

Contents lists available at ScienceDirect

Soil & Tillage Research



journal homepage: www.elsevier.com/locate/still

# Combining no-till with rye (*Secale cereale* L.) cover crop mitigates nitrous oxide emissions without decreasing yield



Andrea Fiorini<sup>a,\*</sup>, Stefania Codruta Maris<sup>a</sup>, Diego Abalos<sup>b</sup>, Stefano Amaducci<sup>a</sup>, Vincenzo Tabaglio<sup>a</sup>

<sup>a</sup> Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122, Piacenza, Italy
<sup>b</sup> Department of Agroecology – Soil Fertility, Aarhus University, Blichers Allé 20, 8830, Tjele, Denmark

#### ARTICLE INFO

Keywords: N<sub>2</sub>O emissions No-till Cover crops Soil organic carbon DNDC model Earthworms

# ABSTRACT

No-till (NT) often increases soil carbon (C) sequestration compared with conventional tillage (CT), yet its net effect on N<sub>2</sub>O emissions is controversial. Cover crops (CCs) adoption is promoted in NT systems because CCs growth curbs nitrate losses via leaching. However, incorporating CC residues into the soil may have positive or negative effects on N2O emissions depending on CC species and agro-ecosystem management. A better understanding of how tillage practices and CC species affect N<sub>2</sub>O emissions is therefore needed for the development of productive agroecosystems that contribute to climate change mitigation. The objectives of this three-year (2015-2017) field experiment on a Udertic Haplustalf soil in the Po Valley were to compare N<sub>2</sub>O emissions and crop yield of soybean under NT and CT, and to examine how contrasting residues from two CCs (rye, Secale cereale L. vs hairy vetch, Vicia villosa Roth) affect N2O emissions in NT soybean and maize. We hypothesized that N<sub>2</sub>O emissions would be lower with NT than with CT and with rye residues than with vetch ones. Nitrous oxide was continuously sampled using automatic chambers during three periods (emergence, N-fixation and maturity) over the soybean-cropping season in 2015 and during the entire cropping maize season in 2017. The DNDC model was calibrated (2015 data) and validated (2017 data), and then used to estimate the annual cumulative N<sub>2</sub>O emissions in different treatments. Overall, N<sub>2</sub>O emissions in NT were 40-55% lower than in CT, for both in situ measurements (Period I) and modelled estimations. These differences could be ascribed to the higher waterfilled pore space (WFPS) and soil nitrate availability in CT than in NT. No-till also increased SOC content (28%; 0-5 cm) and earthworm abundance (5 times) compared with CT. Within NT systems, N<sub>2</sub>O emissions were 20–36% lower with rye CC than with vetch CC (P < 0.05), which was a consequence of the lower availability of soil mineral N under rye than under vetch due to the high C/N ratio of rye residues. Yield of soybean and maize under NT was higher with rye CC than with vetch CC. The combination of NT and rye CC that led to the lowest N<sub>2</sub>O emissions and highest yields should be recommended in the Po Valley region.

### 1. Introduction

Nitrous oxide (N<sub>2</sub>O) is a major greenhouse gas (GHG), with a global warming potential 265 times that of carbon dioxide over 100 years (IPCC, 2014), and is the largest ozone-depleting substance emitted by human activities (Ravishankara et al., 2009). Agricultural soils are the largest source of N<sub>2</sub>O, accounting for 45% of the total current emissions (Cayuela et al., 2017) and an estimated contribution of 59% of total emissions by 2030 (Hu et al., 2015). The majority of N<sub>2</sub>O emissions are produced through denitrification and nitrification, which are mainly controlled by substrate availability, such as ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), labile carbon (C), and oxygen concentration (Beheydt et al.,

2008). As soil-crop management (e.g. tillage intensity, nitrogen (N) fertilization, irrigation, and crop residue retention) regulates these soil factors, agriculture has a diversity of means to mitigate  $N_2O$  emissions (Abalos et al., 2013; IPCC, 2014).

Conservation tillage systems (*i.e.* no-till and reduced tillage) have been largely promoted as suitable practices to offset GHG emissions due to their ability to sequester C in soils (Tabaglio et al., 2009). However, reports on the effect of no-till (NT) on N<sub>2</sub>O emission have been contradictory: some studies found that N<sub>2</sub>O fluxes were higher in NT than in conventional tillage (CT) under imperfectly drained clay-loam soils when NT increased soil compaction (Ball et al., 1999); in long-term field experiments, under well-drained soils, NT did not increase

\* Corresponding author. *E-mail address:* andrea.fiorini@unicatt.it (A. Fiorini).

https://doi.org/10.1016/j.still.2019.104442

Received 19 October 2018; Received in revised form 19 August 2019; Accepted 30 September 2019 Available online 22 October 2019 0167-1987/ © 2019 Elsevier B.V. All rights reserved. (Jantalia et al., 2008) or even decreased N2O emissions (Omonode et al., 2011). Soil compaction increases during the transition from CT to NT (Alvarez and Steinbach, 2009; Soane et al., 2012; Fiorini et al., 2018). This tends to decrease soil porosity (Palm et al., 2014), leading to anoxic conditions that promote N<sub>2</sub>O losses (Linn and Doran, 1984). Conversely, the increase of soil organic matter (SOM) concentration and macro aggregate water-stability in the uppermost soil layer, under NT, may decrease anaerobic conditions and improve soil gas diffusivity, resulting in lower N<sub>2</sub>O emission than under CT (Mutegi et al., 2010; Plaza-Bonilla et al., 2014). No-till can also increase earthworm abundance, which in turn may increase N<sub>2</sub>O emissions by increasing N mineralization (Lubbers et al., 2013), or decrease N<sub>2</sub>O due to their burrowing activity that improves water infiltration and decreases water content of soil in the upper layers. Further studies are needed to shed light on the conditions under which NT represents a viable option to simultaneously increase SOC while mitigating N<sub>2</sub>O emissions (van Kessel et al., 2013).

Adoption of NT is highly debated, particularly for its potential negative effects on crop yield (Pittelkow et al., 2015). However, combining NT with other practices of conservation agriculture, such as the use of cover crops (CCs), could improve crop production. Cover crops may affect N<sub>2</sub>O emissions by regulating key soil factors (Mitchell et al., 2013), including the availability of mineral N and C sources for the soil microbial communities, soil pH, soil structure and microbial community composition (Abalos et al., 2014; Maris et al., 2018). Selecting adequate CC species may decrease soil mineral N and water content, thus reducing N<sub>2</sub>O emissions. For instance, legume CCs, having N rich residues, decrease N fertilizer need in subsequent crops, thus potentially lowering N2O emissions. On the other hand, non-legume CCs may offer a better option than legumes to capture excess  $NO_3^-$  in the soil, increasing plant biomass and improving soil structure, which in turn decrease N<sub>2</sub>O (Barthes et al., 2006). The chemical composition of CC residues significantly affects N<sub>2</sub>O emissions (Aulakh et al., 2001; Millar and Baggs, 2004; Garcia-Ruiz and Baggs, 2007): a low C/N ratio (e.g. legume crops) may increase N<sub>2</sub>O emission compared with a high C/N ratio (e.g. grasses) (Toma and Hatano, 2007; Petersen et al., 2011).

The objectives of this study were to measure the effect of contrasting tillage systems (NT and CT) on N<sub>2</sub>O emissions during soybean (*Glycine max* L. Merr.), and to examine how CC residues (rye *vs* hairy vetch) affect N<sub>2</sub>O emissions under NT during soybean and maize (*Zea mays* L.) cultivation. The following hypotheses were tested: (i) N<sub>2</sub>O emission are lower under NT with rye CC than in CT without CCs, and (ii) N<sub>2</sub>O emissions under NT are higher with a legume (hairy vetch) CC than with a grass (rye) CC, due to the low C/N ratio of the legume residue. Nitrous oxide measurements were carried out during two field monitoring campaigns and cumulative fluxes were estimated using the DNDC model (Li et al., 1992), which was previously calibrated and validated with field data as explained below. The DNDC has been extensively used to simulate N<sub>2</sub>O emissions, particularly under contrasting agronomic practices (*e.g.* Uzoma et al., 2015; Abalos et al., 2016).

# 2. Materials and methods

#### 2.1. Site and soil characteristics

A two-year measuring campaign was carried out on a long-term field study (initiated in 2010) at the CERZOO experimental station, in Piacenza (45°00′21.6″N, 9°42′27.1″E; altitude 68 m), Po Valley, Northern Italy. This site is representative of intensive agriculture in northern Italy. It is characterised by a temperate climate with an annual mean temperature of approximately 12.2 °C, and precipitation of 778 mm. Rainfall and air temperature during the sampling period were monitored with an automatic meteorological station located at the experimental field.

The soil is a fine, mixed, mesic Udertic Haplustalf (Soil Survey Staff,

2014), with a silty clay loamy texture (sand 122, silt 462, and clay 416 g kg<sup>-1</sup>) in the upper layer (0–30 cm), well drained and non-saline. Detailed soil physico-chemical characterization and classification are reported in Fiorini et al. (2018). The main physico-chemical properties at the beginning of the experiment were: pH 6.8, soil organic carbon 12.8 g kg<sup>-1</sup>, total N 1.2 g kg<sup>-1</sup>, available P 32 mg kg<sup>-1</sup>, exchangeable K 294 mg kg<sup>-1</sup>, and cation exchange capacity 30 cmol<sup>+</sup> kg<sup>-1</sup>.

# 2.2. Experimental design, treatments and crop management

The field experiment was established in 2010 to compare: (i) conventional tillage (CT), which included autumn plowing (30 cm depth) with crop residues incorporation into the soil and two spring rotating harrowing (15 cm) to provide a suitable seedbed, and (ii) no-till (NT), consisting of direct sowing on untilled soil and residue retention on the soil surface. During the non-cropping season, CCs were sown in NT plots right after harvesting of the previous main crop (October). The experiment was laid out in a randomized complete block design, with four blocks (replicates) and three treatments: CT, NT with hairy vetch (*Vicia villosa* Roth) as CC (NT vetch), and NT with rye (*Secale cereale* L.) as CC (NT rye). The plot size was  $1430 \text{ m}^2$  ( $65 \text{ m} \times 22 \text{ m}$ ) with buffer rows of 4 m between plots. Treatment characteristics and main farm operations during the experiment are shown in Tables 1 and 2.

Between 2015 and 2017, the crop sequence was a three-year crop rotation, with soybean (*Glycine max* L. Merr.), winter wheat (*Triticum turgidum* L. var. *durum*), and maize (*Zea mays* L.) as cash crops (Table 1). In 2015 the main crop was soybean (maturity group 1-), which was planted in May and harvested at the end of October. Eighteen days before planting soybean, both CCs were terminated in NT plots by spraying 2.41 ha<sup>-1</sup> of Glyphosate. No fertilizer was applied during the CCs and soybean cropping seasons (Table 1). Durum winter wheat was sown on November 19th. Nitrogen fertilizer (ammonium nitrate [AN]; 27% N) was applied on February 25th, 2015 at a rate of 170 kg N ha<sup>-1</sup>. Wheat harvesting took place at the beginning of July 2016. In NT plots, rye and vetch were seeded on October 12th, 2016, and terminated with 2.41 ha<sup>-1</sup> of Glyphosate, 17 days before planting maize (FAO 300) (Table 1).

Nitrogen fertilizer was applied to maize in two side dressings: 81 kg N ha<sup>-1</sup> were applied at the growth stage V2-V3, and 119 kg N ha<sup>-1</sup> at V5-V6. Harvest took place in the last week of September (Table 1). In both years, soybean and maize were sprinkler-irrigated to prevent water stress (Table 1). The irrigation water doses were estimated from the crop evapotranspiration (etc) of the preceding week (net water requirements). This was calculated daily as etc = Kc X ETo, where ETo is reference evapotranspiration calculated by the FAO Penman-Monteith method (Allen et al., 1998) using data from the meteorological station located in the experimental field. Crop coefficients (Kc) were calculated as a function of thermal time using an equation developed by Martínez-Cob (2008) in the case of maize, and by Payero and Irmak (2013) for soybean, obtained under the same climatic conditions as our experiment. Thermal time was computed as the cumulative daily difference between daily mean air temperature and a basal air temperature of 8 °C (Kiniry, 1991). The crop irrigation requirements (CIR) were determined weekly as the difference between the *etc* and the effective precipitation, which was estimated as 75% of total weekly precipitation (Dastane, 1978). The irrigation amount applied to the maize and soybean crops was equal to the CIR (Table 1). In the NT plots, after harvesting soybean (2015) and maize (2017), crop residues were left on the soil surface (the whole plant aboveground biomass except the grain biomass).

#### 2.3. Gas sampling and quantification

In 2015, N<sub>2</sub>O emissions were measured under CT, NT vetch (NT vetch-15) and NT rye (NT rye-15) in three periods during soybean cropping season: (i) from May 15th to June 11th (Period I), during the *emergence phase* (vegetative growth), CT vs NT rye-15; (ii) from June

Field management activities in 2015 and 2017. Crop (main crop and cover crop [CC]), tillage system, operation, nitrogen fertilizer dose, and irrigation are reported.

Crop	Tillage system	Operation	Date (dd/mm/yy)	Gas sampling day (n°)	Fertilizer (kg N ha <sup>-1</sup> )	Irrigation (mm)
Rye CC	NT	Seeding	21/10/2014			
-		Fertilization	13/03/2015			
		Harvesting	20/04/2015			
		Herbicide treatment	21/04/2015			
Vetch CC	NT	Seeding	21/10/2014			
		Fertilization	13/03/2015			
		Harvesting	20/04/2015			
		Herbicide treatment	21/04/2015			
Soybean	NT	Planting	08/05/2015			
		Irrigation	21/05/2015	6 (Period I)		10
		Herbicide treatment	30/05/2015	15 (Period I)		
		Irrigation	08/06/2015	23 (Period I)		25
		Irrigation	26/06/2015	14 (Period II)		35
		Irrigation	10/07/2015	27 (Period II)		40
		Fungicidal treatment	27/07/2015	10 (Period III)		
		Irrigation	29/07/2015	12 (Period III)		40
		Fungicidal treatment	10/08/2015	25 (Period III)		
		Harvesting	01/10/2015			
		Herbicide treatment	16/10/2015			
	CT	Planting	08/05/2015			
		Irrigation	21/05/2015	6 (Period I)		10
		Herbicide treatment	30/05/2015	15 (Period I)		
		Irrigation	08/06/2015	23 (Period I)		25
		Irrigation	26/06/2015	14 (Period II)		35
		Irrigation	10/07/2015	27 (Period II)		40
		Fungicidal treatment	27/07/2015	10 (Period III)		
		Irrigation	29/07/2015	12 (Period III)		40
		Fungicidal treatment	10/08/2015	25 (Period III)		
		Harvesting	01/10/2015			
		Herbicide treatment	16/10/2015			
Rye CC	NT	Seeding	12/10/2016			
		Fertilization	09/01/2017			
		Harvesting	31/03/2017			
		Herbicide treatment	07/04/2017			
Vetch CC	NT	Seeding	12/10/2016			
		Fertilization	09/01/2017			
		Harvesting	31/03/2017			
		Herbicide treatment	07/04/2017			
Maize	NT	Planting	24/04/2017			
		Irrigation	06/05/2017	1		20
		Herbicide treatment	23/05/2017	17		
		Fertilization	26/05/2017	19	81	
		Irrigation	28/05/2017	20		25
		Irrigation	08/06/2017	29		35
		Fertilization	10/06/2017	31	119	
		Irrigation	21/06/2017	39		40
		Irrigation	05/07/2017	50		40
		Irrigation	18/07/2017	57		40
		Harvesting	28/09/2017	110		

12th to July 9th (Period II), during the N-fixation phase (vegetative growth), NT rye-15 vs NT vetch-15; and (iii) from July 17th to August 13th (Period III), during the maturity phase (reproductive growth), CT vs NT rye-15. The initial plan was to cover a complete growth cycle of soybean (2015), but N<sub>2</sub>O flux measurements could not be completed from the middle of August to the beginning of October 2015 (soybean) due to instrumental failure. However, our measurements showed that N<sub>2</sub>O emissions had returned to background levels by early August, which is consistent with the results reported in most field studies conducted under similar conditions (e.g. Almaraz et al., 2009; Negassa et al., 2015; Ladan and Jacinthe, 2017). On November 13th 2015, winter wheat was sown in all plots following the established crop rotation (Table 1), and therefore no measurements assessing CC residue effect on N2O during 2016 were undertaken. In 2017, the field monitoring campaign (May 5th - November 15th) started 11 days after planting maize (which occurred 28 days after CCs termination) (Table 1) and gas sampling took place under NT rye (NT rye-17) and NT vetch (NT vetch-17) over the entire cropping season and during the early postharvest period (Table 3).

The flux of N<sub>2</sub>O from the soil to the atmosphere was measured with the automatic chamber method coupled to the SASSFLUX system (Static Automatic Sampler for Soil FLUX measurements, Ecometrics, I) (Perego et al., 2016). The chambers (40 cm length  $\times$  40 cm width  $\times$  20 cm height) consisted of a boxshaped lid of transparent Plexiglas, placed on a steel frame inserted into the soil at a depth of 5 cm (1 day before the start of the experiment), and fixed tightly to the chamber. Each chamber delimited a measuring soil surface of  $1225 \text{ cm}^2$  ( $35 \text{ cm} \times 35$ cm). Each lid had two air sampling ports (one inlet and one outlet), opened and closed automatically by motors controlled by a computer. The chamber lids closed over the metal frames three times per hour. A small fan, installed under the lids, mixed the air within the chamber during flux measurements (Perego et al., 2016). Air samples collected from the chambers were passed to the analysers using a membrane pump through an inflow Teflon tube (4 mm inner diameter). After the analysis, a second Teflon tube redirected the sampled air into the chamber, in order to avoid pressure alterations. Each chamber lid remained closed for about 4 min per measuring cycle and one N<sub>2</sub>O concentration was measured every second. The N2O analyser was

Amount of nitrous fertilizer (Urea N 46%) applied to each treatment during soybean (2015) and maize (2017).

Year	Treatment	Topdress fertilization (kg N ha <sup><math>-1</math></sup> )
2015	СТ	0
	NT rye-15	0
	NT vetch-15	0
2017	NT rye-17	200
	NT vetch-17	200

Treatments: CT: conventional tillage; NT rye-15: no-tillage with rye cover crop residues (in 2015); NT vetch-15: no-tillage with vetch cover crop residues (in 2015); NT rye-17: no-tillage with rye cover crop residues (in 2017) + 200 kg N ha<sup>-1</sup>; NT vetch-15: no-tillage with vetch cover crop residues (in 2017) + 200 kg N ha<sup>-1</sup>.

calibrated in the laboratory with a standard  $N_2O$ -air mixture cylinder before the beginning of each date of measurements. The proper operation of the SASSFLUX systems was controlled weekly until the end of the field experiment. A detailed description of SASSFLUX structure and functionality is reported in Perego et al. (2016).

Two chamber collars were installed in each plot. The collars were positioned approximately 10 cm apart from the planted row (soybean or maize). No vegetation was allowed to grow within the chamber. During all sampling campaigns, the four automatic chambers of the SASSFLUX system were moved from a collar to another on a weekly basis, thus collecting flux measures from all the experimental plots.

The N<sub>2</sub>O emission flux ( $F_{N2O}$ , nmol m<sup>-2</sup> s<sup>-1</sup>) through the soil surface was calculated using the equation described by Perego et al. (2016):

$$F_{N2O}(t) = \frac{dN_2O}{dt} \times \frac{P}{R \times T} \times \frac{V}{A}$$
(1)

Where the scalar variation of N<sub>2</sub>O concentration on time (dN<sub>2</sub>O dt<sup>-1</sup>, ppb s<sup>-1</sup>) during the lid closure is related to the volume (V, m<sup>3</sup>), surface area (A, m<sup>2</sup>), pressure (*P*, Pa) and temperature in the chamber (*T*, °K), and to the universal gas constant ( $R = 8.3144 \text{ m}^3 \text{ Pa K}^{-1} \text{ mol}^{-1}$ ).

The flux of N<sub>2</sub>O was determined by measuring the increase in N<sub>2</sub>O concentration (above the ambient air concentration) in each enclosed chamber over a determined period of time (Chadwick et al., 2014). A best-fit linear regression was used to determine  $dN_2O/dt$ . To minimize nonlinearity in the  $dN_2O/dt$  calculation and the risk of underestimating the N<sub>2</sub>O flux, only the first 300 s of each chamber measurement were used (Chadwick et al., 2014). Cumulative N<sub>2</sub>O emissions (kg ha<sup>-1</sup>) were calculated by linear interpolation as the mean of the cumulative fluxes of the chambers times the number of days between two adjacent sampling events (using the trapezoid rule) (Maris et al., 2016).

#### 2.4. Soil and plant biomass analyses

To determine bulk density (0–30 cm) and SOC (0–5 and 5–15 cm), soil samples were collected on May 6th, 2015 and on April 20th, 2017, before soybean and maize planting, respectively. Four randomly-selected undisturbed soil core samples were collected from each plot, using a steel auger of 5 cm diameter. Soil bulk density was determined according to the cylinder method (Gómez-Paccard et al., 2015), while samples for SOC determination were air dried, ground with a rubber pestle, sieved to 2 mm and analyzed with the Walkley-Black method. The soil water content at field capacity and wilting point was estimated by the van Genuchten-Mualem soil hydraulic model based on measured soil texture information (Van Genuchten, 1980).

After starting gas measures, soil samples were collected every 7 days over the entire cropping seasons (both in 2015 and 2017, under soybean and maize, respectively), using a self-constructed steel tube sampler (3 cm diameter). In detail, four undisturbed soil cores were randomly sampled in each plot to determine soil nitrate (NO3<sup>-</sup>-N) and ammonium (NH4<sup>+</sup>-N) concentrations, as well as water-filled pore space (WFPS) in the top 0–10 cm soil layer. To determine  $NO_3^{-}$ -N and  $NH_4^{+}$ -N concentrations, 10 g of homogeneously mixed soil was extracted with 50 ml of HCl (1 M) and pipetted into 96-well quartz microplates. NO<sub>3</sub><sup>-</sup>-N was then analyzed with dual wavelength UV spectroscopy (275, 220 nm) on acidified (HCl 1 M) samples. NH<sub>4</sub>-N, was measured through colorimetric Berthelot reaction (Rhine et al., 1998) based on a 96-well microplate format and read with a microplate reader (Biotek Synergy 2, Winooski, VT, USA) (Ferrarini et al., 2017). Gravimetric water content of soil was determined by drying soil samples at 105 °C until constant weight (Fiorini et al., 2018). The WFPS was calculated as the ratio between soil volumetric water content and total soil porosity. Total soil porosity was calculated by measuring the bulk density of the soil (CT and NT) according to the relationship: soil porosity = 1 - (soil bulk)density / 2.65); assuming a particle density of 2.65 Mg m<sup>-3</sup> (Danielson et al., 1986). Water-filled pore space was measured every time when soil samples were taken to determine soil nitrate (NO3-N) and ammonium (NH4+-N) concentrations. In detail, WFPS measurements occurred every two weeks during the soybean cropping cycle, and weakly during the maize cropping cycle until the second topdress fertilization (Fig. 6), while afterwards every two weeks. A temperature probe inserted 10 cm into the soil was used to measure soil temperature.

Grain yield and above-ground biomass weight were measured by harvesting three randomly selected  $4.0 \times 1.4$  m squares from each plot. Above-ground biomass was manually cut at the soil level and weighed. Grain and straw were also separated for both soybean and maize. The dry weight biomass of soybean and maize (grain and straw), vetch, and rye was gravimetrically determined by drying biomass at 70 °C until constant weight. The C/N ratios of the collected biomass were

Table 3	
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Timing for gas sampling and modelling for each period and year (2015 and 2017)

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Year	Period number	Measured treatment	Start of sampling	End of sampling	Duration of the sampling period (days)	Modeled treatment	Start of model run	End of Model run	Duration of the modeled period (days)		
2015	1	CT and NT rye-15	15/05/2015	11/06/2015	28	CT and NT rye-15	15/05/2015	11/06/2015	28		
	2	NT rye-15 and NT vetch-15	12/06/2015	09/07/2015	28	NT rye-15 and NT vetch-15	12/06/2015	14/07/2015	28		
	3	CT and NT rye-15	17/07/2015	13/08/2015	28	CT and NT rye-15	15/07/2015	13/08/205	28		
	1	-			-	CT	01/01/2015	31/12/2015	365		
		-			-	NT rye-15	01/01/2015	31/12/2015			
		-			-	NT vetch-15	01/01/2015	31/12/2015			
2017	1	NT rye-17 and NT vetch-17	05/05/2017	28/09/2017	110	NT rye-17 and NT vetch-17	05/05/2017	28/09/2017	110		
	1	NT rye-17 and NT vetch-17	29/09/2017	14/11/2017	45	NT rye-17 and NT vetch-17	29/09/2017	14/11/2017	45		
		-	-	-	-	NT rye-17 and NT vetch-17	01/01/2017	31/12/2017	365		

determined by Dumas combustion method with an elemental analyzer (VarioMax C:NS, Elementar, Germany). N inputs due to aboveground biomass of cover crop residues (rye and vetch) were calculated by multiplying the weight of each biomass fraction by its N concentration.

The yield-scaled  $N_2O-N$  emissions were calculated according to van Groenigen et al. (2010), and Grassini and Cassman (2012) as follows:

Yield-scaled N<sub>2</sub>O-N emission = cumulative N<sub>2</sub>O-N emission/grain yield (kg N<sub>2</sub>O-N  $Mg^{-1}$  grain yield). (2)

Soil samples were collected for earthworms (*Lumbricidae*) count in October when their activity was highest. In 2015, eight soil blocks (four in CT and four in NT) of  $20 \times 20 \times 20$  cm were sampled using the hand sorting method (VSA, FAO, 2008); the living earthworms were washed, kept in a refrigerator for 48 h, weighed and counted. In 2017, the same method was used to sample earthworms, but only four soil blocks of  $20 \times 20 \times 20$  for NT were sampled. The mean number of individuals was calculated according to VSA, FAO (2008).

#### 2.5. Model description, calibration and validation

The DNDC (DeNitrification-DeComposition) model was used to simulate and evaluate the cumulative flux of  $N_2O$  for the entire soybean and maize cropping season under different tillage systems (CT vs NT rye and vetch-15) and with different cover crops in NT (NT vetch-15 vs NT rye-15 and NT vetch-17 vs NT rye-17). Additionally, the model was run to predict the cumulative flux of  $N_2O$  for the entire year in 2015 and 2017. The DNDC model (version 95; http://www.dndc.sr.unh.edu), which simulates soil C and N cycling, is based on sub-models for soil and climate, crop growth, and organic matter decomposition (Li et al., 1992, 1994). Major soil processes for  $N_2O$  production such as nitrification and denitrification are included in other sub-models (Li et al., 2006). Soil temperature, water content, water flow, water uptake by plants, nitrification and denitrification are described and calculated on either a daily or hourly basis within the model, while DNDC outputs are provided on a daily basis (Congreves et al., 2016).

The following field data were used as input to calibrate the model: (1) local meteorological data (daily maximum and minimum air temperature, precipitation, wind speed and relative humidity) in 2015; (2) main soil physical and chemical properties in the topsoil (0–30 cm) (*e.g.* soil texture, pH, C/N ratio, concentration of total N) and selected properties at 0–10 cm soil depth in 2015 (*e.g.* SOC, soil bulk density, water filled pore space at field capacity and wilting point); and (3)

agricultural management information for soybean (*e.g.*, residue rate and C/N ratio of previous cover crop; crop parameters and yield, tillage, fertilization, irrigation, planting and harvesting dates) (Tables 2 and 4).

Data used to validate the model were: (1) local daily meteorological data (daily maximum and minimum air temperature, precipitation, wind speed and relative humidity) in 2017; (2) selected soil physical and chemical properties at 0-10 cm soil depth in 2017 (e.g. SOC, soil bulk density, water filled pore space at field capacity and wilting point); (3) agricultural management information for maize (e.g., residue rate and C/N ratio of previous cover crop; crop parameters and yield, tillage, fertilization, irrigation, planting and harvesting dates). To calibrate the input parameters iteratively we tested a set of different values, ranging from  $\pm 10\%$  of the measured value. The parameters that were calibrated for our experimental field were: maximum biomass production (limited to a range based on regional corn production potential), biomass C:N ratios, water demand, and thermal degree days to maturity. During the calibration of one parameter, all the others were kept constant. For each parameter value, a statistical analysis was performed to verify if the new value improved the model's ability to predict N2O emissions. The parameter values that gave the smallest root mean square error (RMSE) and correlation coefficients (R) that were closest to 1 were chosen as the final values (Abalos et al., 2016).

# 2.6. Statistical analysis and statistical criteria for model performance evaluation

The normal distribution of the *in situ* N<sub>2</sub>O-N flux data was verified using the Shapiro-Wilk test; this was carried out with the JMP 12 statistical software (SAS Institute, 2014). When necessary, in order to fulfill the assumption of normality, data were log transformed prior to analysis. *F* test was performed with the Statistical Analysis System (SAS) software (SAS Institute, 2014). A one-way ANOVA was used to test whether the cumulative N<sub>2</sub>O emissions over the field monitoring periods depended on the tillage systems (CT *vs* NT rye-15) and cover crops (NT rye-15 *vs* NT vetch-15 and NT rye-17 *vs* NT vetch-17). Significant differences among treatment means (grain yield and yield scaled N<sub>2</sub>O emission in 2015) were further examined using Tukey's multiple range test at the 0.05 probability level. Data in the tables and figures are shown as average values  $\pm$  standard errors.

Correlation analysis was used to test the relationship between *in situ* N<sub>2</sub>O-N emissions and the soil abiotic factors (*i.e.* soil temperature, WFPS,  $NO_3^{-}$ -N and  $NH_4^{+}$ -N) and cover crop residue variables (dry

Table 4

DNDC input data for conventional tillage in 2015 (CT), no-till with rye cover crops in 2015 (NT rye-15) and in 2017 (NT rye-17), and no-till with vetch cover crops in 2015 (NT vetch-15) and 2017 (NT vetch-17).

Input data	2015		2017		
	СТ	NT rye-15	NT vetch-15	NT rye-17	NT vetch-17
Climatic conditions					
Latitude and longitude (degree)	45º5'N. 9º69'E	45°5'N. 9°69'E	45°5'N. 9°69'E	45°5'N. 9°69'E	45º5'N. 9º69'E
Slope	0	0	0	0	0
N concentration in rainfall (mg N $1^{-1}$ )	0.001	0.001	0.001	0.001	0.001
Annual increasing rate of atmospheric CO <sub>2</sub> concentrations (ppm)	2	2	2	2	2
Average daily temperature (°C)	15	15	15	15	15
Yearly accumulated precipitation (mm)	590	590	590	536	536
Soil proprieties (0-10 cm)					
Soil texture	silt clay				
Clay content (%)	46.00	46.00	46.00	46.00	46.00
Soil pH	6.80	6.80	6.80	6.80	6.80
Soil bulk density (g cm <sup>-3</sup> )	1.33	1.24	1.24	1.18	1.18
WFPS at field capacity	0.60	0.57	0.52	0.53	0.51
WFPS at wilting point	0.09	0.12	0.11	0.11	0.10
Initial organic C content at surface soil (kg C kg $^{-1}$ )	0.01	0.02	0.02	0.02	0.02
Initial nitrate at surface (mg N kg $^{-1}$ )	12.05	3.34	13.20	14.91	26.15
Soil C/N	10.19	10.08	10.29	10.08	10.29

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matter, N concentration and rate of total N retained in the biomass). Correlations were assessed using the non-parametric Spearman rank coefficient ( $\rho$ ). A P-value of 0.05 was used as the threshold for statistical significance.

The model performance was measured by quantifying the discrepancy between modelled and measured values of soil N<sub>2</sub>O emissions. Several statistical metrics were calculated including the root mean square error (RMSE), model efficiency (ME), correlation coefficient (R) and the bias was expressed as a percentage using the relative error (E). RMSE measures absolute prediction error, but in a quadratic sense, and is therefore more sensitive to outliers. Positive ME values indicate good performance and *vice versa*. A RMSE equal to 0 and an ME equal to 1 indicate a perfect fit. The significance of the RMSE and E were determined by comparing them to the values of RMSE and E that would be obtained at the 95% confidence interval of the replicated values (RMSE<sub>95</sub> and E<sub>95</sub>) (Smith and Smith, 2007). Annual and crop season (soybean and maize) cumulative fluxes for model outputs were calculated as the sum of simulated daily fluxes.

# 3. Results

# 3.1. Environment, soil temperature and water-filled pores

During the soybean cropping season (from May 8th to October 1st,

2015), mean daily temperature ranged from 9.5 to 37.8 °C and the cumulative rainfall was 173 mm (Fig. 1a). The corresponding values during the maize cropping season were 7.1 to 36.5 °C and 206 mm. In 2015, soil temperature was generally slightly lower under NT (0.5–1 °C) than under CT (Fig. 1b). In 2017, soil temperature was measured only in NT plots and it was recorded from April to mid-November (Fig. 1b). The lowest soil temperatures occurred at the beginning of April and at the beginning of October, (7 and 11 °C, respectively). The warmest soil temperatures in 2017 occurred in June, (26 °C) (Fig. 1b).

In Period I of the soybean cropping cycle, WFPS values were significantly higher for CT (60%) than for NT rye-15 (57%), but the differences were small and the values never exceeded 63% (Table 5). In Period II, mean WFPS values were 55 and 53% for NT rye-15 and for NT vectch-15, respectively (Table 5). In Period III, WFPS was on average 55 and 52% for NT rye-15 and for CT (Table 5). In all periods, WFPS values followed the same temporal pattern (Fig. 2a–c) and no significant differences between treatments were found during these periods (Table 5). During the maize cropping cycle, the corresponding values ranged from 42 to 63% under NT rye-17 and from 36 to 64% under NT vecth-17 (Fig. 2d); no statistically significant differences were found between these cover crop treatments (Table 5).



**Fig. 1.** Evolution of daily precipitation (bars) and average daily temperature (line) of the field site in 2015 and 2017 (a); evolution of soil temperature (0–10 cm): for CT and NT rye + vetch-15 treatments in 2015 and for NT rye + vetch-17 treatment in 2017 (b).

Analysis of variance of WFPS (0–10 cm; %), nitrate and ammonium concentration in the soil (0–10 cm; mg $NO_3^{-}$ -N kg <sup>-1</sup> and mg $NH_4^{+}$ -N kg	<sup>1</sup> ), soil organic carbon
(0–5 and 5–15 cm; g kg <sup>-1</sup> ), bulk density (0–30 cm; Mg m <sup>-3</sup> ), and earthworm abundance (n° m <sup>-2</sup> ) in 2015 and 2017.	

Year	Crop planted	Period	Treatment	WFPS (%)	NO <sub>3</sub> -N (mg kg <sup>-1</sup> )	$NH_4$ -N (mg kg <sup>-1</sup> )	SOC (g l	(g <sup>-1</sup> )	Bulk density (Mg m <sup>-3</sup> )	Earthworms (n° $m^{-2}$ )
				0-10 cm	0-10 cm	0-10 cm	0-5 cm	5-15 cm	0-30 cm	·
2015	Soybean	I - Vegetative growth	CT	60.31a	9.44a	0.37a	12.58b	12.19	1.33a	67b
		(Emergence phase)	NT rye-15	57.32b	4.74b	0.18b	17.55a	13.17	1.24b	367a
			Significance	*	*	*	*	n.s.	*	*
		II - Vegetative growth (N-	NT rye-15	54.75	5.17	0.19	17.55	13.17	1.24	-
		fixation phase)	NT vetch-15	52.64	5.24	0.18	18.44	14.02	1.24	-
			Significance	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-
		III - Reproductive growth	CT	52.15	4.77	0.18	12.58b	12.19	1.33a	67b
		(Maturity phase)	NT rye-15	54.85	4.91	0.17	17.55a	13.17	1.24b	367a
			Significance	n.s.	n.s.	n.s.	*	n.s.	*	*
2017	Maize	Crop season + postharvest	NT rye-17	53.04	17.76b	0.66	22.20	14.25	1.18	-
			NT vetch-17	51.08	21.56a	0.76	20.25	14.50	1.18	-
			Significance	n.s.	*	n.s.	n.s.	n.s.	n.s.	-

SOC: soil organic carbon. Within columns, means followed by the same letter are not significantly different according to F test (P = 0.05); \*Significant at the 0.05 probability level; ns: not significant.



**Fig. 2.** Soil water-filled pore space (WFPS) (0–10 cm) for all treatments for each period of soybean (a, b, and c) and for maize (d). Treatment abbreviations refer to conventional tillage (CT), no-till with cover crop of rye in 2015 (NT rye-15) and 2017 (NT rye-17), and no-till with cover crop of vetch in 2015 (NT vetch-15) and 2017 (NT vetch-17).

3.2. Soil Mineral N, organic carbon concentration, bulk density and earthworms

Soil  $NO_3^{-}$ -N concentration was significantly higher in CT than in NT rye-15 in Period I of the soybean cycle (Fig. 3a; Table 5), while in Periods II and III, no differences were found between treatments (Fig. 3b, c). During the maize cropping cycle, soil  $NO_3^{-}$ -N concentration (Fig. 3d) was significantly higher in NT vetch-17 than in NT rye-17 (Table 5).

During soybean cropping cycle, soil  $NH_4^+$ -N concentration in NT rye-15 was generally below 0.64 mg kg<sup>-1</sup> over Period I, and mean  $NH_4^+$ -N concentration was significantly lower in NT (0.18 mg kg<sup>-1</sup>) than in CT (0.37 mg kg<sup>-1</sup>) (Table 5 and Fig. 4a). No significant differences in soil  $NH_4^+$ -N concentration were found between NT rye-15 and NT vetch-15 in Period II and between CT and NT rye-15 in Period III (Table 5 and Fig. 4b, c). During maize cropping cycle, soil  $NH_4^+$ -N concentration was not different between NT vetch-17 and NT rye-17 (Table 5 and Fig. 4d).

NT rye-15 significantly increased SOC during the soybean cropping cycle in the 0–5 cm soil layer compared with CT (Period I and III), but not in the 5–15 cm soil layer (Table 5). In detail, mean SOC concentration in the 0–5 cm soil layer for NT rye-15 was 17.55 g kg<sup>-1</sup>, which was 28% higher than for CT (12.58 g kg<sup>-1</sup>). In maize, there were no significant differences in SOC (both at 0–5 and 5–15 cm depth) between cover crop treatments (Table 5).

Soil bulk density in the 0–30 cm soil layer was higher in CT than in the NT rye-15 during Period I and III of soybean cropping cycle (Table 5). The earthworm abundance under NT was significantly higher than under CT. In Period II of the soybean and during the maize cropping season (2017), no significant differences in soil bulk density were found between NT rye-17 and NT vetch-17 (Table 5).

# 3.3. Biomass production and N uptake by cover crops, and grain yield of cash crops (soybean and maize)

In both years, the C/N ratio was significantly higher in rye residues than in vetch residues (Table 6). In addition, rye tended to accumulate more aboveground biomass than vetch, although no significant differences in dry matter (DM) were found between NT-rye and NT-vetch treatments (Table 6). The N concentrations in vetch CC residues were significantly higher than in rye CCs and in turn the total N in NT vetch



**Fig. 3.** Soil nitrate concentration  $(NO_3^-N)$  (0–10 cm) for all treatments for each period of soybean (a, b, and c) and for maize (d). Treatment abbreviations refer to conventional tillage (CT), no-till with cover crop of rye in 2015 (NT rye-15) and 2017 (NT rye-17), and no-till with cover crop of vetch in 2015 (NT vetch-15) and 2017 (NT vetch-17).



**Fig. 4.** Soil ammonium concentration  $(NH_4^+ -N)$  (0–10 cm) for all treatments for each period of soybean (a, b, and c) and for maize (d). Treatment abbreviations refer to conventional tillage (CT), no-till with cover crop of rye in 2015 (NT rye-15) and 2017 (NT rye-17), and no-till with cover crop of vetch in 2015 (NT vetch-15) and 2017 (NT vetch-17).

biomass markedly exceeded that of NT rye (Table 6).

Soybean yield was significantly affected by the tillage systems and CCs (Table 7), being significantly higher for NT rye-15 than for CT and NT vetch-15 (Table 7). Regarding maize yield, a significant difference between CC treatments (NT rye-17 and NT vetch-17) was observed.

# 3.4. Field measurements of nitrous oxide emissions

# 3.4.1. Nitrous oxide emissions during soybean crop season (2015)

The magnitude of daily  $N_2O-N$  fluxes varied among the three periods tested during soybean cropping cycle (Fig. 5a–f). As reported above, Period I (*emergence phase*) and Period III (*maturity phase*) had different tillage systems (CT vs NT rye-15), while Period II (*N-fixation phase*) received different CC residues application (NT vetch-15 and NT rye-15) (Fig. 5c, d; Table 7). In all periods, the largest N<sub>2</sub>O-N fluxes generally appeared after irrigation events (Fig. 5a–f).

In Period I, N<sub>2</sub>O-N fluxes ranged from 1.08 to 537.06 g ha<sup>-1</sup> d<sup>-1</sup> in CT and from 0.63 to 323.53 g ha<sup>-1</sup> d<sup>-1</sup> in NT rye-15 (Fig. 5a, b). The first and highest peak occurred on day 8 after monitoring started, while a second minor peak was measured on day 26, in both CT and NT rye-15 (Figs. 5a, b). Both peaks occurred 1 day after irrigation events (Table 1). Cumulative N<sub>2</sub>O-N emissions at the end of Period I were

C/N ratio, dry matter content (Mg  $ha^{-1}$ ), N concentration (%) and total N applied (kg N  $ha^{-1}$ ) with cover crop residues (rye and vetch) in 2015 and 2017.

Year	Cover crop residues	Treatment	C/N ratio	Dry matter (Mg ha <sup>-1</sup> )	N concentration (%)	Total N (kg N ha <sup>-1</sup> )
2015	Rye	NT rye-15	47a	3.12	1.17b	37.06b
	Vetch	NT vetch-15	16b	2.09	3.90a	80.70a
		Significance	*	n.s.	*	*
2017	Rye	NT rye-17	32a	2.34	3.01b	69.83b
	Vetch	NT vetch-17	13b	1.97	4.61a	91.25a
		Significance	*	n.s.	*	*

Within columns. means followed by the same letter are not significantly different according to *F* test (p = 0.05); \*Significant at the 0.05 probability level; ns: not significant.

# significantly lower in NT rye-15 than in CT (Table 7).

In Period II, N<sub>2</sub>O-N fluxes ranged from -3.72 to 47.89 g ha<sup>-1</sup> d<sup>-1</sup> in NT rye-15 and from 0.78 to 78.46 g ha<sup>-1</sup> d<sup>-1</sup> in NT vetch-15 (Fig. 5c, d). N<sub>2</sub>O-N fluxes followed the same pattern for both treatments, and the peak of N<sub>2</sub>O-N fluxes was measured 16 days after the start of measurements, 1 day after irrigation was applied (35 mm) (Table 1). In NT rye-15, soil acted as a sink for N<sub>2</sub>O, which led to negative fluxes from day 8 to day 14 after sampling started (Fig. 5c, d). Cumulative N<sub>2</sub>O-N emissions did not differ between NT rye-15 and NT vetch-15 in this period (Table 7).

In Period III larger N<sub>2</sub>O-N fluxes were observed in CT than in NTrye-15 for most days (Fig. 5e, f). N<sub>2</sub>O-N fluxes ranged from 0.39 to 107.85 g ha<sup>-1</sup> d<sup>-1</sup> in CT and from -0.56 to 28.95 g ha<sup>-1</sup> d<sup>-1</sup> in NT rye-15 (Fig. 5e, f). Day 18 and day 17 after the start of measurements corresponded to the emission peaks in CT and in NT rye-15 respectively during this period (Fig. 5e, f). No significant differences in cumulative N<sub>2</sub>O emissions between the two treatments were found in Period III (Table 7).

During the soybean cropping cycle (Period I, II and III) the cumulative N<sub>2</sub>O emissions from all the treatments were positively correlated with soil temperature (Table 8). Moreover, the cumulative  $N_2O$ -N emissions from both treatments (NT rye-15 and CT) were positively correlated with WFPS and  $NO_3^-$ -N concentration, but only in Period I (Table 8).

3.4.2. Nitrous oxide emissions during maize cropping season and postharvest period (2017)

Over the entire sampling period, fluxes ranged between -5.87 and 717.98 g  $N_2O$ -N ha<sup>-1</sup> d<sup>-1</sup> in NT rye-17, and between -3.01 and 986.43 g  $N_2O$ -N ha<sup>-1</sup> d<sup>-1</sup> in NT vetch-17 (Fig. 6a, b). The first large  $N_2O$ -N peak was measured on day 2 after monitoring started in both treatments (Fig. 6a, b). This occurred right after the first irrigation event (Table 1) and remained the largest peak in NT rye-17 over the entire monitoring campaign (Fig. 6a). The second large peak of  $N_2O$ -N took place in both treatments following mineral fertilizer application, at day 38 (Fig. 6a, b). This second  $N_2O$ -N peak was 77% lower than the first one in NT rye-17 (Fig. 6a), while in NT vetch-17 the first and second peaks were in the same range (Fig. 6b). The cumulative  $N_2O$ -N emissions over the entire maize cropping season were significantly lower from NT rye-17 than from NT vetch-17 (Table 7).

During the postharvest (fallow) period, no notable N<sub>2</sub>O-N flux peaks were observed (Fig. 6a and b) for any treatment (NT rye-17 and NT vetch-17). The cumulative N<sub>2</sub>O-N emissions during this period (45 up to 165 days of the total monitoring session) accounted only for 3% of NT rye-17 and 5% of NT vetch-17 cumulative N<sub>2</sub>O-N emissions over the entire maize cropping season (Table 7). Significant positive correlations between cumulative N<sub>2</sub>O-N emissions and soil temperature and soil NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N concentration were found (Table 8).

# 3.5. Model evaluation

Temporal patterns of simulated and measured daily N<sub>2</sub>O-N fluxes during both soybean and maize cropping cycles were in good agreement (Figs. 5, 6). Model simulations and field observations of N<sub>2</sub>O-N emissions showed similar flux peaks after irrigation, rainfall events and fertilizer applications (fertilizer was applied only in 2017) (Table 1;

# Table 7

Measured and estimated cumulative N<sub>2</sub>O-N emission from each treatment, period and entire year, in 2015 and 2017. Mean values ± standard errors.

Year				Cumulative emission measured (kg ha $^{-1}$ )	Cumulative emission modelled $(kg ha^{-1})$	Grain yield $(Mg ha^{-1})$	Yield scaled -N <sub>2</sub> O emission (kg $N_{2}O_{2}N_{3}O_{2}N_{3}O_{2}N_{3}O_{3}O_{3}O_{3}O_{3}O_{3}O_{3}O_{3}O$
	Crop planted	Period	Treatment	N <sub>2</sub> O-N	(kg int )	(ivig ita )	N20-IN ING grain yield)
2015	Soybean	Vegetative growth	CT	2.36 ± 0.27a	2.47	-	-
		(Emergence phase)	NT rye-15	$1.41 \pm 0.21b$	1.45	-	-
			Significance	*	-	-	-
		Vegetative growth (N-	NT rye-15	$0.29 \pm 0.06$	0.24	-	-
		fixation phase)	NT vetch-15	$0.34 \pm 0.10$	0.33	-	-
			Significance	n.s.	-	-	-
		Reproductive growth	CT	$0.54 \pm 0.12$	0.46	-	-
		(Maturity phase)	NT rye-15	$0.15 \pm 0.03$	0.12	-	-
			Significance	n.s.	-	-	-
		Crop season	CT	-	4.50	3.30b	1.38a
			NT rye-15	-	2.04	3.87a	0.52c
			NT vetch-15	-	2.61	3.22b	0.82b
			Significance	-	-	*	*
		Entire year	CT	-	4.91	-	-
			NT rye-15	-	2.19	-	-
			NT vetch-15	-	2.77	-	-
2017	Maize	Crop season	NT rye-17	6.94 ± 0.87b	6.85	11.40a	0.60b
			NT vetch-17	11.12 ± 1.71a	10.88	10.37b	1.08a
			Significance	*	-	*	*
		Postharvest period	NT rye-17	$0.43 \pm 4.84$	0.19	-	-
			NT vetch-17	$0.23 \pm 2.04$	0.51	-	-
			Significance	n.s.	-	-	-
		Entire year	NT rye-17	-	7.91	-	-
			NT vetch-17	-	12.41	-	-

Within columns, means followed by the same letter are not significantly different according to F test (P = 0.05); \*Significant at the 0.05 probability level; ns: not significant.



Fig. 5. Measured (field) and estimated (model) daily fluxes of N<sub>2</sub>O for each period of soybean and for all treatments. Treatment abbreviations refer to conventional tillage (CT), no-till with cover crop of rye (NT rye-15), and no-till with cover crop of vetch (NT vetch-15). Vertical lines indicate the standard error.

Fig. 6a, b). The total bias and error differences between measured and simulated  $N_2O$ -N fluxes were within the 95% confidence levels during the calibration and validation stages (Table 9). Positive values (ranging from 0.51 to 0.80) of modelling efficiency were also obtained (Table 9). The DNDC-simulated daily  $N_2O$ -N fluxes had correlation coefficients with the measured values ranging from 0.8 to 0.9 (Table 9). There were strong variations for the relative error in simulating daily fluxes of  $N_2O$ . The model generally overestimated the magnitude of  $N_2O$  fluxes during the experiment (Table 9). However, underestimations and overestimations in daily emission values occurred both for soybean and maize (Figs. 5a–f; 6 a, b).

#### 3.6. Simulated annual nitrous oxide emissions

In 2015, the simulated annual N<sub>2</sub>O-N emissions were 4.91, 2.77 and 2.19 kg N<sub>2</sub>O-N ha<sup>-1</sup> in CT, NT vetch-15, and NT rye-15, respectively (Table 7). Therefore, on average CT increased two-fold the cumulative

N<sub>2</sub>O-N emissions compared with NT over the entire year. In 2017, the simulated annual emissions were 12.41 kg N<sub>2</sub>O-N ha<sup>-1</sup> for NT vetch-17, and 7.91 kg N<sub>2</sub>O-N ha<sup>-1</sup> for NT rye-17; that means that vetch residues increased N<sub>2</sub>O-N emissions by 57% compared with rye under NT.

# 3.7. Cover crop residues, yield-scaled N<sub>2</sub>O emissions

In both years, we found a negative correlation between cumulative *in situ*  $N_2O$ -N emissions and the C/N ratio of CC residues (Table 8). No correlations were observed between  $N_2O$ -N emissions and dry matter content in CC residues, N concentration or total N retained in the biomass of CCs (Table 8).

When cumulative N<sub>2</sub>O-N losses were related to grain yield of soybean in 2015, NT rye-15 and NT vetch-15 significantly decreased yieldscaled N<sub>2</sub>O emissions compared with CT (Table 7). In 2017, yield-scaled N<sub>2</sub>O emissions during maize cropping cycle were significantly affected by the CCs (Table 7): NT vetch-17 and NT rye-17 had 0.60 and 1.08 kg



Fig. 6. Measured (field) and estimated (model) daily fluxes of N<sub>2</sub>O during maize cropping cycle. Treatment abbreviations refer to no-till with cover crop of rye (NT rye-17), and no-till with cover crop of vetch (NT vetch-17). Vertical lines indicate the standard error.

Spearman rank correlation coefficients between mean air temperature, WFPS, nitrate and ammonium content in the soil (0-10 cm) and cover crop residues  $(C/N \text{ rate, dry matter, N concentration, total N amount (kg N) retained in the biomass) with cumulative N<sub>2</sub>O-N emissions per each period in 2015 and 2017.$ 

	N <sub>2</sub> O-N						
Variable	2015	2015					
	Period 1	Period 2	Period 3				
Mean air T	0.146	0.144	0.013	-0.075			
Soil T	0.411*	0.455*	0.314*	0.234*			
WFPS	0.553*	-0.318	-0.196	0.494*			
NO <sub>3</sub> -N	0.471*	-0.313	0.142	0.551*			
NH4 <sup>+</sup> -N	-0.230	0.160	0.211	0.450*			
C/N CCs	-	-0.885*	-	-0.667*			
D.M. CCs		-0.368	-	-0.149			
N conc. CCs	-	0.152	-	0.072			
Total N amount (kg) retained in the	-	0.265	-	0.396			
CCs biomass							

WFPS: water-filled pore space; D.M. CCs: dry matter content in cover crop residue (rye and vetch); N conc.: nitrogen concentration in cover crop residues (rye and vetch); Total amount N (kg ha<sup>-1</sup>) retained the biomass of CCs (rye and vetch). \* indicates statistical significance (*P-value* < 0.05).

#### Table 9

Statistical analyses of the model performance simulating daily fluxes of  $\mathrm{N}_2\mathrm{O}$  emissions.

2015		2017		
СТ	NT rye-15	NT vetch- 15	NT rye-17	NT vetch- 17
0.89	0.81	0.84	0.88	0.80
80.00	143.06	78.59	88.51	132.14
150.27	525.36	122.00	222.99	208.51
274.93	143.89	605.13	38.54	86.50
0.80	0.57	0.65	0.77	0.51
	2015 CT 0.89 80.00 150.27 274.93 0.80	2015           CT         NT rye-15           0.89         0.81           80.00         143.06           150.27         525.36           274.93         143.89           0.80         0.57	2015           CT         NT rye-15         NT vetch-15           0.89         0.81         0.84           80.00         143.06         78.59           150.27         525.36         122.00           274.93         143.89         605.13           0.80         0.57         0.65	2015         2017           CT         NT rye-15         NT vetch- 15         NT rye-17           0.89         0.81         0.84         0.88           80.00         143.06         78.59         88.51           150.27         525.36         122.00         222.99           274.93         143.89         605.13         38.54           0.80         0.57         0.65         0.77

R = correlation coefficient; RMSE = root mean square error; E = relative error; EF = modeling efficiency.

of N<sub>2</sub>O-N emitted per Mg of grain, respectively (Table 7).

### 4. Discussion

# 4.1. Drivers of N<sub>2</sub>O emission dynamics

We found that irrigation and mineral N-fertilizer application were the major determinants of N<sub>2</sub>O temporal dynamics in all treatments as measured *in situ* (Figs. 5a–f; 6 a, b). Accordingly, water and soil mineral N availability were the main limiting factors for N<sub>2</sub>O-producing processes during our experiment. This pattern was well captured by the DNDC model simulations (Figs. 5a–f; 6 a, b), and it is consistent with results reported in previous studies (*e.g.* Smith et al., 2008; Ludwig et al., 2011; Uzoma et al., 2015; Abalos et al., 2016).

The effect of water was intense immediately after the first irrigation and rainfall events in both years, leading to major  $N_2O$  peaks, and decreased gradually with time (Figs. 5a–f and 6 a, b). During the soybean cropping cycle,  $N_2O$  peaked in both tillage systems in Period I (emergence phase), as irrigation and rainfall events rewetted the dry soil increasing WFPS from 53 to 63%. Similar trends have been previously reported on irrigated cropping systems (Halvorson et al., 2008), showing that the addition of water to dry soils activates the microbial populations producing pulses of  $N_2O$  (Sanchez-Martín et al., 2010). This is a consequence of nitrate accumulation in soil also in dry conditions (Davidson et al., 1990) as mineralization occurs and is released with readily available carbon (Davidson et al., 1987). Subsequent

precipitation or irrigation events lead to N<sub>2</sub>O fluxes (i) by stimulating N<sub>2</sub>O production as a consequence of denitrification processes (Maris et al., 2018), and (ii) by enhancing the "piston effect", with irrigationwater pushing out N<sub>2</sub>O trapped in the soil (Machefert et al., 2004). Other irrigation or rainfall events occurred in Period II (N-fixation phase) and Period III (maturity phase), contributing to the rewet of deep soil layers, but this led to minor pulses of N<sub>2</sub>O in all treatments (Fig. 5c-f). These lower fluxes of N<sub>2</sub>O-N were most likely related to the low availability of mineral N for soil microorganisms in Period II (Nfixation phase) and III (maturity phase), due to the high plant N uptake after the emergence phase. The importance of the simultaneous availability of water and soil mineral N for N<sub>2</sub>O emissions is further shown by the contrasting emission patterns observed for the first and second fertilizer-N applications in maize (Fig. 5a, b). The first application (81 kg N ha<sup>-1</sup>; May 26<sup>th</sup>, 2017) did not result in any peak of N<sub>2</sub>O-N probably because no rainfall or irrigation event occurred immediately after N distribution (Table 1). In contrast, the second N application (119 kg N ha<sup>-1</sup>, June 10<sup>th</sup>, 2017) resulted in a fast and large peak of N<sub>2</sub>O-N, which was promoted by an increase in soil water content due to rainfall and irrigation in both NT treatments (NT vetch-17 and NT rve-17) (Fig. 5a, b).

Our findings suggest that both nitrification and denitrification processes contributed to the emission of N<sub>2</sub>O during the experiment. For example, during the first N<sub>2</sub>O-N peak of 2015, soil NO<sub>3</sub><sup>-</sup>-N concentration was sufficiently high (  $> 20 \text{ mg NO}_3^-$ -N kg<sup>-1</sup> for 21 days) and WFPS reached c. 60%, thereby favoring N<sub>2</sub>O production by denitrifiers (Maris et al., 2015). The positive correlation of  $N_2O$ -N emissions and soil NO3<sup>-</sup>-N concentration during maize cropping season (Table 8) confirms the importance of denitrification processes (Davidson, 1991; García-Marco et al., 2014). Furthermore, negative N<sub>2</sub>O-N fluxes were measured on several occasions (Fig. 5c, d), and this effect is generally attributed to denitrification, in which N<sub>2</sub>O may be reduced to N<sub>2</sub> (Merino et al., 2004; Chapuis-Lardy et al., 2007; Abalos et al., 2013; Maris et al., 2018). This occurred when low availability of NO<sub>3</sub><sup>-</sup>-N  $(< 5 \text{ mg N kg}^{-1} \text{ in Period III; Fig. 3b})$  favoured the use of other electron acceptor sources by denitrifying bacteria, such as N2O produced in soil (Okereke, 1993). However, the strong positive correlation between N<sub>2</sub>O and NH<sub>4</sub><sup>+</sup> (Table 8) showed that nitrification was also a major process leading to N<sub>2</sub>O fluxes, and that the continuous drying-wetting cycles during summer irrigated maize can result into favourable WFPS conditions for both nitrification and denitrification processes (Fig. 2d and Table 5) (Bateman and Baggs, 2005). As these soil processes may occur in close proximity, a relevant part of  $NO_3^-$  formed by nitrification in an aerobic zone can diffuse to an anaerobic zone and then be denitrified into N2O (Khalil and Baggs, 2005; Stehfest and Bouwman, 2006). In our experiment the low soil NH4<sup>+</sup> concentration in both years illustrates that nitrification indeed took place rapidly providing substrate for denitrifiers, and also that substantial losses via NH<sub>3</sub> volatilization may have occurred immediately after the surface application of urea as fertilizer (Soares et al., 2012).

The N<sub>2</sub>O-N emissions during the postharvest period in 2017 represented only a small fraction of the total crop season cumulative emissions (ranging from 0.23 to 0.43 kg N<sub>2</sub>O-N ha<sup>-1</sup>; Table 7). Low N<sub>2</sub>O-N emission during this period could be attributed to the low mineral N concentration and labile C sources from CC residues decomposition, which decreased the activity of nitrifying and denitrifying microorganisms (Davidson and Verchot, 2000). Moreover, soil temperature was positively correlated with N<sub>2</sub>O emissions, and therefore the low soil temperature during this period might have contributed to decrease N-mineralization rates, and the processes of nitrification and denitrification, thus decreasing N<sub>2</sub>O production (Ussiri and Lal, 2013).

# 4.2. Role of no-till on N<sub>2</sub>O emissions

As hypothesized, NT consistently decreased  $N_2O-N$  emissions (40–55%) compared with CT, for both *in situ* measurements (Period I)

and modelled estimations (Table 7). These results are in agreement with earlier findings (*e.g.* Jacinthe and Dick, 1997; Parkin and Kaspar, 2006; Grandy et al., 2006; van Kessel et al., 2013; Perego et al., 2016; Gillette et al., 2017).

Our study identified several mechanisms through which NT decreased N2O-N emissions. Firstly, tillage may promote SOM decomposition (Perego et al., 2019) thereby increasing labile organic C sources and mineral N availability for heterotrophic processes such as denitrification (Ruan and Robertson, 2013). In our experiment, SOC was indeed 28% lower under CT than under NT (0-5 cm; Table 5), and soil NO<sub>3</sub><sup>-</sup>-N concentration was almost three times higher in CT than in NT at the beginning of Period I (Fig. 3a), suggesting higher SOM decomposition after plowing (Gómez-Paccard et al., 2015). Secondly, high SOC concentration and low soil bulk density in NT compared with CT probably improved soil aggregation, porosity, aeration, and water infiltration in the 0-10 cm soil layer (Plaza-Bonilla et al., 2014; Gómez-Paccard et al., 2015), presumably inducing a reduction of anaerobic conditions (Garcia-Marco et al., 2016), resulting in lower N<sub>2</sub>O emission under NT (Chatskikh and Olesen, 2007; Van Kessel et al., 2013; Forte et al., 2017). Moreover, increased water infiltration under NT could have been favored by the abundance of continuous biopores, as suggested by the higher abundance (around 5 times) of earthworms measured in NT than in CT, which is in agreement with Jordan et al. (1997). Third, tillage loosened the soil and decreased crop residue density on the soil surface, both of which allowed faster soil warming up than in NT, leading to slightly warmer soil temperature (0.5-1 °C) under CT (Fig. 1b). This higher temperature may have increased to some extent mineralization rates as well as nitrification and denitrification processes under CT (Bateman and Baggs, 2005; Zhu et al., 2013).

# 4.3. Cover crop residues regulate $N_2O$ emissions during subsequent maize cropping season

Confirming our second hypothesis, N<sub>2</sub>O emissions were lower (1.6 times) during the maize cropping cycle (2017) when rye was used as CC than with vetch (Table 7). These results are in agreement with the studies of Constantinides and Fownes (1994); Millar and Baggs (2004) and Basche et al. (2014), who measured significantly higher N<sub>2</sub>O emissions from legumes (cumulative N2O emission of those studies varied from 8.51 kg N ha<sup>-1</sup> to 12.53 kg N ha<sup>-1</sup>) than from non-legumes (cumulative  $N_2O$  emission of those studies varied from 4.24 kg N ha<sup>-1</sup> to 8.90 kg N ha  $^{-1}$ ) as CCs. The higher emissions in NT vetch than in NT rye were most likely caused by the higher concentration of NO3-N measured under vetch than under rye (Table 5; Fig. 3d). This may be a consequence of some N fixed by legumes (by establishing a symbiosis with N-fixing microorganisms) being released to the soil mineral N pool during the period of active CC growth (Parkin and Kaspar, 2006), to become available for N2O-producing processes. Once the CCs are terminated, the decomposition of their residues further regulates the availability of soil mineral N. Specifically, the C/N ratio of plant residues regulates whether mineral N is immobilized by soil microorganisms (C/N ratio > 20-30) or released into the soil through mineralization (C/N ratio < 20-30) (Snyder et al., 2009; Abalos et al., 2013; Maris et al., 2018). Our results show that vetch residue decomposition (low-C/N = 15) probably released N contributing to increased N<sub>2</sub>O emissions, but it is unlikely that rye induced N immobilization, as crop yields for both soybean and maize were the highest when rye was used as cover crop (Table 7). Another possibility is that an initial N immobilization occurred due to the presence of rye residues, but the mineral N pool was not exhausted (which would hamper main crop growth), leading to a better synchronization between soil N supply and the rate of plant N uptake. Finally, the more acquisitive and deeper rooting systems of the non-legume CC (rye) may have also contributed to the lower soil mineral N and N<sub>2</sub>O emissions (McCracken et al., 1994; Abalos et al., 2018).

During the soybean cropping cycle (2015), although the emissions

tended to be higher from vetch treatment (0.34 kg  $N_2$ O-N ha<sup>-1</sup> vs  $0.29 \text{ kg N}_2\text{O-N ha}^{-1}$  from rye treatment), there were no significant differences in N<sub>2</sub>O-N emissions between the two CCs (Table 7). A possible explanation for these results is that N-fertilizers were not applied to the cash crop, as it is common practice with soybean. Adding a complementary source of mineral N via fertilizers stimulates residue mineralization (Garcia-Ruiz and Baggs, 2007; Abalos et al., 2013). The lack of fertilization probably limited the release of N from the CC residues, thereby lowering soil mineral N availability and associated N<sub>2</sub>O emissions and masking the differences between the two CC species. This is corroborated by the higher vields obtained with rve: the higher N applied with vetch residues (81 kg N ha<sup>-1</sup> vs 37 kg N ha<sup>-1</sup> from rye) than with rve residues (Table 6) did not lead to higher yield due to the limited role of N mineralization in Period II of the soybean cropping cycle. The higher yields obtained with rye also confirm that residues with high C/N ratios may release nutrients gradually, matching effectively the timing of soil N supply and plant N uptake under our experimental conditions.

#### 4.4. Implications for agricultural management

Expressing N<sub>2</sub>O emissions on a yield basis provides valuable information for estimating the environmental effects of intensive agricultural production systems (Van Groenigen et al., 2010; Venterea et al., 2011). The studies directly reporting yield-scaled N<sub>2</sub>O emissions in grain production systems provide a range of values varying over approximately one order of magnitude (Venterea et al., 2011). In the present study, yield-scaled emissions (0.52–1.38 kg N<sub>2</sub>O-N Mg<sup>-1</sup> grain) (Table 7) were in the same range of those found by Qin et al. (2012) and by Gagnon et al. (2011) (0.41-2.00 kg N<sub>2</sub>O-N Mg<sup>-1</sup> grain) for irrigated maize-soybean rotation and maize monoculture under similar climate and soil conditions. Nevertheless, the vield-scaled N<sub>2</sub>O emissions in our study were relatively higher than those previously found (i) under maize-soybean cropping system in Adviento-Borbe et al. (2007) (0.07–0.51 kg N<sub>2</sub>O-N Mg<sup>-1</sup> grain), probably because more favourable cropping conditions (e.g. 18.2 g kg<sup>-1</sup> organic C) than that of our experiment increased maize-soybean yields and in turn decreased yieldscaled values; and (ii) under maize monoculture in Mosier et al. (2006) (0.06–0.31 kg  $\rm N_2O\text{-}N~Mg^{-1}$  grain), due to the optimum crop management practices used (e.g. lower rate of N applied, deep N fertiliser placement in tilled soil) during their experiment, which further decreased N<sub>2</sub>O emissions.

During the soybean cropping cycle, higher crop grain yield and lower soil N<sub>2</sub>O emissions resulted in lower yield-scaled N<sub>2</sub>O emissions under NT than under CT (Table 7). Specifically, the yield-scaled N<sub>2</sub>O emissions in NT with vetch and rye as CCs were 41 and 62% lower than in CT, respectively (Table 7). These results show that replacing CT with NT combined with winter CCs can be a feasible alternative to mitigate N<sub>2</sub>O emissions from agricultural soils without yield penalties, corroborating earlier findings by Mosier et al. (2006); Huang et al. (2014) and Bayer et al. (2016). In addition, our study demonstrates that a shift towards conservation agriculture would have additional benefits such as promoting C sequestration *via* increases in SOC (at least in the upper soil layer) and enhancing the abundance of earthworms (Table 5), which are soil ecosystem services, including the development of soil structure and water regulation (Blouin et al., 2013).

It was found that vetch as CC increased  $N_2O$ -N emissions (by 22% in 2015 and by 37% in 2017), decreased grain yield (by 17% in 2015 and by 9% in 2017), and thereby increased yield-scaled  $N_2O$  emissions (by 37% in 2015 and by 45% in 2017) relative to rye (Table 7). Based on these results, the use of non-legume CCs should be promoted as they may provide an optimum balance between greenhouse gas emissions and agronomic productivity. Additionally, non-legume cover crops under a NT system could decrease N leaching losses compared with CT and legume CCs under NT (Gabriel et al., 2012). If a legume CC is used,

N fertilizers should be applied taking into account the expected rate and timing of available N from mineralization of their N-rich CC residues.

# 5. Conclusions

Nitrous oxide and yield-scaled  $N_2O$  emissions were lower for NT than for CT (c. 51%); in addition, NT increased SOC concentration and earthworm abundance. This shows that replacing CT with NT can be a feasible alternative to mitigate  $N_2O$  emissions from agricultural soils without yield penalties, while concurrently improving the net greenhouse gas balance of the agroecosystem and enhancing essential ecosystem services provided by earthworms. As we hypothesized, non-legume cover crops such as rye should be promoted to further elicit the benefits of NT, since they can decrease yield-scaled emissions compared with legume cover crops as shown in our study, besides being able to reduce N-leaching losses as repeatedly shown in other studies. If a legume CC is used, N fertilizers should be applied considering the expected rate and timing of available N from mineralization of CC residues.

# Acknowledgments

This work was supported by the Foundation Romeo and Enrica Invernizzi (Milan, Italy). We would like to thank colleagues, technicians and students from the Agronomy group of the Department of Sustainable Crop production (Università Cattolica del Sacro Cuore of Piacenza), for their assistance during gas sampling and all the experimental procedures. Diego Abalos has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 656632.

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