghum.

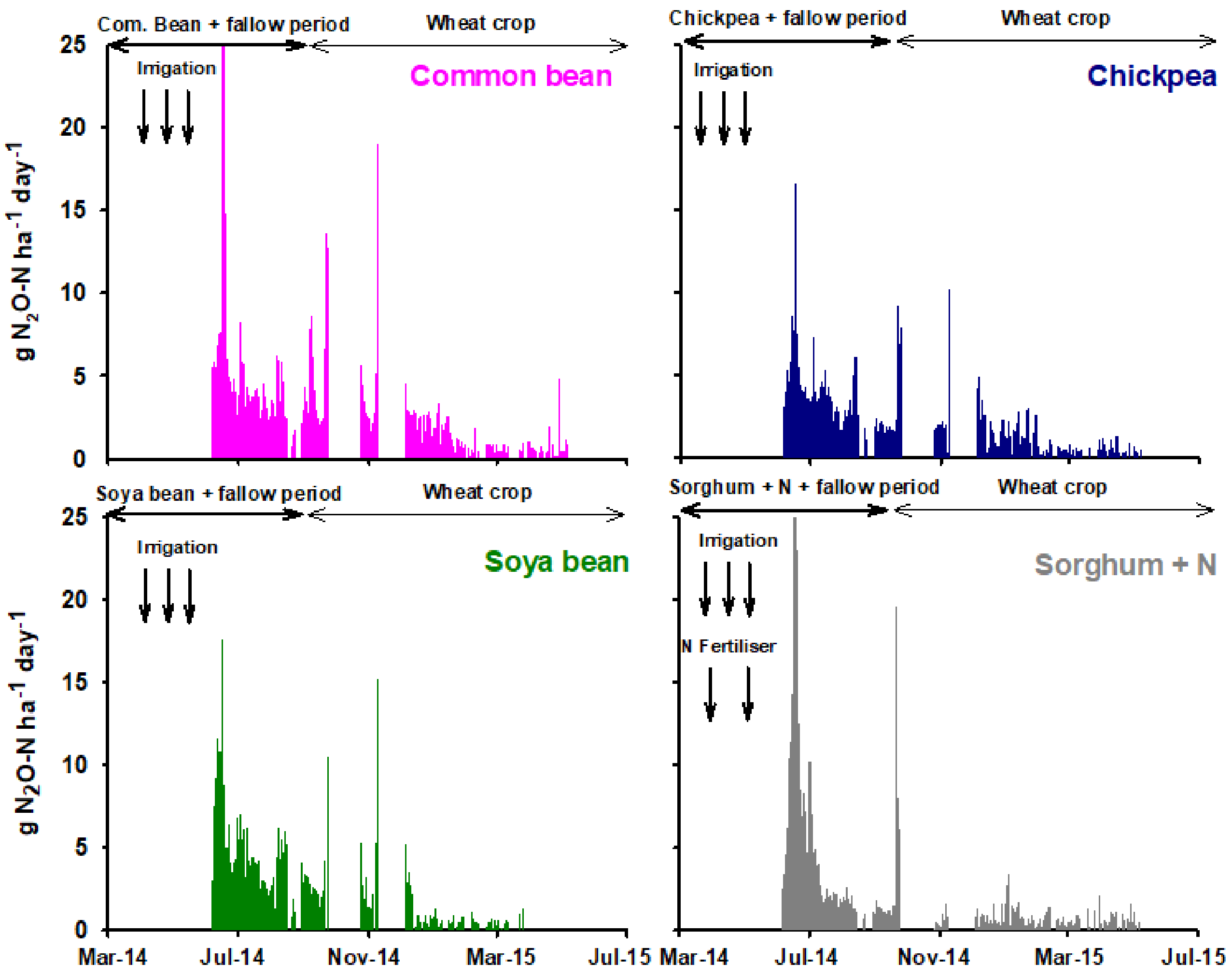

Figure 2 Daily N_2O emissions for crops sown in May and for experiment A (2014-2015). Pre-crops are common bean, chickpea soya bean and sorghum. Measurements were performed 4 times a day. Amounts of N fertilizer (for sorghum) and irrigated water (for all crops) are given in part 2.2.

During the wheat crop period, following all pre-crops, mean N_2O emissions were respectively 1.2 and 1.4 g N ha^{-1} d^{-1} for the pre-crop sown in March and May, respectively (Figure 1 and Figure 2). Minimal and maximal emission values were respectively 0.2 and 56.9 g N ha^{-1} d^{-1} for wheat following all the pre-crops sown in March 2014, and 0.1 and 39.3 g N ha^{-1} d^{-1} for wheat following all the pre-crops sown in May 2014. The effects of date ($P < 0.001$) and pre-crops ($P < 0.001$) were highly significant for the pre-crops sown in March. The effects of date ($P < 0.001$) and pre-crops ($P < 0.001$) were equally highly significant for the pre-crops sown in May. The highest emissions were measured for wheat following legume pre-crops (mean value 1.6 g N ha^{-1} d^{-1}) than wheat following sorghum (mean value 0.9 g N ha^{-1} d^{-1}).

3.2 Daily Emissions for Experiment II

During the pre-crop and fallow period, N_2O emissions varied from 0.2 to 37.4 g N ha^{-1} d^{-1} for the crop sown in March 2016, with an average N_2O emission of 2.5 g N ha^{-1} d^{-1} (Figure 3). 67% of N_2O emissions were less than 2.0 g N ha^{-1} d^{-1} with a heterogeneous distribution of values. Higher N_2O

emission peaks were measured during the rainy period (spring-beginning the summer of 2016 2016). Higher peaks of N_2O were measured for fertilized crops (barley or sorghum) after fertilizer inputs. The effects of date ($P < 0.001$), pre-crop ($P < 0.001$) and date*pre-crop interaction ($P < 0.001$) were highly significant. The main differences were observed during the period from 11 June to 30 June 2016 when higher N_2O emissions were measured for barley compared to the legume pre-crops.

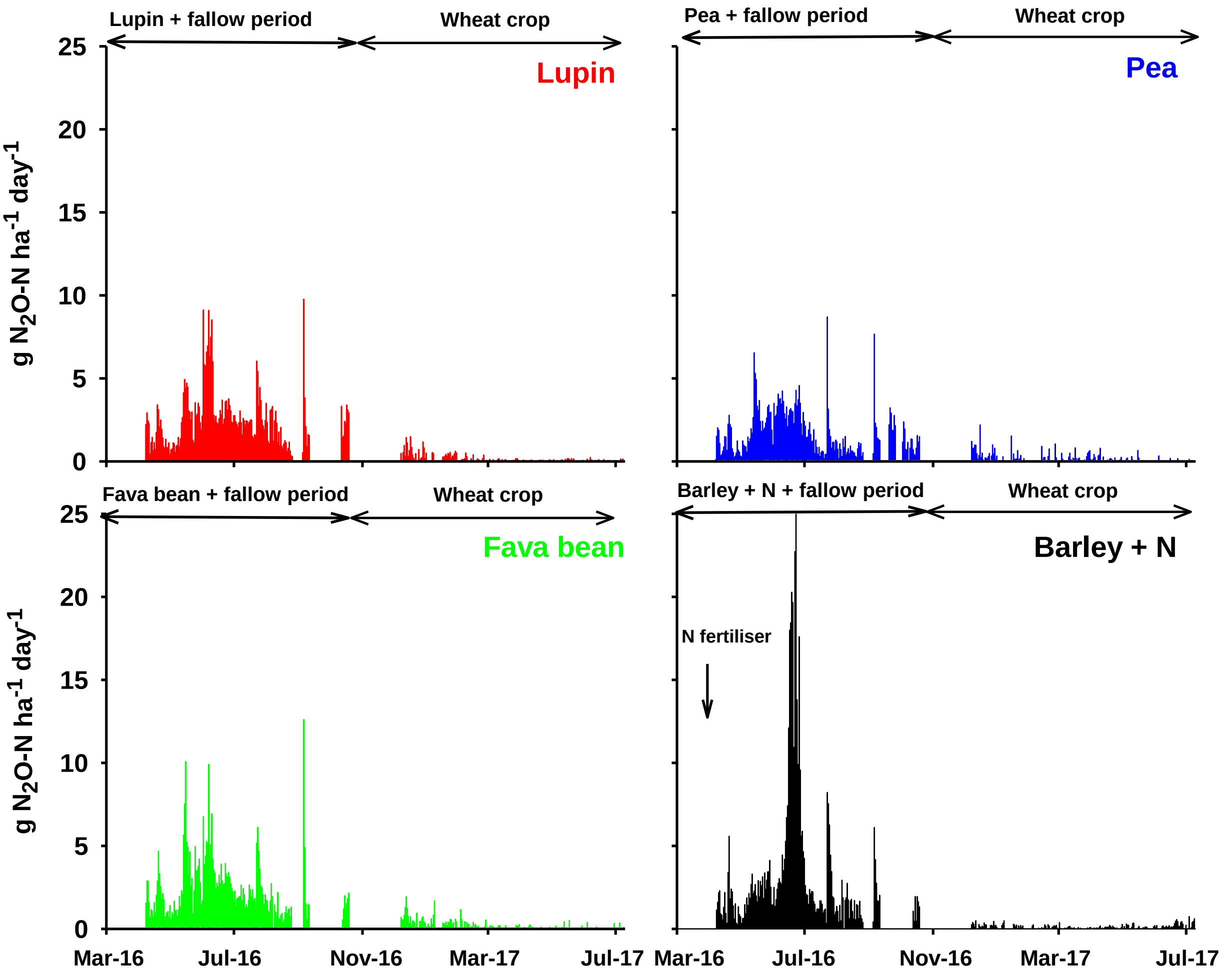

Figure 3 Daily N_2O emissions for crops sown in March and for experiment II (2016-2017). Pre-crops are lupin, fava bean, pea and barley. Measurements were performed 4 times a day. Amounts of N fertilizer (for barley) and irrigated water (for all crops) are given in part 2.2.

For pre-crops sown in May 2016, the mean N_2O emission during the pre-crop + fallow period was 2.9 g N ha^{-1} d^{-1} with minimal and maximal N_2O emissions of 0.1 and 32.1 g N ha^{-1} d^{-1}, depending on the pre-crop and date (Figure 4). 70% of emissions were less than 2.0 g N ha^{-1} d^{-1}. The effects of date ($P < 0.001$) and pre-crop ($P < 0.001$) were highly significant with higher emissions measured for sorghum compared to legume pre-crops.

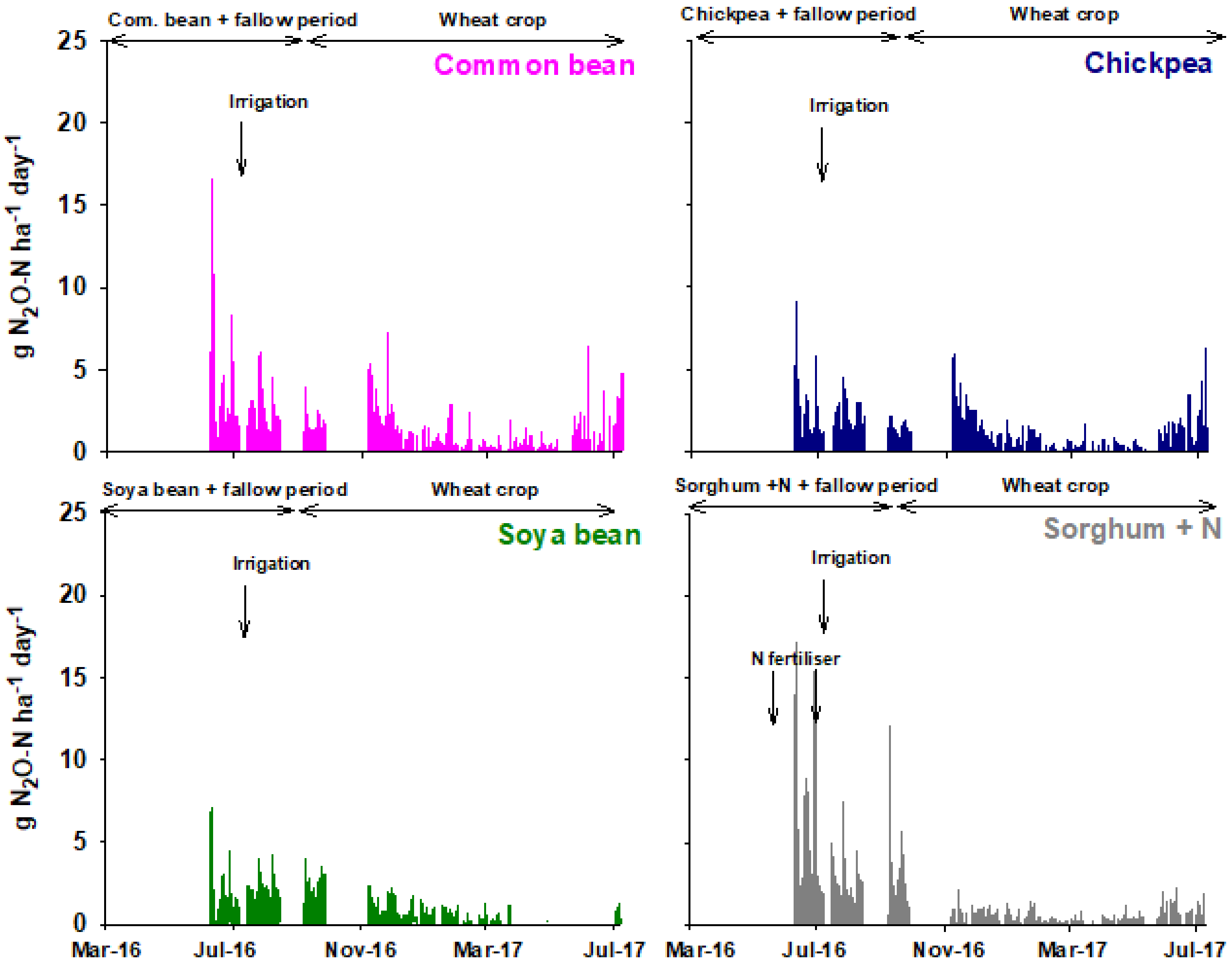

Figure 4 Daily N_2O emissions for crops sown in May and for experiment II (2016-2017). Pre-crops are common bean, chickpea, soya bean and sorghum. Measurements were performed 4 times a day. Amounts of N fertilizer (for sorghum) and irrigated water (for all crops) are given in part 2.2.

During wheat cultivation in the second experiment, mean N_2O emissions were 0.4 and 1.2 g N ha^{-1} d^{-1} for the pre-crop sown in March and May, respectively (Figure 3 and Figure 4). Minimal and maximal emission values were respectively 0.1 and 5.1 g N ha^{-1} d^{-1} for all the pre-crops sown in March 2016 and 0.1 and 12.5 g N ha^{-1} d^{-1} for all the pre-crops sown on May 2016. The effects of date ($P < 0.001$) and pre-crops ($P < 0.001$) for pre-crops sown in March and the effects of date ($P < 0.001$) and pre-crops ($P < 0.001$) for pre-crops sown in May were highly significant. N_2O emissions were slightly higher for wheat following common bean and chickpea compared to wheat following sorghum or Soya bean. Lower N_2O emissions were measured for wheat following barley compared to legume pre-crops (i.e., lupin, fava bean, and pea).

3.3 Cumulative Emissions for Both Experiments

Cumulative N_2O emissions for the two years of both experiments are presented in Figure 5 and Figure 6, differentiating both measurement periods. The contributions of N_2O emitted by pre-crops during the pre-crop + fallow period are also indicated. The N_2O emitted during the fallow following the cultivation of species sown in March was higher than species sown in May, due to a long fallow period in the first case. It appears that the fallow period contributed more to N_2O emitted during the pre-crop + fallow period in experiment I compared to experiment II. Emissions were significantly higher for an experiment I compared to experiment II ($P < 0.001$). For both experiments, most N_2O emissions occurred during the pre-crop + fallow period ($P < 0.001$). The kind of crops (i.e., spring crops sown in March vs. summer crops sown in May) did not influence cumulative N_2O emissions ($P = 0.54$). During the pre-crop + fallow period, differences in cumulative N_2O emissions between pre-crops were only observed for experiment II. Cumulative emissions for fertilized barley were significantly higher than a pea and sorghum were significantly higher than chickpea and soybean (Figure 6). During the second year (wheat crop) of experiment II significantly higher emissions were measured after legume pre-crops were sown in May (Figure 6).

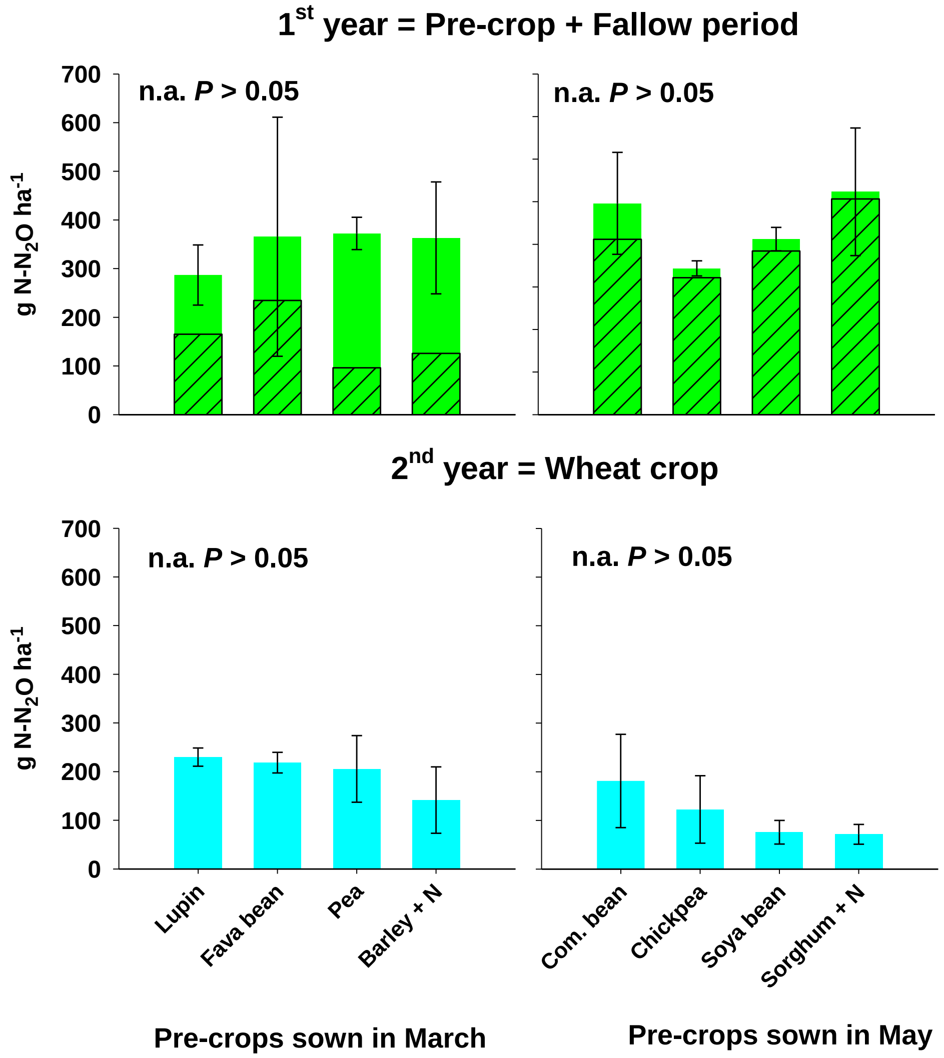

Figure 5 Cumulative emissions for crops sown in March and May for experiment I (2014-2015) and for pre-crop + fallow period, and wheat crop. Hatched bars indicate N_2O emitted during legume crops. Pre-crops are lupin, fava bean, pea, barley, common bean, chickpea soya bean and sorghum. Bars for fertilized crops (barley and sorghum) are surrounded in black. LSD tests were performed separately for each period and kind of crops (i.e. crops sown in March and crops sown in May) to test the effect of pre-crops ($P < 0.05$). Upper and lower bars correspond to standard deviation values.

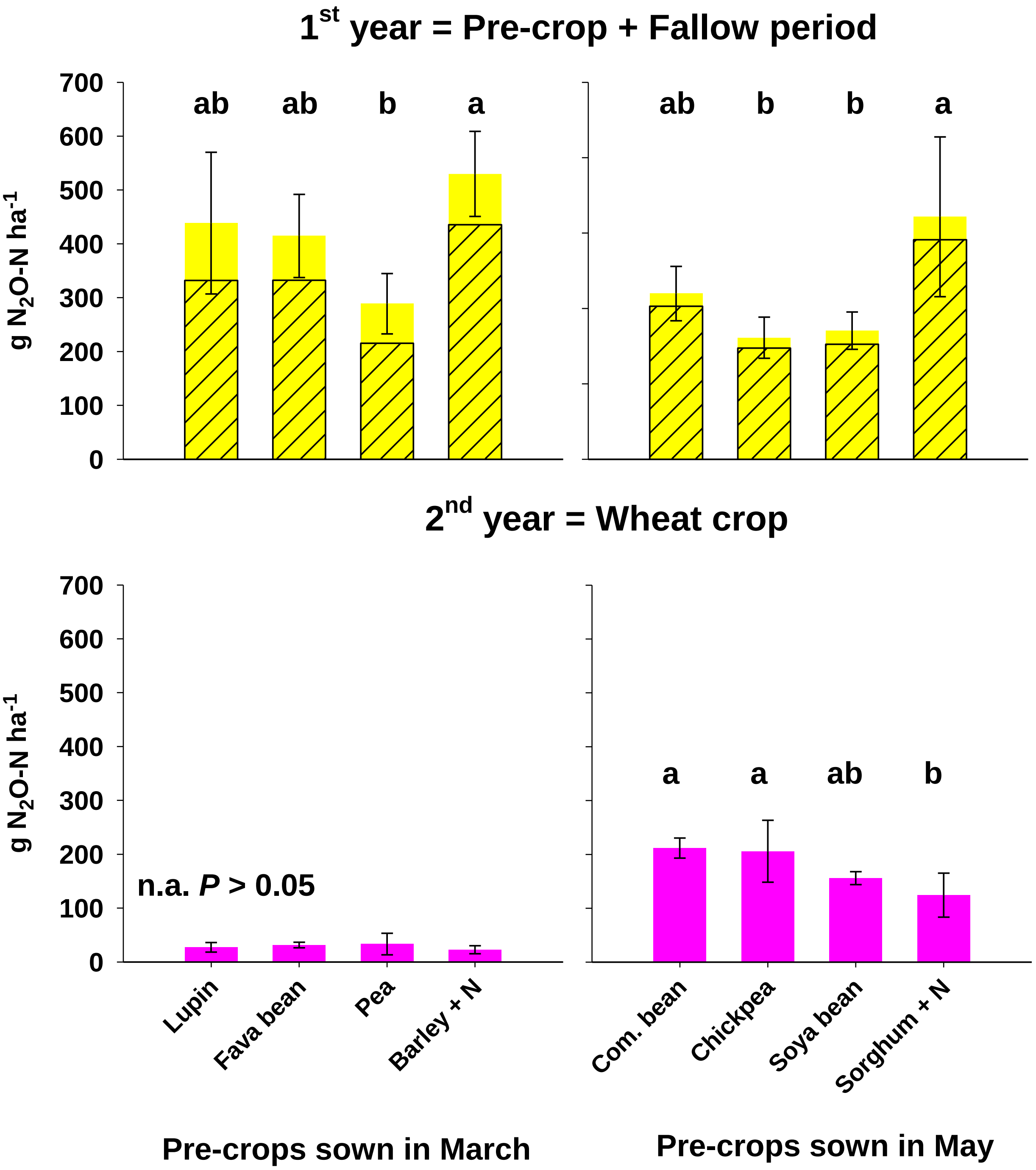

Figure 6 Cumulative emissions for crops sown in March and May for experiment II (2016-2017) and for both periods (pre-crop + fallow and wheat crop). Hatched bars indicate N_2O emitted during legume crops. Pre-crops are lupin, fava bean, pea, barley, common bean, chickpea soya bean and sorghum. LSD tests were performed separately for each period and kind of crops (i.e. crops sown in March and crops sown in May) to test the effect of pre-crops ($P < 0.05$). Upper and lower bars correspond to standard deviation values.

Considering the 2-year rotation (i.e., pre-crop + fallow period + wheat crop) the cumulative emission of Experiment I (mean value (± SD) = 551.1 ± 140.4 g N-N_2O ha^{-1} was significantly higher ($P < 0.001$) than for Experiment II (429.6 ± 100.2 g N-N_2O ha^{-1}). The kind of crops (i.e., spring crops sown in March vs. summer crops sown in May) did not affect cumulative N_2O emissions ($P = 0.54$). No significant differences ($P > 0.10$) were observed between pre-crop treatments.

4. Discussion

Daily N_2O emissions measured during the two experiments were very low (<1-2.0 g N_2O ha^{-1} d^{-1}) in most cases. Nevertheless, daily peaks reached 130.0 g N_2O ha^{-1} d^{-1}. N_2O emissions were of the same order of magnitude as those measured at the same experimental site and in similar tilled cropping systems, with values ranging from 0.0 to 30.0-50.0 g N_2O ha^{-1} d^{-1} [18, 20]. Although our measurements represent on average only 60% of the days in each period, N_2O emissions appear to be of the same magnitude as measurements made under comparable pedoclimatic conditions in Eastern France [21] or under crop rotations including legumes [4, 22, 23]. These N_2O emissions appear lower than those generally measured for different crop covers with or without legumes and with or without N (e.g. [24-26]). Likewise, cumulative N_2O emissions (mean value 360.0 g N-N_2O ha) during the pre-crop + fallow period, mainly due to N_2O emitted during pre-crop cultivation, appear relatively low compared to other studies [4, 24, 27] and suggest that this experimental site is probably low emitting.

Indeed, soil N_2O emissions vary considerably depending on many factors including pedoclimatic conditions (e.g. [24, 27, 28]), in particular soil temperature, moisture and rainfall [23, 29]. In both experiments, higher N_2O emissions occurred during rainy periods. It is also generally admitted that N_2O emissions originate from both anaerobic respiration such as denitrification [25, 30] and nitrification under aerobic conditions [31, 32], and could therefore occur in very variable soil moisture conditions. N fertilization (both level and form) is also a key parameter determining the amounts of N_2O emitted by soils [9, 26]. For N-fertilized crops, N_2O emissions are usually positively related to the quantities of nitrogen supplied [8, 28, 33]. Nevertheless, several authors [27, 34] have also shown that N_2O emissions are governed together by the amount of nitrogen contained in the soil and by numerous variables (e.g., WFPS, pH, organic matter levels, tillage practices), making it difficult to compare emission levels for contrasted experimental conditions.

Numerous studies have generally observed higher N_2O emissions for fertilized crops than grain legumes [4, 7, 22, 35]. Except for barley in 2016, we observed only small or no differences in N_2O emissions between fertilized cereals and legumes. This was probably due (i) to the moderate or suboptimal level of N fertilization provided to cereals in environmental conditions unfavorable for high emissions and (ii) the high variability observed for cumulative emissions calculated for the pre-crop + fallow period in experiment I. Thus our first hypothesis (i.e., "N_2O emissions are lower during legume crops compared to N fertilized cereals") was not systematically validated since fertilized cereals induced comparable N_2O emissions compared to legume species, except for some pre-crop sown in March or May for experiment II.

Differences of N_2O emissions among legume crops were slight or non-existent in this study during the pre-crop + fallow period, by studies [15] that compared N_2O emissions for several grain or forage legume species. Other authors have observed differences in N_2O emissions among legume species or cultivars varying according to their capacity to fix nitrogen [12]. Also, the context of low emissions for this experimental site and the relatively high variability of the measurements probably contributed to the inability to highlight differences between species.

During wheat cultivation, cumulative N_2O emissions were lower than during the pre-crop period (average N_2O emissions of 129.0 g N-N_2O ha^{-1}) but, surprisingly, there were no differences between treatments. The probable explanation for this lack of significant difference in the two experiments was related to (i) the context of the low-emitting experimental site and (ii) the variability of N_2O emission measurements for some treatments. Our second hypothesis was therefore not validated, except for pre-crop treatments sown in May in experiment II (2016-17). After legume crops, N_2O emissions are generally higher than those measured during the cultivation period [13, 36]. During the intercropping period and the following crop, pre-crop residues decompose and provide variable amounts of nitrogen to the soil through mineralization, depending on the biochemical characteristics of the residues (i.e., N content or C:N ratio) [7, 37, 38].

In this study, the amounts of N residues incorporated in the soil as well as their C:N ratio were different among legume species and cereals (Table S3), but these characteristics did not affect N_2O emissions. However, in the current experiment [20], the amounts of N mineralized from crop residues were related to wheat N uptake. In addition, these authors showed by modeling that some significant amounts of nitrate were lost by leaching during the fallow period and may explain the low N_2O emissions during wheat cultivation with probably insufficient amounts of nitrate remaining in the soil to induce higher N_2O emissions which may in turn explain the absence of differences between experimental treatments. Under these conditions, it can be assumed that gaseous losses by denitrification (N_2 + N_2O) are unlikely to have greatly affected the N amounts available for wheat uptake.

Based on these experimental results and taking into account the complex context of low N_2O emissions from the soil, and the field experiments carried out under very contrasting climatic conditions and under-fertilized cereals, the interest in legume cultivation compared to cereals was demonstrated for only one of the field experiments concerning reducing N_2O emissions by soil. This absence or weak of differences between the cumulative emissions for the different experimental treatments during the 2-year rotation is probably explained by low emission values, moderate N fertilization of cereals and variability of measurements. However, numerous studies have shown the value of introducing pulses to reduce N_2O emissions across the cropping system, whether by grain legumes [7, 36], forage legumes [39, 40], cover-crop legumes [38, 40] or intercropped with a non-legume species [34, 41]. Legumes allow reducing the use of synthetic fertilizers during their growth through symbiotic fixation, and on the following crop by providing nitrogen from the mineralization of nitrogen-rich residues [42-44].

Conversely, the manufacture of synthetic fertilizers and their use in agriculture has a high impact on the use of energy resources and on the production of greenhouse gases [45]. Therefore, the introduction of legumes in cropping systems is recognized as a lever for mitigating greenhouse gas emissions [15, 46-48].

However, research is still needed to: i) clarify the origin of N_2O emissions (nitrification vs. [denitrification), ii) quantify these emissions under different pedoclimatic conditions, and iii) propose management methods for leguminous plants that make the best use of the nitrogen fixed during their cultivation and released when the residues are incorporated into the soil. As already implemented [23, 43, 44, 49, 50], the use of crop models is highly relevant to address these issues in future research.

Acknowledgments

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Author Contributions

B. Nicolardot measured the N_2O emissions and managed the data, M Guinet and A.S. Voisin performed the field experiments and C. Hénault managed the data. All the authors contributed to writing the paper.

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Competing Interests

The authors have declared that no competing interests exist.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Figure S1: Daily rainfall (grey bars), irrigation (blue bars) and mean daily temperature (red line) for the pre-crop + fallow period and during the wheat crop in Experiment I (A) and Experiment II (B).
2. Table S1: Soil characteristics in Experiments I and II.
3. Table S2: Pre-crops and wheat characteristics and management in Experiments I and II (from [17]).
4. Table S3: Amounts, N contents and C:N ratios of pre-crops residues incorporated in soil before wheat sowing in Experiments I and II (from [17]).

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