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Relationship between tillage management and DMPSA nitrification inhibitor efficiency

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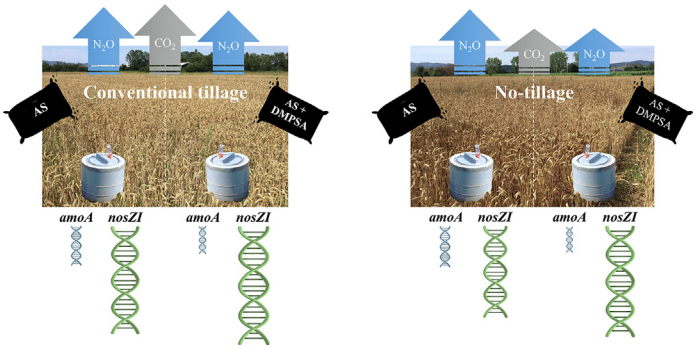
HIGHLIGHTS

- Nitrification inhibitor DMPSA was tested under tillage (CT) and no-tillage (NT).
- N_2O emissions due to fertilization were completely abated by DMPSA in NT.
- DMPSA decrease *amoA* and induce *nosZI* gene abundances.
- Crop yield and quality were not affected by DMPSA application.
- NT was more sustainable than CT in terms of CO_2 emissions.

GRAPHICAL ABSTRACT



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ABSTRACT

Agricultural sustainability is compromised by nitrogen (N) losses caused by soil microbial activity. Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) produced as consequence of nitrification and denitrification processes in soils. Nitrification inhibitors (NI) as 3,4-dimethylpyrazole-succinic acid (DMPSA) are useful tools to reduce these N losses from fertilization. The objective of this work was to test the efficiency of DMPSA in two different tillage management systems, conventional tillage (CT) and no-tillage (NT), in a winter wheat crop under Humid Mediterranean conditions. N fertilizer was applied as ammonium sulphate (AS) with or without DMPSA in a single or split application, including an unfertilized treatment. GHG fluxes (N_2O, CO_2 and CH_4) were measured by the closed chamber method. *amoA* and *nosZI* genes were quantified by qPCR as indicators of nitrifying and denitrifying populations. Nitrification was inhibited by DMPSA in both CT and NT, while the higher water filled pore space (WFPS) in NT promoted a better efficiency of DMPSA in this system. This higher efficiency might be due to a greater N_2O reduction to N_2 as result of the *nosZI* gene induction. Consequently, DMPSA was able to reduce N_2O emissions down to the unfertilized levels in NT. Provided that NT reduced CO_2 emissions and maintained crop yield compared to CT, the application DMPSA under NT management is a promising strategy to increase agro-systems sustainability under Humid Mediterranean conditions.

1. Introduction

Human population is growing at a rate of 1.1% per year, and it is expected to reach a total population size of 9,722 million people in

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2050 and 11,184 million in 2100 (UN, 2017). Thereby, agriculture will need to grow in order to satisfy all system requirements. For 2050, previsions are that agriculture will need to increase its production by 50% respect to 2012 to satisfy human demand (FAO, 2017). In 2007, cereals accounted for 60% of global fertilizer use (100 million Mg of nutrients NPK) and this trend is expected to continue. Cereals production is projected to reach 3,009 million Mg in 2050, when, specifically, wheat average yield would reach 3.8 Mg ha^{-1} in a 225 million ha harvested area (Alexandratos and Bruinsma, 2012).

Growth of crops is often limited by the availability of nitrogen (N), making essential the use of N fertilizers in crops production. However, the N applied can be lost by nitrate leaching, ammonia volatilization and by microbial soil processes, causing environmental problems. The main microbial N-transforming processes contributing to nitrous oxide (N_2O) formation are nitrification and denitrification (Braker and Conrad, 2011). Both processes occur simultaneously under most soil conditions, although the dominant process depends on many different factors such as soil characteristics (pH, texture, carbon availability, ammonium/nitrate ratio, soil texture and aeration or microbial activity) and environmental conditions (rainfall and temperature) (Dobbie and Smith, 2003; Del Prado et al., 2006; Menéndez et al., 2012). Nevertheless, soil moisture has a key role regulating the contribution of both processes to soil N_2O emissions, being nitrification the preferential source of N_2O fluxes from well-aerated soils, while denitrification dominates under oxygen-limited conditions (Fowler et al., 2009).

In 2010, the agricultural sector was responsible of 24% of total anthropogenic greenhouse-gases (GHG) emissions (IPCC, 2014). One of the most important GHG is N_2O, with a global warming potential 265 times higher than carbon dioxide (CO_2) in a 100-year time horizon (IPCC, 2014). Fowler et al. (2009) estimate that agriculture is responsible of the emission of 1.7–4.8 Mg N_2O–N y^{-1}, which represents around 19% of total N_2O global source and 49% of anthropogenic N_2O emissions. In order to mitigate N losses from agriculture, agronomic research must focus on the rational use of N fertilizer by means of developing better agronomic practices that help to prevent leaching and gaseous losses, being at the same time key tools to obtain maximum profitability in terms of yield and quality, i.e., getting better efficiency. Among these possible tools, the use of a no-tillage (NT) management and the use of nitrification inhibitors (NI) are promising strategies.

Conventional tillage (CT) managements imply more time of use of machinery with its fuel-associated costs, CO_2 emissions and soil disturbance. Thus, NT and reduced-tilling practices are being recommended to reduce costs and preserve the environment (Sánchez-Girón et al., 2004; Álvaro-Fuentes et al., 2014; Guardia et al., 2016). In fact, the use of conservation tillage systems such as reduced tillage or NT has been pointed out as one of the most promising strategies to enhance soil organic carbon stocks in dryland areas due to its beneficial effect on soil water storage (Plaza-Bonilla et al., 2015). However, effects of NT practices in terms of GHG emissions and yield are variable. Not all soils and climates are appropriate for NT because of its special requirements in terms of climatology and soil compaction (Holland, 2004). Effects on carbon sequestration are variable depending on soil properties, climatology and even sampling depth (Soane et al., 2012). Moreover, different fertilizations, combined with different reduced or NT managements, can result in different responses (Venterea et al., 2005), even increasing net GHG emissions in poorly aerated soils by increasing N_2O emissions even at the same time being CO_2 sinks (Rochette, 2008). Implementation of these managements is increasing in winter-sown crops in south-western Europe because, under these conditions, it improves water conservation, increases soil carbon stocks and maintains or increases yields (Soane et al., 2012). Specifically, under rainfed Mediterranean conditions, it has been demonstrated that long-term NT is a good practice to reach an equilibrium between agricultural productivity and N_2O emissions (Cantero-Martínez et al., 2016; Plaza-Bonilla et al., 2018) and leads to increased yields in cereals in arid or semi-arid conditions due to a higher water storage capacity (Lampurlanés et al., 2016).

In order to decrease N losses, NIs have been developed to reduce bacteria nitrification avoiding ammonium transformation to nitrate and subsequent leaching. So N is maintained in soils during more time, extending its availability for plant uptake. The nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP), has been proved to be effective reducing N_2O emissions (Weiske et al., 2001; Menéndez et al., 2012; Huérfano et al., 2015, 2018). 3,4-dimethylpyrazole-succinic acid (DMPSA) is the newest of this kind of products and, due to its non-polarity, has the capacity to be combined with a larger range of fertilizers such as calcium ammonium nitrate (CAN) or diammonium phosphate (DAP), while maintaining/improving the availability of the reactive compound (DMP) in soils (Pacholski et al., 2016). To date, just a few studies have analyzed DMPSA behavior. Under laboratory conditions, Torralbo et al. (2017) found that N_2O emissions were practically suppressed when DMPSA was applied, while Volpi et al. (2017) found reductions of 50%. In field trials, reductions $\geq$ 50% on N_2O emissions have been found in a maize-ryegrass rotation (Huérfano et al., 2018), in a winter wheat crop (Huérfano et al., 2016) and in irrigated maize (Guardia et al., 2017, 2018a). However, findings of Volpi et al. (2017) under controlled conditions revealed that DMPSA performance could depend on soil type.

There is even less information about the effects of DMPSA on crop yield and quality parameters. Guardia et al. (2018b), in a two years field experiment with wheat, did not observe effects on any parameter in the first year, while, in the second, DMPSA decreased grain N content. Also in a two years field experiment with wheat, Huérfano et al. (2016) did not see effects on yield or grain protein content. Other studies with other crops as a maize-ryegrass rotation (Huérfano et al., 2018) or irrigated maize (Guardia et al., 2017) also found that DMPSA application maintained in general all yield components.

Determining agro-system responses when different tillage practices and new nitrification inhibitors are simultaneously used is an important step in crop management research that will allow to develop strategies in order to increase agricultural efficiency and achieve the crop yield and quality demanded by the market. The aim of this work was to study the response of the application of DMPSA in terms of GHG emissions, with special interest in N_2O and soil microorganisms populations associated to it; as well as yield parameters of a winter wheat crop. This response was evaluated under conventional and no-tillage systems.

2. Materials and methods

2.1. Experiment setup

This work was conducted during a crop season (2016–2017) of winter wheat (*Triticum aestivum* L., var. Cezanne) in Arkaute, northern Spain (42°51′N, 2°37′W, 530 m above sea level). Soil characteristics of the upper horizon (0–30 cm) are compiled in Table 1. Daily precipitation and mean temperatures are shown in Figure S1.

To compare conventional tillage (CT) and no-tillage (NT) managements, two randomized complete blocks designs were established with four replicates and an individual plot size of 40 m^2 (8 × 5 m). The plots, whose previous crop was also wheat, were conditioned with a seedbed preparation consisting in mechanical tillage (disk and moldboard plow) for CT. For NT plots, spontaneous weeds were desiccated with glyphosate-based herbicide before

Table 1
Physical and chemical properties of the soil (0–30 cm depth).

Soil texture			Soil chemical properties								
Sand (%)	Silt	Clay	pH[a]	C:N	N[b] (g kg^{-1})	Organic matter[c]	Carbonate[d]	P[e] (mg kg^{-1})	Mg[d]	K[d]	Ca[d]
43.4	24.7	31.9	8.0	8.15	1.6	21.2	9.8	59.0	92.4	167	6,356

[a] pH (1:2.5 soil:water).
[b] N Kjeldahl digestion (Keeney and Nelson, 1982).
[c] Organic matter (Walkley and Black, 1934).
[d] $CaCO_3$, Mg, K (MAPA, 1994).
[e] P (Watanabe and Olsen, 1965).

direct sowing. Wheat was sown at a density of 220 kg seeds ha^{-1} in both managements on December 1st 2016 and harvested on July 18th 2017. Disc harrow was applied again on CT treatments on August 29th 2017 in order to prepare the soil for the next crop.

Fertilization rate was 180 kg N ha^{-1} applied as ammonium sulphate 21% (AS) split in two applications of 60 kg N ha^{-1} at beginning of tillering stage (GS21) and 120 kg N ha^{-1} at stem elongation stage (GS30) according to the Zadoks growth scale (Zadoks et al., 1974). Provided that Huérfano et al. (2015) showed that the application of nitrification inhibitors allows to apply the fertilizer in a single application without effects on yield, we also have included a single application treatment of 180 kg N ha^{-1} at beginning of tillering stage (GS21).

Within each design (CT or NT), five treatments were applied: I) Control without N fertilization (C); II) AS in a single application (1AS); III) AS in split application (2AS); IV) AS combined with the nitrification inhibitor DMPSA in a single application (1ASD) and V) AS combined with DMPSA in a split application (2ASD). All plots were supplied with 85 kg ha^{-1} K (K_2SO_4) at GS21 stage. Fertilizer combined with DMPSA inhibitor was provided by EuroChem Agro Iberia S.L. DMPSA rate was 0.8% of the NH_4^+-N applied with the fertilizer. Treatments application dates and rates are detailed in Table 2.

2.2. Soil mineral nitrogen and water contents

To measure ammonium (NH_4^+) and nitrate (NO_3^-) content, three soil subsamples (3 cm diameter × 30 cm depth) were taken randomly in each plot before sowing, every week during a month after fertilizer applications, and before harvesting. From the homogenized soil from each plot, 100 g fresh soil was extracted with 200 mL 1 M KCl. These extracts were filtered through Whatman n°1 filter papers (GE Healthcare, Little Chalfont, Buckinghamshire, UK) to remove particles and, secondly through Sep-Pak Classic C18 Cartridges 125 Å pore size (Waters, Milford, MA, USA) to eliminate organic matter. The resultant solutions were used to determine NO_3^- content by ultraviolet spectrophotometry as described by Cawse, (1967) and NH_4^+ by the Berthelot method (Patton and Crouch, 1977).

Table 2
Nitrogen application rates as ammonium sulphate (kg N ha^{-1}) and growth stage.

Treatment	Tillering (GS21)[a]	Stem elongation (GS30)[a]
	(kg N ha^{-1})	(kg N ha^{-1})
C	0	0
1AS	180	0
1ASD	180	0
2AS	60	120
2ASD	60	120

C = unfertilized control; 1AS = ammonium sulphate 21% (single application); 2AS = ammonium sulphate 21% (split application); 1ASD = ammonium sulphate 21% + 3,4-dimethylphyrazole succinate (DMPSA) (single application); 2ASD = ammonium sulphate 21% + 3,4-dimethylphyrazole succinate (DMPSA) (split application).
[a] Zadoks growth scale (Zadoks et al., 1974).

The rest of the soil sample was oven-dried to determine soil water content, which is expressed as the percentage of water filled pore space (WFPS). WFPS was calculated as in Linn and Doran (1984): WFPS = (soil gravimetric water content × bulk density) × (1 − bulk density/particle density))$^{-1}$, by using a particle density of 2.65 Mg m^{-3} and bulk densities determined for each tillage management resulting in values of 1.13 Mg m^{-3} and 1.35 Mg m^{-3} for CT and NT respectively.

2.3. Greenhouse gas emissions measurements

N_2O, methane (CH_4) and CO_2 soil emissions were measured using the close chamber method (Chadwick et al., 2014). The chambers, with a diameter of 20 cm, were inserted into the soil at the beginning of the experiment and just removed to allow managements events. Sampling frequency was 3 times per week after each fertilization along 2 weeks, reducing frequency to 2 times per week in the next 2 weeks and 1 time per week in the following 2 weeks. In the remaining time sampling was conducted every 2 weeks. Taking into account the diurnal variation of emissions (Baggs and Blum, 2004), sampling was performed between 10 a.m. and 13 p.m. To account for soil heterogeneity, four chambers were placed in each plot and two of them were sampled each day alternatively. Gas samples were taken just after closing the chambers and after 45 min. 20 mL of gas were taken from each chamber and stored at overpressure in pre-evacuated 12 mL glass vials. The linearity of the fluxes was checked regularly along the experiment. Samples were analyzed in a gas chromatograph (GC) (Agilent, 7890A) equipped with an electron capture detector for N_2O detection, with a methanizer to measure CO_2 by reducing it to CH_4, and a flame ionization detector for CH_4 determination. A capillary column (IA KRCIAES 6017:240 °C, 30 m × 320 μm) was used, and samples were injected by means of a headspace autosampler (Teledyne Tekmar HT3) connected to the GC. Standards of N_2O, CO_2 and CH_4 were analyzed at the same time.

Gas emission rates were calculated taking into account the gas concentration variation from the beginning to the end of the 45 min. Cumulative emissions during the sampling period were estimated using the trapezoidal rule integration (linear interpolation and numerical integration between sampling times) (Levy et al., 2017). Cumulative N_2O and CH_4 emissions were converted to CO_2 equivalents following the recommendations of IPCC (2007), using a global warming potential (GWP) factor of 265 for N_2O and 28 for CH_4.

Soil temperature (10 cm depth) was measured before sampling of gaseous emissions. Air temperature was measured 3 times during the 45 min. gas-sampling period to get the average.

2.4. Nitrogen-cycle-related microbial abundance

Soil cores from 2AS and 2ASD treatments of both CT and NT managements were collected at 0–30 cm depth 8 and 19 days after the first fertilization, and 12 and 31 days after the second. After

homogenization, subsamples were weighted, frozen in liquid nitrogen and stored at −80 °C until use. DNA was extracted from 0.35 g FW of soil using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA) including some modifications described in Harter et al. (2014).

Extracted DNA concentration and quality were determined by spectrophotometry with a NanoDrop® 1000 (Thermo Scientific, Walthman, MA, USA). Quantitative polymerase chain reactions (qPCR) were performed using SYBR® Premix Ex Taq™ II (Takara-Bio Inc.) and gene-specific primers (Supplementary Table S1) to amplify and quantify total bacteria abundance (16S rRNA), nitrification-involved *amoA* gene and denitrification-involved *nosZI* gene. Each sample was quantified in triplicate using the StepOnePlus™ Real-Time PCR System and data analysis was carried out by StepOnePlus™ Software 2.3 (Thermo Scientific). Standard curves (log gene copies number per reaction volume versus log N) were prepared from serial dilutions of 10^7 to 10^2 gene copies μL^{-1} of linearized plasmids with insertions of the target genes, and the copy number of target genes per gram of dry soil was calculated according to a modified equation detailed in Behrens et al. (2008): [(number of target gene copies per reaction × volume of DNA extracted)/(volume of DNA used per reaction × gram of dry soil extracted)]/DNA concentration.

2.5. Crop yield parameters

A harvested surface of 12 m^2 (1.5 m × 8 m) per plot was used for grain yield determination, being adjusted to 12% moisture content. A surface of 0.45 m^2 per plot was measured to calculate the number of tillers per m^2, number of grains per ear and dry weight of 1000 grains. Total grain N content was analyzed by the Kjeldhal procedure (A.O.A.C., 1980) with a Kjeltec Auto sampler System 1035 analyzer (Tecator) after grinding the grain through a 1 mm screen. Grain protein content was calculated as 5.7 times the total N content (Teller, 1932).

2.6. Statistical analysis

Data were statistically evaluated by one-way ANOVA using the Duncan's multiple-range test for separation of means between treatments, and the Student-*T* test or Mann-Whitney-*U* test were carried out to specifically compare two treatments with the statistical software SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp). In all cases, significant differences are expressed at $P < 0.05$. Additional details have been included in figure legends.

3. Results

3.1. Soil mineral N

NH_4^+, NO_3^- contents and their ratio (NO_3^-/NH_4^+) along all the experiment are shown in Table 3. After N fertilization, NH_4^+ content increased in both CT and NT systems. Although ASD treatments tended to show higher NH_4^+ content than AS in both CT and NT, only in three cases differences were statistically significant. DMPSA

Table 3
Soil NH_4^+ (kg NH_4^+-N ha^{-1}), NO_3^- (kg NO_3^--N ha^{-1}) content and ratio (NO_3^--N/NH_4^+-N) at 0–30 cm depth before fertilization and at different days after fertilization (daf) until harvest.

		Pre fertilization	First fertilization (15th March)	Second fertilization (4th April)				
		14th March	8 daf	19 daf	11 daf	21 daf	30 daf	72 daf
AMMONIUM								
CT	C	15	18	20	23	25	17	18
	1AS	15	81	56	47	41	24	18
	1ASD	14	111#	90	73	73	36	22
	2AS	19	38	29	86	82	39	18
	2ASD	18	53	33	91	63	55#	17
NT	C	28*	29*	23	31	26	33*	16
	1AS	26*	112*	80	36	75	36*	23
	1ASD	22*	100	91	82#	79	46	23*
	2AS	28*	35	32	89	66	58	22
	2ASD	24	44	40	73*	106	88	25*
NITRATE								
CT	C	26	21	20	31	38	28	27
	1AS	15	49	102	163	140	124	18
	1ASD	23	55	93	117	83	69	27
	2AS	21	30	92	124	79	144	26
	2ASD	20	29	71	89	68	83#	17
NT	C	20	15	6*	15	13*	11*	9.2
	1AS	13	29	56	46*	49*	44*	13
	1ASD	9	24*	39#*	38*	26#*	21#*	15
	2AS	18	28	41*	57*	58	51*	22
	2ASD	15	22	27*	23#*	39#*	30#*	33
NO_3^-/NH_4^+								
CT	C	1.23	0.98	1.27	1.05	1.51	1.68	1.33
	1AS	0.75	0.62	1.96	3.98	3.40	4.81	0.96
	1ASD	1.23	0.49	1.06	1.65#	1.11#	1.94#	1.30
	2AS	1.12	0.82	3.84	1.48	0.80	3.70	1.47
	2ASD	1.20	0.55#	2.23	0.99#	1.09	1.52#	1.10
NT	C	0.69	0.54	0.26	0.47*	0.52*	0.33*	0.55*
	1AS	0.49	0.26*	0.74*	1.29*	0.74*	1.23*	0.58
	1ASD	0.41	0.25*	0.45*	0.51#*	0.34#*	0.50#*	0.84
	2AS	0.64	0.77	1.30*	0.65*	1.08	0.78*	1.00
	2ASD	0.65 *	0.50	0.70*	0.32*	0.40*	0.37#*	1.36

Hash (#) indicates significant differences ($P < 0.05$; n = 4; Mann-Whitney-*U* test) induced by DMPSA application respect to AS. Asterisk (*) indicates significant differences ($P < 0.05$; n = 4; Mann-Whitney-*U* test) between CT and NT within a fertilizer treatment.
CT = conventional tillage; NT = no-tillage; C = unfertilized control; 1AS = ammonium sulphate (single application); 2AS = ammonium sulphate (split application); 1ASD = ammonium sulphate + DMPSA (single application); 2ASD = ammonium sulphate + DMPSA (split application).

effects were much more obvious in the case of NO_3^- in NT management, where this content was significantly lower in both NT–2ASD and NT–1ASD treatments up to 30 days after fertilization (daf) and 50 daf respectively (Table 3). NO_3^- content tended to be lower in both CT–1ASD and CT–2ASD, although significant differences were only observed 30 daf in 2ASD. Most part of significant differences observed between CT and NT treatments are referred to NO_3^- content, usually showing CT higher soil NO_3^- content than NT.

3.2. Gaseous emissions, soil WFPS and temperature

3.2.1. Soil WFPS and temperature

Soil WFPS (30 cm depth) and soil temperature (10 cm depth) are shown in Fig. 1. WFPS ranged between a minimum of 31% and a maximum of 78% in CT, whereas values ranged between 41% and 100% in NT. WFPS was higher in NT than in CT in 40 of 42 sampling days, with an average value for the whole experimental period of 58% in NT, which was higher than the average value of 44% in CT. By periods (Table 5), soil water content was higher in the pre-fertilization and 1st fertilization, showing values above 60% WFPS, and it decreased below 50% in the 2nd fertilization and post-harvest periods. Soil temperature ranged between a minimum of 2 °C and a maximum of 21 °C without differences between CT and NT.

3.2.2. Nitrous oxide emissions

Daily N_2O emissions (Fig. 1) ranged from −1.77 to 10.84 g N_2O-N ha^{-1} d^{-1} in CT and from −1.14 to 16.75 g N_2O-N ha^{-1} d^{-1} in NT. These maximum daily rates occurred in 1AS treatment in both CT and NT managements. Nevertheless, maximums for DMPSA treatments only reached 6.09 g N_2O-N ha^{-1} d^{-1} in CT (CT-1ASD) and 6.79 g N_2O-N ha^{-1} d^{-1} in NT (NT-2ASD).

Differences between CT and NT in total cumulative N_2O emissions (from sowing to the last sampling day) (Table 4) were statis-

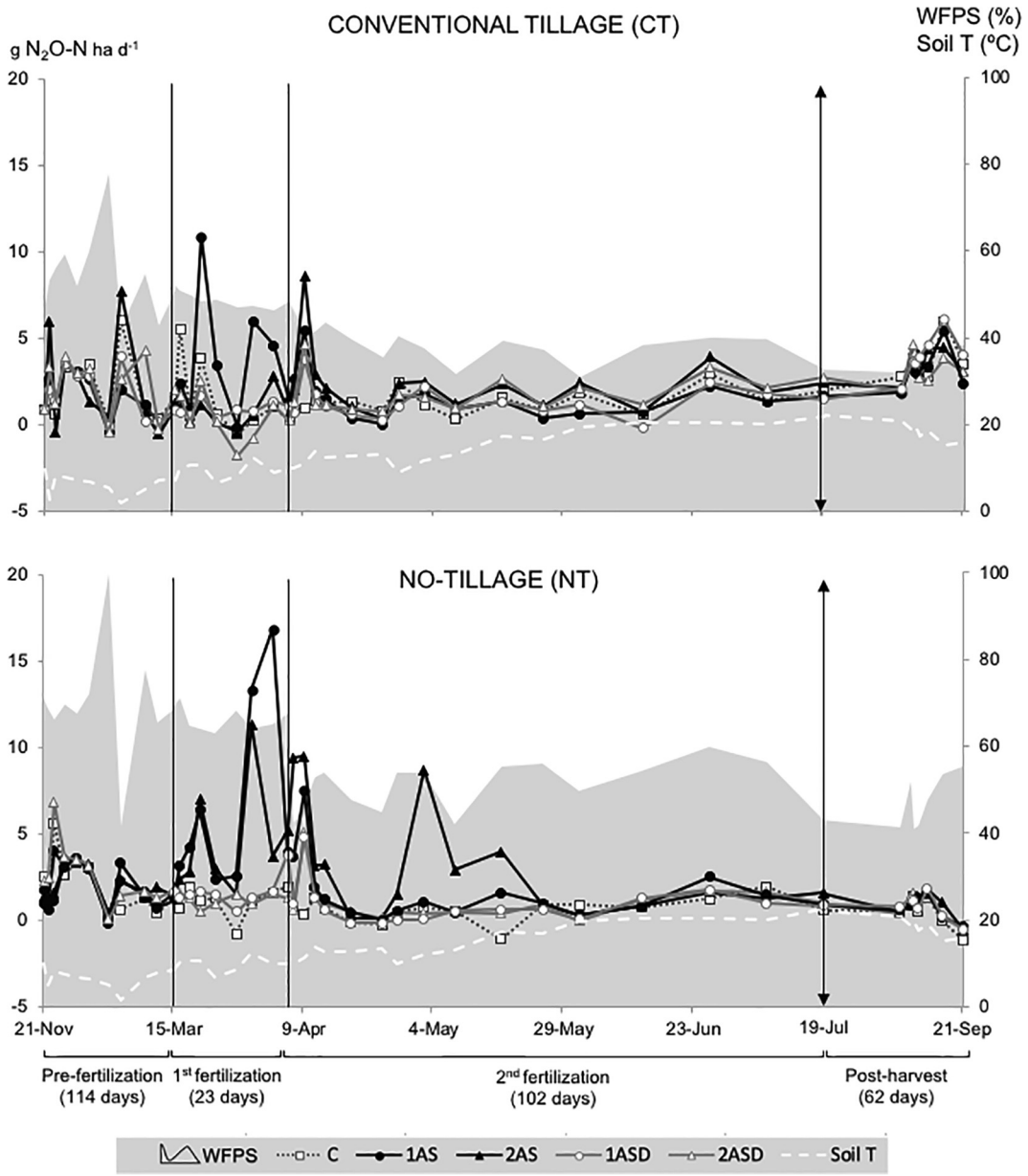

Fig. 1. N_2O daily emissions, WFPS (0–30 cm depth) and soil temperature (0–10 cm depth) in conventional tillage (CT) and no-tillage (NT). Vertical lines indicate fertilizer applications and harvest (arrows). C = unfertilized control; 1AS = ammonium sulphate (single application); 2AS = ammonium sulphate (split application); 1ASD = ammonium sulphate + DMPSA (single application); 2ASD = ammonium sulphate + DMPSA (split application).

Table 4
Total cumulative emissions (from sowing to the end of the experiment) of N_2O, CO_2 and CH_4; Emission Factor (EF) N yield scaled N_2O emissions (YSNE), Global Warming Potential (GWP) and Greenhouse Gas Intensity index (GHGI) from sowing until the end of the experiment.

		N_2O (g N_2O-N ha^{-1})				EF (%)	YSNE (g N_2O-N/kg N uptake)			CO_2 (kg CO_2-C ha^{-1})			CH_4 (g CH_4-C ha^{-1})			GWP (Mg CO_2-eq ha^{-1})			GHGI (kg CO_2eq ha^{-1}/kg yield ha^{-1})		
CT	C	625	ab				17.7	a		6812	a		−432	a		6.97	a		3.36	a	
	1AS	546	ab			0	6.6	b		5984	b		−512	a		6.12	b		1.28	b	
	1ASD	521	ab			0	6.2	b		6171	b		−568	a		6.20	b		1.38	b	
	2AS	659	a			0.02	5.2	b		6056	b		−459	a		6.22	b		1.35	b	
	2ASD	619	ab			0	5.9	b		6424	ab		−493	a		6.58	ab		1.36	b	
NT	C	328	B		(−47%)		7.2	A	(−59%)	4078	A	(−40%)	−280	A	(35%)	4.16	A	(−40%)	1.65	A	(−51%)
	1AS	547	A			0.12	5.4	AB		3814	A	(−36%)	−206	A	(60%)	3.95	A	(−35%)	0.91	B	(−29%)
	1ASD	396	B	[−28%]	(−24%)	0.04	4.1	B	(−34%)	4002	A	(−34%)	−386	A		4.10	A	(−34%)	0.92	B	(−33%)
	2AS	617	A			0.16	5.3	AB		4094	A	(−32%)	−393	A		4.25	A	(−32%)	0.84	B	(−38%)
	2ASD	429	B	[−31%]	(−31%)	0.06	4.6	B	(−21%)	4359	A	(−32%)	−282	A		4.47	A	(−32%)	1.00	B	(−26%)

Different letters within a column and management (CT = lowercase; NT = capital) indicate significant differences between treatments using the Duncan Test ($P < 0.05$; n = 4). Values in square brackets indicate significant differences in percentage between DMPSA and AS ($P < 0.05$; n = 4; Student-T test). Values in round brackets indicate significant differences in percentage between CT and NT within each treatment ($P < 0.05$; n = 4; Student-T test).
CT = conventional tillage; NT = no-tillage; C = unfertilized control; 1AS = ammonium sulphate (single application); 2AS = ammonium sulphate (split application); 1ASD = ammonium sulphate + DMPSA (single application); 2ASD = ammonium sulphate + DMPSA (split application).

Table 5
Cumulative N_2O emissions (g N_2O-N ha^{-1}), mean Water Filled Pore Spaces (WFPS) (%) and mean Soil Temperature (°C) for each period.

		Pre-fertilization Sowing-Tillering (114 days)		1st fertilization Tillering-Stem elongation (23 days)			2nd fertilization Stem elongation-Harvest (102 days)				Post-harvest (62 days)		
CT	C	266.8	a	25.1	b		145.3	ab			188.0	ab	
	1AS	177.6	a	86.0	a		126.1	b			156.7	a	
	1ASD	196.6	a	20.7	b	[−76%]	126.1	b			177.3	a	
	2AS	250.7	a	19.1	b		213.1	a			176.4	a	
	2ASD	255.6	a	6.2	b		190.9	ab			166.7	a	
	WFPS (%)	*55.3*		*48.4*			*38.5*				*35.0*		
	Soil T (°C)	*6.3*		*9.7*			*15.3*				*18.2*		
NT	C	207.5	A	23.4	C		68.4	C			29.4	B	(−84%)
	1AS	216.7	A	162.5	A	(47%)	123.1	B			44.9	AB	(−71%)
	1ASD	232.0	A	34.9	C	[−79%]	80.1	C	[−35%]	(−36%)	49.3	AB	(−72%)
	2AS	234.1	A	108.6	B	(82%)	213.3	A			61.4	A	(−65%)
	2ASD	268.2	A	26.2	C	[−76%]	96.3	C	[−55%]	(−50%)	44.2	AB	(−74%)
	WFPS (%)	*70.3*		*66.0*			*50.8*				*47.5*		
	Soil T (°C)	*6.5*		*10*			*15.4*				*17.9*		

Different letters within a column and management (CT = lowercase; NT = capital) indicate significant differences using the Duncan Test ($P < 0.05$; n = 4). Values in square brackets indicate significant differences in percentage between DMPSA and AS ($P < 0.05$; n = 4; Student-T test). Values in round brackets indicate significant differences in percentage between CT and NT within each treatment ($P < 0.05$; n = 4; Student-T test).
CT = conventional tillage; NT = no-tillage; C = unfertilized control; 1AS = ammonium sulphate (single application); 2AS = ammonium sulphate (split application); 1ASD = ammonium sulphate + DMPSA (single application); 2ASD = ammonium sulphate + DMPSA (split application).

tically significant in ASD and C treatments, being lower under NT. Total cumulative emissions values in AS treatments were similar between CT and NT. However, emissions in the C treatment were double in CT than in NT, and there were no differences between C and AS treatments in CT. Additionally, there were no significant differences due to DMPSA application in CT management, although, by periods, DMPSA showed effect in the 1st fertilization decreasing CT-1AS N_2O losses by 76% (Table 5). As opposite, fertilization clearly increased N_2O losses in both NT-1AS and NT-2AS treatments respect to the Control. However, DMPSA application significantly decreased N_2O emissions in both NT-1ASD and NT-2ASD in a 24% and 31% respectively, being this reduction down to C levels. This reduction induced by DMPSA was higher when considering individual fertilization periods, with reductions of 79% in the 1st fertilization and 55% in the second one (Table 5).

3.2.3. Carbon dioxide emissions

In CT, CO_2 emissions ranged from 0.40 to 44.53 kg CO_2-C ha^{-1}-d^{-1} (data not shown). Total cumulative CO_2 emissions (Table 4) were significantly higher in CT-C than in the rest of the CT-treatments except CT-2ASD. In NT, emissions ranged between 2.48 and 28.65 kg CO_2-C ha^{-1} d^{-1} (data not shown) and total cumulative emissions showed no significant differences between NT-treatments.

Comparison between CT and NT managements provided lower CO_2 emissions in NT than in CT, with these differences being statistically significant in all treatments with an average reduction of 35%. DMPSA application did not significantly affect cumulative CO_2 emissions in any case.

3.2.4. Methane emissions

Methane daily fluxes in CT oscillated between −11.52 and 2.36 g CH_4-C ha^{-1} d^{-1} (data not shown). In the case of NT, fluxes ranged between −11.34 and 2.18 g CH_4-C ha^{-1} d^{-1} (data not shown). Total cumulative fluxes (Table 4) showed that both CT and NT managements acted as CH_4 sinks. Although, in average, soil CH_4 uptake was 37% higher in CT than in NT, only in CT-C and CT-1AS treatments the uptake of CH_4 was significantly higher than in NT-C and NT-1AS respectively (Table 4). DMPSA application did not significantly affect cumulative CH_4 fluxes in any case.

3.2.5. Global warming potential

GWP ranged from 6.12 Mg CO_2-eq ha^{-1} in 1AS up to 6.97 Mg CO_2–eq ha^{-1} in the C treatment under the CT management. It

was significantly higher in the CT-C treatment respect to the rest, except CT-2ASD (Table 4). In the case of NT, GWP ranged from 3.95 Mg CO_2-eq ha^{-1} in 1AS up to 4.47 Mg CO_2-eq ha^{-1} in 2ASD. No significant differences between treatments were found in this management. On the other hand, GWP was significantly reduced in all treatments of NT with respect to CT by 35% in average (Table 4).

3.3. Abundance of nitrification and denitrification genes

No difference in total bacteria abundance (*16S rRNA* gene abundance) was observed due to DMPSA application in any sampling day (Fig. 2-A). DMPSA effect on AOB (in terms of absolute abundance of *amoA* gene) varied between managements and days (Fig. 2-B). After the 1st fertilization, DMPSA application significantly reduced *amoA* gene abundance 8 days after fertilization (daf) in CT management. After the 2nd fertilization, results were similar 12 daf, when only CT-2ASD showed lower *amoA* gene abundance than CT-2AS, while the application of DMPSA significantly decreased *amoA* gene abundance in both CT and NT 31 daf. When analyzing the trend in time, AOB abundance clearly increased with time in both CT-2AS and NT-2AS treatments reaching a maximum on day 31 after the 2nd fertilization, while just a slight increase was observed in 2ASD treatments. DMPSA application also affected

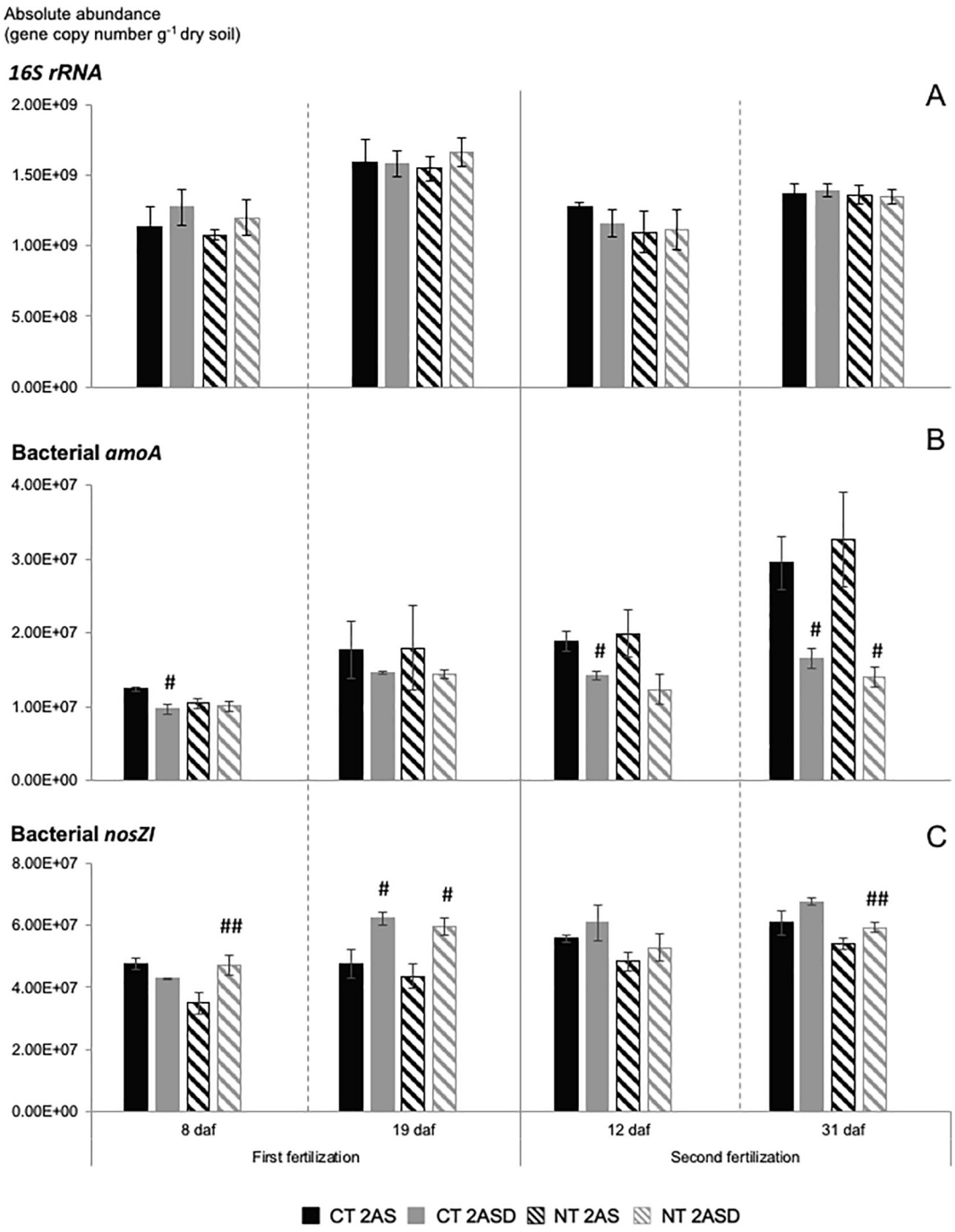

Fig. 2. Total abundance of bacteria (*16S rRNA*) (A), *amoA* (B), and *nosZi* (C) genes, expressed as gene copy number per gram of dry soil, at different days after each fertilization (daf). Hash (#) indicates siginificant effect of DMPSA application ($P < 0.05$ = #; $P < 0.1$ = ##; n = 3; Student-T test). 2AS = ammonium sulphate (split application) 2ASD = ammonium sulphate + DMPSA (split application).

denitrifying bacteria, significantly increasing *nosZI* gene abundance (Fig. 2-C) 8 and 19 days after the 1st fertilization and 31 days after the 2nd fertilization in NT. This trend was only significant 19 days after 1st fertilization in the case of CT.

3.4. Crop yield parameters

Grain yield in the C treatments was 2162 and 2547 kg ha^{-1} for CT and NT respectively (Table 6). Yield was increased by fertilization to an average for all the fertilized treatments of 4713 kg ha^{-1} (CT) and 4617 kg ha^{-1} (NT). Neither fertilized treatments nor management (CT or NT) induced any difference in grain yield in any case. However, some yield components did show significant differences between CT and NT managements depending on treatments (Table 6). The number of tillers per m^2 was higher in NT than in CT, being these differences statistically significant in C, 1AS and 1ASD treatments. The dry weight of 1000 grains was lower in NT than in CT, with significant differences in all treatments except the Control. The harvest index also tended to be lower in NT than in CT, being significantly lower in 1AS and 2ASD. On the other hand, grain protein content was not significantly affected either by the fertilization treatments or soil management (Table 6), except by a slight increase in NT–1ASD with respect to CT–1ASD.

4. Discussion

4.1. No-tillage reduces CO_2 emissions

NT systems tend to decrease CO_2 emissions in dry climates (Álvaro-Fuentes et al., 2008), but not in humid (Huang et al., 2018). In our case, NT management was revealed as the better option in terms of reducing CO_2 emissions, with an average reduction of 35% respect to CT (Table 4). This reduction in CO_2 emissions is in agreement with other studies carried out under Mediterranean conditions (Alvaro-Fuentes and Cantero-Martinez, 2010; Carbonell-Bojollo et al., 2011, 2015), although there are cases in which this effect was not observed (Guardia et al., 2016). Our results indicate that NT practices can effectively increase the soil carbon stock in our climate conditions, Humid Mediterranean. Different studies performed in Spain have shown that CT increases CO_2 emissions due to a greater root respiration, a higher microbial decomposition and liberation of the CO_2 accumulated in soil pores with ploughing (González-Sánchez et al., 2012; Soane et al., 2012). Although higher CO_2 emissions could be expected just after ploughing in CT, differences between CT and NT were constant along all periods (data not shown). Soil CO_2 efflux is a key indicator of both microbial and plant activity, being soil temperature and water content two main variables controlling soil CO_2 emissions (Morell et al., 2011). In order to know to what extent the tillage system could be inducing quantitative changes in the soil microbial population, soil bacterial abundance was quantified as the abundance of bacterial *16S rRNA* gene. The results showed that there were no differences between the total bacterial abundance of both tillage systems (Fig. 2). So, it could be inferred that the differences in soil CO_2 emissions between CT and NT systems could be more related to differences in plants root respiration rather than differences in microbial activity.

Respect to NIs effects on CO_2 emissions, several studies have found variable effects of DMP based NIs. While Huérfano et al. (2016) found no effect of DMPSA on CO_2 fluxes, Guardia et al. (2018a) observed decreased respiration rates. Other studies have demonstrated no effect (Menéndez et al., 2012; Huérfano et al., 2015; Florio et al., 2016) or a decrease (Weiske et al., 2001; Pfab et al., 2012) in CO_2 emissions after DMPP application. In our case, we did not find any effect due to fertilization nor DMPSA application in NT or CT in terms of CO_2 emissions (Table 4) or bacteria population abundance (Fig. 2). The only remarkable difference was that CT fertilized treatments emitted less CO_2 than the unfertilized Control, as also described by Bowden et al. (2000) and Menéndez et al. (2006) after mineral fertilization, which was attributed to a different soil C/N ratio between fertilized and unfertilized treatments.

4.2. Tillage masks the effect of fertilization on N_2O emissions

Two different tillage managements (CT and NT) would result in different soil physicochemical conditions, inducing changes in soil porosity, compaction, moisture and O_2 availability. The differences generated by CT and NT managements in soil physical properties could lead to different gas fluxes. In terms of N_2O emissions, it has been described that variations due to NT practices as an alternative of CT can generate different results depending on other management practices, crop type, soil properties and climatology (Soane et al., 2012). In our study, the use of NT management as a replacement for CT was a valuable strategy to mitigate N_2O emissions, since a reduction of almost 50% in the total cumulative emissions was observed when C treatments were compared (Table 4). However, following fertilization, N_2O emissions were statistically equal between CT-AS and NT-AS treatments. Analysing in more detail the data presented in Table 5, it is observed that the effect exerted by the tillage system on N_2O emissions was a function of the time period analyzed. Thereby, this effect was also dependent

Table 6
Grain yield, yield components and grain protein content of wheat.

		Grain yield (kg ha^{-1})		Tillers m^{-2}		1000 grains DW (g)		Grains ear^{-1}		Harvest index		Grain protein (%)	
CT	C	2162	b	316	b	37.5	a	30.6	b	0.67	a	9.6	b
	1AS	4781	a	439	a	36.5	a	39	ab	0.69	a	12.7	a
	1ASD	4542	a	419	ab	34.4	a	43.4	ab	0.67	a	11.2	ab
	2AS	4650	a	471	a	35.3	a	39.3	ab	0.68	a	12.5	a
	2ASD	4878	a	404	ab	37.5	a	41.2	ab	0.72	a	11.7	ab
NT	C	2547	B	469	B*	35.3	A	35.3	A	0.54	AB	10.6	B
	1AS	4356	A	772	A*	28.3	B*	39.4	A	0.49	B*	13.2	A
	1ASD	4542	A	770	A*	30.7	B*	35.7	A	0.54	AB	12.4	A*
	2AS	5103	A	721	A	27.9	B*	37.3	A	0.61	A	13.2	A
	2ASD	4467	A	615	AB	31.0	B*	37.1	A	0.58	A*	11.7	AB

Different letters within a column and management (CT = lowercase; NT = capital) indicate significant differences using the Duncan Test ($P < 0.05$; n = 4). Significant differences between CT and NT within each treatment are represented by an asterisk (*) ($P < 0.05$; n = 4; Student-T test).
CT = conventional tillage; NT = no-tillage; C = unfertilized control; 1AS = ammonium sulphate (single application); 2AS = ammonium sulphate (split application); 1ASD = ammonium sulphate + DMPSA (single application); 2ASD = ammonium sulphate + DMPSA (split application).

on the WFPS in each management and period. In fact, the tillage management did not exert any difference during the pre-fertilization period, when WFPS was > 55% in both management systems. After the 2nd fertilization and post-harvest, during spring and summer, soil moisture in the CT system remained below a mean WFPS value of 40–45%. In these conditions, CT-C showed soil NO_3^- contents which were around 50% higher than soil NH_4^+ contents (i.e., NO_3^-/NH_4^+ values around 1.5) while in NT-C they were around 50% lower (i.e., NO_3^-/NH_4^+ values around 0.5) (Table 3). These higher NO_3^-/NH_4^+ values in the CT system could be observed as a general trend along the whole experimental period and also in the fertilized treatments when comparing CT with NT within each treatment. This suggests that in the CT system nitrification was taking place in a more efficient manner than in the NT system. In this sense, it has been described that the intensity of nitrification in soils is influenced by soil structure, which regulates aeration (Hoffman et al., 2007), being this structure more aerated in CT respecting to NT. At the low soil WFPS < 40–45% of the CT system in the 2nd fertilization and post-harvest periods, the basal NH_4^+ content of treatment CT-C seems to have been enough to produce relatively high N_2O emissions by means of an efficient nitrification process, which were of the same magnitude of those produced by a higher NH_4^+ quantity in the fertilized AS treatments (i.e., no effect of fertilization was observed on N_2O emissions). Therefore, the tillage management exerted a great effect on soil N emissions by means of lowering the soil water content in comparison to the NT system, thus masking the effect of fertilization on N_2O emissions. Contrarily, in the NT system, the > 40–45% WFPS resulted in conditions where denitrification was a process taking relevance as responsible for N_2O emissions. Under these more denitrifying conditions, lower basal N_2O emissions were observed in NT-C treatment than in CT-C treatment. This was especially well observed in the post-harvest period, when N_2O emissions in NT-C were 84% lower than in CT-C, when mean WFPS for that period was 47.5% in NT with respect to 35% in CT (Table 5). With these lower basal N_2O losses in the unfertilized NT-C, the increase in N_2O emissions induced by fertilization (both 1AS and 2AS treatments) was clearly observed both after the 1st and 2nd fertilizations in the NT system. Moreover, provided that after the 1st fertilization also in the CT system soil water content showed a mean WFPS > 40–45% (48.4% of WFPS), also under this tillage system an induction in N_2O emissions could be observed after fertilization over the low N_2O emissions of the unfertilized CT-C. This induction was observed when the whole N rate of 180 kg N ha^{-1} was applied (1AS treatment), while the application of 60 kg N ha^{-1} (2AS treatment) was not enough to induce an increase effect. Therefore, the induction of N_2O emissions after fertilizer application seems to be due to an induction of the denitrification process. On top of this, soil structure is an additional factor that influences the differential N_2O emissions observed under NT with respect to the CT management. The N_2O produced in the NT system could be retained for more time within the more structured NT soil before reaching the soil surface. This will enhance the probability that N_2O could be reduced to N_2 in the microbial denitrification process. This helps to explain why, when WFPS is lower than 48% (i.e. after the 2nd fertilization and post-harvest periods), the N_2O emissions of NT-C (coming in a greater extent from denitrification) are lower than those of CT-C (predominantly coming from nitrification). Then, the effect of fertilization on N_2O emissions is more clearly observed in the NT system, because it is known that after fertilization the higher NO_3^- content enhances proportionally more the production of N_2O than its reduction to N_2 in the denitrification process (Saggar et al., 2013). Finally, the increase in N_2O emission observed after the application of 180 kg N ha^{-1} in the 1st fertilization of the CT system would also indicate that, above a threshold value of around 47% of WFPS, denitrification seems to have been the process responsible for the increase observed in N_2O emissions.

4.3. DMPSA shows better efficiency reducing N_2O emissions under no-tillage conditions

Although a few studies have been already carried out to analyze the potential of DMPSA to reduce N losses, to our knowledge this is the first study analyzing DMPSA behavior under NT management. Our experiment demonstrated that the application of DMPSA in the NT system, both applied in a single or split application, effectively reduced N_2O emissions by 28% and 31%, respectively. Both NT-ASD treatments were able to reduce emissions down to Control levels. A more exhaustive analysis by periods (Table 5) showed that, in the NT system, the highest reductions occurred in the 23 days length period after 1st fertilization, when average WFPS was 66%, with a DMPSA reduction efficiency of 79% (single application) and 76% (split). After the 1st fertilization, WFPS conditions were in a range between 60% and 70%. These values are related with high emissions episodes (Davidson, 1991; Del Prado et al., 2006), when denitrification should have been also taking place together with nitrification. Bearing in mind that the response to AS application was higher in NT, it is relevant that the application of DMPSA was able to reduce NT-ASD treatments emissions to the same level of CT-ASD treatments, so DMPSA demonstrating to be an efficient tool to offset the response of the NT system to fertilization in terms of N_2O. After the 2nd fertilization, in a 102 days cumulative emissions period, DMPSA reduction in NT-2ASD was 55%, when WFPS decreased down to an average of 51%, making thus conditions less suitable for denitrification. The DMPSA effect inhibiting nitrification was also reflected in NT in the lower soil NO_3^- contents during this 2nd fertilization period (Table 3) as well as in the lower nitrifier bacteria abundance (Fig. 2-B). In this sense, the decrease in *amoA* gene abundance observed 31 daf in NT-2ASD respect to NT-2AS (Fig. 2-B) showed that the effect of DMPSA was stronger at the low WFPS values (< 51%) of the 2nd fertilization rather than at the higher WFPS values (> 60%) of the 1st fertilization. These results are in accordance with those of Torralbo et al. (2017), who found a decrease in AOB abundance at low soil water content values (40% of WFPS) 16 daf and 51 daf, while with higher soil water contents (80% of WFPS) they did find a decrease 16 daf but not 51 daf. Other studies with the other DMP-based nitrification inhibitor (DMPP) have reported similar effects, with a significant reduction in AOB abundance at 40% of WFPS, but no effect at 80% of WFPS (Barrena et al., 2017). DMPSA is considered as a highly specific NI that focuses its activity on nitrifying communities. So, it was expected to find a clear effect on *amoA* gene abundance without affecting total bacterial abundance (*16S rRNA* gene abundance). To our knowledge, this is the first study analyzing DMPSA effect on soil bacteria abundance in field conditions and, as expected, soil total bacterial abundance was not affected by DMPSA application along the field experiment (Fig. 2-A). In this same sense, no other microbial activity except ammonium monooxygenase of nitrifiers should be expected to be affected by the application of DMP-based Nis. However, previous works have reported different responses of *nosZI* gene abundance after DMPP application depending on WFPS as a main factor. In a laboratory experiment, Barrena et al. (2017) found an induction at 80% WFPS that was not observed at 40% WFPS at the same incubation temperature. This induction at 80% WFPS was also observed by Torralbo et al. (2017) 51 daf but not at 40% WFPS in any case, and Duan et al. (2017) did neither find any effect at 50% WFPS. Regarding DMPSA, Torralbo et al. (2017) also demonstrated that DMPSA can stimulate *nosZI* expression 51 daf at 80% WFPS, while 16 daf was not enough time as to have induced a change in this bacterial population. In

our case, it seems that a threshold value of around 48% of WFPS is necessary to induce an increase in *nosZI* gene abundance in the NT system, and also even in the CT system provided that a sufficient time is maintained under these soil water content conditions. Thus, an increase of *nosZI* gen copy number was observed 8, 19 and 31 daf in the case of NT, and also 19 daf in the case of CT when WFPS was higher than 48% (Fig. 2-C). So, *nosZI* induction was better observed at the higher WFPS levels present in the NT management after the 1st fertilization, which matches with the results of Barrena et al. (2017) and Torralbo et al. (2017). These results fully support the argument that in the case of the NT system the decrease observed in N_2O emissions after DMPSA application might be occurring by means of an induced reduction of N_2O up to N_2 as a specific mechanism that is promoted due to the fact that NT induces a higher WFPS with respect to the CT system. It should be remarked that this is the first time that such a *nosZI* gene inducing effect by the application of DMPSA has been described to occur under field conditions instead of under the laboratory incubation studies previously described (Torralbo et al., 2017). It was also demonstrated that the DMPSA applied in the 1st fertilization was still effective after the 2nd fertilization, when NT-1ASD treatment was emitting 35% less N_2O than NT-1AS (Table 5). In fact, DMPSA mitigation effect was observable up to the end of May (43 days after second application) (Fig. 1).

With respect to CT, other field experiments applying DMPSA in CT systems as Huérfano et al. (2018) found reductions up to 32% in a rainfed maize-ryegrass rotation, and reductions up to 58% have been reported in maize under irrigated conditions (Guardia et al., 2017, 2018a; Recio et al., 2018). In our case, and by the contrary to that observed in NT, DMPSA did not cause a significant reduction in N_2O losses under CT conditions when taking into account the whole experimental period (Table 4). This was due to the induction of N_2O emissions caused by the tillage management, which masked the response of N_2O emissions to fertilization, as previously discussed. Recio et al. (2018) also found no significant reduction in total cumulative emissions when DMPSA was applied with CAN, and Huérfano et al. (2016), in our same edaphoclimatic conditions, found that DMPSA was not effective under a CT management in the second of two experimental years, attributing that lack of effectivity to environmental conditions less prone to N_2O losses. However, the mitigation of 56% reported by Huérfano et al. (2016) in the first experimental year of conventional tillage, at an average WFPS of 75% in their fertilizations period, was above ours (28–31% mitigation) under NT management at an average WFPS of 57% in our fertilizations period. This means that, under the same edaphoclimatic conditions, the efficiency of DMPSA reducing N_2O emissions depends on the WFPS level as a main factor, which, while being modulated by the tillage system, will also depend on the environmental conditions of each year and particular season. In fact, in the 23 days period after 1st fertilization, DMPSA strongly reduced N_2O emissions by 76% after applying 180 kg N ha^{-1} in a single application. Nevertheless, the emission levels in all fertilized treatments in CT were much lower than in NT, so, in absolute values, the reduction was not as high as in NT-ASD treatments. In the 102 days period after the 2nd fertilization, we did not observe any significant effect of DMPSA on N_2O emissions, not even in the split application, since the high unfertilized treatment emission was masking the effect of fertilization on N_2O emissions. This was already proposed by Menéndez et al. (2012), who described that the percentages of reduction induced by DMPP are proportional to the percentage of emissions-increase induced by fertilizer application. Nevertheless, there were evidences indicating that DMPSA was inhibiting nitrification during this period of time, as shown by the lower NO_3^-/NH_4^+ values (Table 3) and lower *amoA* gene abundances (Fig. 2-C) 12 and 31 daf in treatment CT-2ASD respect to CT-2AS.

4.4. Effect of the tillage system and DMPSA application on crop yield and quality

In our Humid Mediterranean conditions wheat can obtain higher yields with respect to other areas of Mediterranean climate due to the relatively high precipitation in spring (Fig. S1). In fact, in the humid Mediterranean conditions that prevail in our field site (Alava, Northern Spain), the average yield for winter wheat as reported by the Ministry of Agriculture, Food and Environment (MAGRAMA, 2014) is 5000 kg ha^{-1} approximately, being the usual N rate applied 180 to 220 kg N ha^{-1}, and 182 kg N ha^{-1} the agronomically optimum N fertilizer rate described for the region (Ortuzar-Iragorri et al., 2010). Maximum yields have been described for this region in a range as high as 9000 to 11000 kg grain ha^{-1} for Cezanne variety (Huérfano et al., 2015, 2016). The yield obtained in this field experiment under CT conditions was half of the yield registered by Huérfano et al. (2016) in the same location, with the same Cezanne variety and same CT management. This might be attributed to the fact that these authors registered a slightly higher precipitation (715 mm $year^{-1}$ compared to the 666 mm $year^{-1}$ in our case). Regarding yield components, in CT tillers per m^{-2} and grains per ear were in the same range as in Huérfano et al. (2016), being 1000 grains DW lower in our experiment, which is concomitant with the lower grain yield obtained. However, in our case, grain protein content was higher than in their case, pointing that the lower production favored grain N content. In this sense, it is well known that the increase in grain yield leads to a decrease in the protein to starch ratio in the grain (Triboi et al., 2006).

It is assumed that lower yields could be expected just after adopting NT, while equal yields respect to a CT system can be achieved after a few years (Soane et al., 2012). Moreover, under Mediterranean conditions, low or NT practices usually generate an increase in crop yield (Sanz-cobena et al., 2017). In our case, NT management induced the appearance of more tillers per m^{-2} than CT (Table 6), especially in the case when fertilizer was applied in a single application. The number of tillers per m^{-2} is determined at the beginning of tillering stage (GS21); thus, a higher soil water content in the NT system in that period could have improved plant N absorption, causing the increase observed in the number of tillers, especially in the single fertilization treatment. This was compensated by an opposite effect in the 1000 grains dry weight, whose values is determined later, during the grain-filling period, and were lower in all fertilized treatments of the NT management compared to CT.

Until now, some studies have reported no effect of DMPSA on yield parameters and grain N content of wheat (Huérfano et al., 2015, 2016), except a decrease in grain N content in one of two field experimental years (Guardia et al., 2018b). In our experiment, we did not observe any detrimental or beneficial effect of DMPSA application on any yield component nor on grain protein content (Table 6). In the case of NT, a decrease in the harvest index was detected in the AS single application soil respect to the split. In this point, it is interesting to observe that DMPSA was able to reverse that effect and reach the harvest index values of the split treatments, highlighting that DMPSA could be an efficient tool to join the split fertilizer applications into just one single application while maintaining yield.

4.5. Sustainability factors

When N_2O emissions are referred to the total N harvested (Yield Scaled N_2O emissions; YSNE), our study shows the advantages of the NT management in conjunction with the application of DMPSA, when YSNE resulted to be 21–34% lower than in the CT management (Table 4). While N_2O emissions factors were, in any case,

much lower than the default value of 1% proposed by IPCC (2006), this approach (NT + DMPSA application) demonstrates to be able to reduce it even further in our edaphoclimatic conditions. CH_4 and, specially, CO_2 emissions have also a high importance in terms of sustainability. The Global Warming Potential (GWP) and Greenhouse Gas Intensity (GHGI) (Mosier et al., 2006) factors consider N_2O, CH_4 and CO_2 emissions together (in terms of CO_2-equivalent emissions) to give an account for the global impact of the system. Guardia et al. (2016), considering other associated costs and savings of different tillage systems, already indicated that the NT system decreases GWP under Mediterranean conditions. In our experiment, NT management also showed a much lower GWP and GHGI, with an average reduction of 35% respect to CT. This was mainly due to its capacity to maintain yield and a higher carbon stock, which added to the fact that NT usually leads to lower net costs (Sánchez-Girón et al., 2004) with savings in both labor time and fuel inputs in comparison to CT (Álvaro-Fuentes et al., 2014; Guardia et al., 2016) remarks NT management as a good mitigation strategy.

Respect to NIs effect on CH_4 fluxes, a higher content of NH_4^+ as result of DMPSA application might derive in an inhibition of CH_4 oxidation (Tlustos et al., 1998; Ullah et al., 2008) due to the competition between NH_3 and CH_4 for methane monooxygenase enzyme (Holmes et al., 1995), so increasing CH_4 emissions. Nevertheless, our results are in concordance with other studies showing no statistically significant effects of DMPSA application on CH_4 fluxes (Guardia et al., 2018a, 2018b; Huérfano et al., 2018).

5. Conclusions

The soil management system plays a key role in terms of GHG emissions, being no-tillage an environmentally efficient practice to reduce CO_2 and N_2O emissions, as shown in the unfertilized treatments. However, when fertilizing with ammonium sulphate, N_2O emissions in no-tillage increased up to the levels of conventional tillage. The higher soil WFPS in no-tillage resulted in DMPSA being more effective under this management, where its application was able to avoid fertilization-induced N_2O emissions. This higher efficiency might be due to a greater N_2O reduction to N_2 as consequence of the *nosZI* gene induction observed. Therefore, we conclude that the use of a no-tillage management combined with DMPSA is a promising strategy to increase sustainability of the rainfed agricultural systems under our Humid Mediterranean conditions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contribution

Mario Corrochano-Monsalve: Investigation, Verification, Formal analysis, Visualization, Writing- Original Draft. Ximena Huérfano: Investigation, Verification, Formal analysis, Visualization, Writing- Original Draft. Sergio Menéndez: Investigation, Writing – Rewiew & Editing. Fernando Torralbo: Investigation, Writing – Review & Editing. Teresa Fuertes- Mendizábal: Investigation, Writing – Review & Editing. José María Estavillo: Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition. Carmen González-Murua: Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition. Mario Corrochano-Monsalve and Ximena Huérfano contributed equally to this work. All the authors contributed to the discussion of the results and the final edition of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.134748.

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