Soil N_2O emissions after perennial legume termination in an alfalfa-wheat crop rotation system under Mediterranean conditions

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Abstract

Agricultural activities are potential sources of greenhouse gas (GHG) emissions, and nitrous oxide (N_2O) is one of the most important non-carbon-dioxide GHGs. Perennial legumes such as alfalfa (*Medicago sativa* L.) have potential roles for reduction of soil GHG emissions as part of crop rotation systems. However, the implications of perennial legume termination by tillage and subsequent soil incorporation of the residues for reduced GHG emissions have been poorly examined in Mediterranean environments. With the aim to assess the magnitude of soil N_2O emissions (important for the definition of mitigation strategies) after perennial legume termination in alfalfa-wheat crop rotation systems in a Mediterranean environment, we defined the hypothesis that alfalfa termination by tillage with incorporation of the crop residues will increase soil N_2O emissions during the subsequent wheat season. To test this hypothesis, closed static chambers were used in a field-plot experiment, using a complete randomised block design with three replicates. Soil N_2O emissions were monitored across 33 sampling dates from October 2017 to July 2018, as a comparison between an original 6-year-old alfalfa field ('continuous alfalfa') and alfalfa termination followed by wheat ('alfalfa+wheat'). The soil N_2O emission fluxes varied markedly across the treatments and throughout the monitoring period (from -0.02 ± 0.01 to 0.53 ± 0.14 g N-N_2O ha^{-1} h^{-1}, and from 0.02 ± 0.07 to 0.37 ± 0.11 g N-N_2O ha^{-1} h^{-1} for continuous alfalfa and alfalfa+wheat, respectively), generally following the changes in soil temperature. Several soil N_2O emission peaks were recorded for both treatments, which mainly coincided with rainfall and with increased soil water content. In the 2 months following alfalfa termination, alfalfa+wheat showed higher cumulative weekly soil N_2O emissions compared to continuous alfalfa. Following alfalfa termination for alfalfa+wheat, the increased cumulative weekly soil N_2O emissions appeared to be due to asynchrony between nitrogen (N) released into the soil from mineralisation of the alfalfa residues and N uptake by the wheat. Despite these initial high soil N_2O emissions for alfalfa+wheat, the seasonal cumulative soil N_2O emissions were not significantly different (0.77 ± 0.09 *vs* 0.85 ± 0.18 kg N-N_2O ha^{-1} for continuous alfalfa and alfalfa+wheat, respectively). These data suggest that legume perennial crop termination in alfalfa–wheat rotation systems does not lead to significant loss of N_2O from the soil. The alfalfa termination by tillage performed in autumn might, on the one hand, have slowed the mineralisation process, and might, on the other hand, have synchronised the N release by the mineralised crop residues, with the N uptake by the wheat reducing the soil N_2O emissions.

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Introduction

Many recent studies have indicated that increased greenhouse gas (GHG) emissions into the atmosphere are linked to human activities and to land use and management (Stehfest and

Bouwman, 2006; Wang and Fang, 2009; Reay *et al*., 2012; Smith *et al*., 2014; Cayuela *et al*., 2017; Francioni *et al*., 2019). Nitrous oxide (N_2O) is one of the most relevant non-carbon-dioxide GHGs (Forster *et al.*, 2007), with a global warming potential 265-fold that of carbon dioxide (CO_2) over a 100-year time horizon (Smith *et al.*, 2014). Evaluation of the magnitude of the agricultural N_2O emissions and definition of the possible mitigation strategies are important, as the soil is the largest natural source of N_2O (Stehfest and Bouwman, 2006; Van Groenigen *et al.*, 2010) and agriculture is responsible for around 60% of N_2O emissions (Syakila and Kroeze, 2011; Reay *et al.*, 2012). Apart from climate conditions, many other factors have key roles in the nitrogen (N) cycle, and consequently on soil N_2O emissions from agriculture, such as N fertiliser type and application rate, crop type, crop residue type and timing of incorporation, tillage type (Signor and Cerri, 2013), cropping system (Signor and Cerri, 2013; Autret *et al*., 2019) and soil physicochemical properties, which include its organic carbon content, pH and texture (Stehfest and Bouwman, 2006). Soil N_2O emissions generally increase with higher clay content of the soil (Lesschen *et al.*, 2011), compared to sandy soil, due to higher levels of anaerobic microsites (Signor and Cerri, 2013). As N_2O is one of the by-products of microbial nitrification and denitrification processes, the fertiliser type and application rate affect the soil N_2O (Malhi *et al.*, 2010; Sanz-Cobena *et al.*, 2017; Volpi *et al.*, 2018; Tenuta *et al.*, 2019), to increase the emissions, especially at N input rates higher than the crop requirements (Kim *et al.*, 2013). The type of crop residues and the C:N ratio (which is low in alfalfa crop residues) also affect soil N_2O emissions (Gomes *et al.*, 2009; Lin *et al.*, 2013). For example, Toma and Hatano (2007) reported that the incorporation of crop residues with low C:N ratio in a Grey Lowland soil in Hokkaido (Japan) resulted in high soil N_2O emissions, due to rapid mineralisation of residues, and to the resulting suitable conditions for nitrification and denitrification processes (Huang *et al.*, 2004). According to these data, Signor and Cerri (2013) reported a close relationship between low C:N ratio of residues and production of N_2O, due to reduced N immobilisation and N increase into the soil. The crop residue type is not the only aspect that affects the soil N_2O emission, but also the way these residues are returned to the soil, although there remain some uncertainties about their effects on N_2O emissions that should be investigated (Shan and Yan, 2013). In the literature, the effects of tillage practices have been widely reported in terms of the soil organic N mineralisation, nitrification and denitrification processes, and consequently the N_2O soil emissions. As reported by Abalos *et al.* (2016), by improving soil aeration and reducing soil aggregation, soil tillage can enhance crop residue mineralisation and increase N availability for the nitrification and denitrification processes, with N_2O soil emissions increasing some 10-fold after soil tillage. Similar data were reported by Estavillo *et al.* (2002), who showed that soil tillage promotes mineralisation of soil organic N, with the subsequent release of N_2O. This process and the consequent soil N_2O emissions can be affected by the tillage timing and depth. Higher soil N_2O emissions appear to be linked to summer tillage, compared to autumn tillage (Ball *et al.*, 2007; Krauss *et al.*, 2017), and also to deeper tillage (Forte *et al.*, 2017).

Legumes are widely used as an alternative to chemical fertilisers because of their N fixation, which provides a N_2O emissions mitigation role for perennial systems (Abalos *et al.*, 2016). This also results from reduction of other direct and indirect N_2O emissions that come, for example, from chemical fertiliser production and transport (Aguilera *et al.*, 2013). Adoption of the best agronomic practices that take into account all of the factors that can affect N_2O emissions, combined with the use of specific mitigation strategies, can thus lead to reduced soil N_2O emissions.

Alfalfa (*Medicago sativa* L.) is one of the most important forage crops worldwide (Tesfaye *et al.*, 2006). It represents one of the most used perennial legumes in organic farming systems, with continually increasing areas of cultivation throughout the world (Willer and Lernoud, 2017). However, very few studies have investigated the effects of perennial legume termination on soil N_2O emissions (*e.g*., Westphal *et al.*, 2018; Tenuta *et al.*, 2019), while crop residues (Jensen *et al.*, 2012) might also have a key role in such emissions (Basche *et al*., 2014; Autret *et al*., 2019).

Taking into account the mitigation of soil N_2O emissions by perennial legumes through N fixation, the detrimental effects of soil tillage in this respect and the GHG impact on cropping systems (Autret *et al.*, 2019), we hypothesise that alfalfa termination by tillage will increase soil N_2O emissions, which would cancel out the positive effects of the perennial legume in terms of mitigation of N_2O soil emissions. With this regard, the aim of the present study was to assess the magnitude of soil N_2O emissions after perennial legume termination in alfalfa-wheat crop rotation systems in a Mediterranean environment.

Materials and methods

Site description

The site was located in a hilly area of the Marche region (Ancona Province, central Italy) (43° 33' N, 13° 25' E; 100 m a.s.l.; SW exposure; 23% slope) where alfalfa-wheat rotation is one of the most typical crop rotation systems (Monaci *et al.,* 2017; Francioni *et al.,* 2020). In this territory, long-term alfalfa fields are mainly grazed during the winter by the transhumant flocks of sheep, which are generally moved to permanent mountain grasslands during the summer (Budimir *et al.*, 2018). The bioclimate was the temperate oceanic sub-Mediterranean variant (Agnelli *et al.*, 2008), with mean annual precipitation of 788 mm and mean annual temperature of 14.6°C (Figure 1).

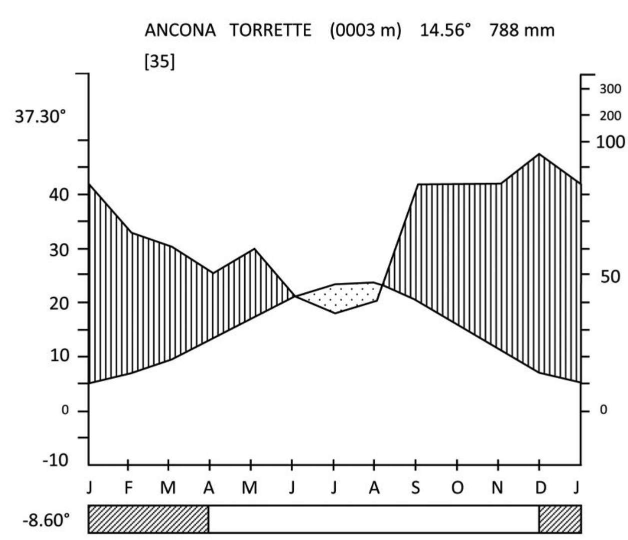

Figure 1. Walter-Lieth climate diagram of the study area (Walter and Lieth, 1960).

The meteorological data recorded by a weather station located 0.3 km from the study site indicated cumulative rainfall of 890 mm and mean air temperature of 13.5°C over the experimental period (October 2017-July 2018).

The soil of the study site was classified as Inceptisol according to the United States Department of Agriculture soil taxonomy system (Smith, 2014). Soil ancillary measurements were carried out at the beginning of the trial (*i.e.*, October 2017), following the guidelines of the Italian Ministry of Agriculture and Forestry (DM 13/09/99 GU 248), which is the Italian official reference for soil chemical analyses. Soil samples were collected at a depth of 0.1 and 0.4 m (Table 1), following the non-systematic 'W' pattern described by Paetz and Wilke (2005).

Experimental design

This study was conducted from October 2017 to July 2018. In October 2017, an area that was homogeneous for soil, crop vegetation and topographic conditions was identified in a 6-year-old alfalfa field. The experimental area was fenced off to prevent any disturbance. A complete randomised block design with three replicates and individual plots of 25 m^2 (2.5×10 m) was applied to measure the soil N_2O emissions under the following defined treatments (Table 2): i) Continuous 6-year-old alfalfa ('continuous alfalfa'): the original alfalfa field was mowed at the initial flowering stage (*i.e.*, 11 May, 4 July 2018) using a bar mower (cutting height, 5 cm), and a standard rake to collect and remove the cut herbage immediately after mowing; ii) Alfalfa termination followed by durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.) ('alfalfa+wheat'): the alfalfa was terminated at the beginning of October 2017 using a spading machine (*i.e.*, 11 October) followed by two uses of a rotary harrow (*i.e.*, 16 October, 21 November 2017), with the alfalfa residues (mean dry matter content, 2.70±0.23 t ha^{-1}) incorporated into the soil (0.20 m depth). The durum wheat plots were sown at the end of November 2017 (*i.e.*, 23 November) in rows (sowing rate, 400 seeds m^{-2}) and were manually harvested at the beginning of July 2018 (*i.e.*, 4 July), in a central plot area of 2 m^2. Manual weeding was performed twice in the second half of May 2018 (*i.e.*, 17, 24 May), with *Convolvulus arvensis* L. and *Papaver rhoeas* L. as the main species removed.

As the effects of N fertilisation on soil N_2O emissions are well known and have been demonstrated in many studies (Wei *et al.*, 2010; Aguilera *et al.*, 2013; Liu *et al.*, 2015), N fertilisation was not applied. This allowed isolation of the effects of only the alfalfa termination on the soil N_2O emissions.

Soil temperature and water content measurements

In each experimental unit, soil temperature and water content were measured from October 2017 to July 2018, for a total of 33 recordings for each variable. At each N_2O sampling, soil temperature was determined using hand-held digital thermometers equipped with a stainless steel probe (Model: 620-0909, VWR International, Italy) inserted to a depth of 0.1 m. Soil samples were taken with a manual auger at 0.1 m depth, and used to determine the soil water content (SWC) using the oven-dried method (105°C, to constant weight).

Nitrous oxide sampling, analysis and calculation

Nitrous oxide was measured using closed static chambers, as described by Parkin and Venterea (2010). The chambers were made of polyvinyl chloride (height, 0.15 m; diameter, 0.25 m) and were equipped with a thermometer to measure the variations in the internal temperature during the sampling period. Two polyvinyl chloride base rings (pseudo-replicates) per plot (n=6 chambers per treatment) were permanently installed in the soil (depth, 0.1 m), to explore spatial heterogeneity (Krauss *et al.*, 2017); these were only removed for soil tillage, after which they were immediately re-installed (Ghimire *et al.*, 2017).

Gas samples were collected between 9:00 a.m. and 12:00 a.m. (standard time) (Krauss *et al.*, 2017) every 3 or 4 days, from tillage (11 October 2017) to sowing (23 November 2017), and after any rain, and later at about every 15 days (Volpi *et al.*, 2018). Before each sampling, the above-ground parts of the plants inside the chambers were clipped off (Westphal *et al.*, 2018), to avoid disturbance to the soil N_2O emissions. The chambers were placed in

Table 1. Basic properties of the soil at the 0-0.1 m and 0.1-0.4 m sampling depths.

Sample depth (m)	pH	Carbon:nitrogen ratio	Texture (g kg^{-1}) Sand	Silt	Clay	Soil organic matter (g kg^{-1})	Total organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})	Humic + fulvic acids (g kg^{-1})	Cation exchange capacity [meq (100 g)$^{-1}$]	Field capacity (%)	Wilting point (%)
0-0.1	8.11	8.40	363.00	383.00	254.00	14.97	8.60	1.03	5.17	22.53	24.60	17.93
0.1-0.4	8.14	8.60	358.33	383.67	258.00	15.50	8.80	1.03	4.77	22.63	24.45	17.69

Table 2. Management practices applied to the different treatments during the study period.

Year	Management practice Type	Soil depth (m)	Time of treatment (Julian day) Continuous alfalfa	Alfalfa + wheat
2017	Spading	0.20	na	284
	Harrowing 1	0.15	na	289
	Harrowing 2	0.15	na	325
	Sowing	0.03	na	327
2018	Mowing/ raking 1	na	131	na
	Weeding 1	na	na	137
	Weeding 2	na	na	144
	Harvesting	na	na	185
	Mowing/ raking 2	na	185	na

na, not applicable.

position for 45 min, during which time four gas samples were withdrawn from the headspace of each chamber (30 mL each, at 15 min intervals). The gas samples were injected into 30 mL glass pre-evacuated vials sealed with a butyl rubber septum (Parkin and Venterea, 2010).

In a following step, the N_2O concentrations were determined using gas chromatography (GC8A; Shimadzu Corporation, Kyoto, Japan) with an electron capture detector.

N_2O fluxes were calculated starting from the change in chamber headspace N_2O concentration (concentration *vs* time), using linear regression analysis (Vitale *et al.*, 2018). The linearity of the headspace concentration of N_2O was previously checked over the adopted closure period (45 min) (all fluxes were screened for potential nonlinearity). According to Gelfand *et al*. (2016) and Koga *et al.* (2017), N_2O fluxes were calculated as:

$$F = \frac{M}{V0}\frac{P}{P0}\frac{(273+T0)}{273+T}\,h\frac{dC}{dt} \quad (1)$$

where T_0, P_0 and V_0 are the absolute air temperature, atmospheric pressure and molar volume under standard conditions, respectively, M is the molecular weight of gas X, P is the pressure outside the chamber, $\frac{dC}{dt}$ is the slope of the curve of gas X concentration variation with time (ppm h^{-1}), and h is the height of the chamber from the base ring to the top.

The cumulative weekly soil N_2O emissions were calculated by linear interpolation between the successive measurements (Ball *et al.*, 2007), and determined by summing the daily fluxes over periods of 7 days.

The seasonal cumulative soil N_2O emissions were calculated by linear interpolation (Gelfand *et al.*, 2016), assuming a linear flux change between sampling days (Abalos *et al.*, 2016; Volpi *et al.*, 2016, 2018; Westphal *et al.*, 2018), and summing over the whole experimental period.

Statistical analysis

According to Krauss *et al.* (2017), the soil N_2O emissions of the two pseudo-replicates per plot underwent arithmetic averaging before the statistical analysis was performed. The soil N_2O emissions are presented as fluxes (N-N_2O g ha^{-1} h^{-1}), cumulative weekly emissions (N-N_2O kg ha^{-1}) and seasonal cumulative emissions (N-N_2O kg ha^{-1}).

Prior to any analysis, all of the data were tested for normal distributions (Shapiro-Wilk tests) and homogeneous variance (Levene's tests), and when necessary for sphericity (Mauchly's tests). Where assumptions were met, repeated measures ANOVA was carried out to determine the effects of time (within factor), treatment (between factors) and their interactions (time×treatment), with one-way ANOVA carried out to determine the differences within each sampling date. Conversely, where assumptions were not met, Wilcoxon signed-rank tests were used instead of repeated measures ANOVA, and Kruskal-Wallis ANOVA instead of one-way ANOVA. Significance was assumed for all of the tests at the limiting value of $P<0.05$, unless otherwise indicated. Both the parametric and non-parametric tests were carried out using SPSS Statistics, version 25.0 (SPSS Inc., IBM, Chicago, IL, USA).

Results

Nitrous oxide fluxes

The soil N_2O fluxes varied markedly across the treatments and throughout the monitoring period (Figure 2C). These ranged from –0.02±0.01 to 0.53±0.14 g N-N_2O ha^{-1} h^{-1} for continuous alfalfa, and from 0.02 ± 0.07 to 0.37 ± 0.11 g N-N_2O ha^{-1} h^{-1} for alfalfa+wheat (Figure 2C). According to the Wilcoxon signed-rank tests, the difference scores in terms of the daily soil N_2O emissions between continuous alfalfa and alfalfa+wheat were approximately symmetrically distributed, as assessed using a histogram with the superimposed normal curve. This test did not highlight any statistically significant median increase in soil N_2O fluxes for alfalfa+wheat (0.13 g N_2O-N ha^{-1} h^{-1}) compared to continuous alfalfa (0.09 g N_2O-N ha^{-1} h^{-1}; z=1.97; P=0.053).

During the study period, out of the 33 sampling dates, alfalfa+wheat had higher soil N_2O emissions for 22 of the sampling dates, and continuous alfalfa for 11. One-way ANOVA highlighted differences between the treatments for only two dates: 21 December 2017, about 2 months after alfalfa termination for alfalfa+wheat, and 5 July 2018, the day after the wheat harvest (alfalfa+wheat) and the second alfalfa mowing (continuous alfalfa) (Figure 2C). On the sampling dates for which the homogeneity of variance of the data was not met, Kruskal-Wallis H tests highlighted that for 1 December 2017 and 20 June 2018, the soil N_2O emissions were different between the two treatments, as assessed by visual inspection of the boxplot. In particular, on 1 December 2017, the distributions of the soil N_2O emission levels were significantly higher for alfalfa+wheat compared to continuous alfalfa ($H(1)$ =3.857, P=0.05); while for 20 June 2018, the distributions of the soil N_2O emission levels were not significantly different between these treatments ($H(1)$ =0.048, P=0.827).

Both treatments showed fluctuations in their fluxes during the whole study period (Figure 2C). Several soil N_2O emission peaks were recorded for both treatments, mainly from October to December 2017 for alfalfa+wheat, and from the end of May to the end of June 2018 for continuous alfalfa.

During the first period (*i.e.*, October-December 2017), the alfalfa+wheat treatment was characterised by a peak on the first sampling day (0.28±0.07 g N_2O-N ha^{-1} h^{-1}), and then after a strong drop, another peak at the end of October 2017 (0.37±0.11 g N_2O-N ha^{-1} h^{-1}; on 24 October). Then, another peak was recorded on 17 November 2017 (0.24±0.05 g N_2O-N ha^{-1} h^{-1}), which was followed by a marked drop before a peak on 5 December 2017 (0.16±0.04 g N_2O-N ha^{-1} h^{-1}). Soil N_2O emissions peaks recorded during this period coincided with important rainfall events that occurred immediately before the N_2O emission pulses (Figure 2A) and with increasing soil water content (Figure 2B). During this first monitoring period, the soil N_2O fluxes followed the soil temperature dynamics (Figure 2B), except for the sampling dates on which rainfall and N_2O pulses occurred.

Between January and the end of February 2018, both treatments showed fluctuations and similar soil N_2O emission fluxes, except for 3 January 2018, when a peak of soil N_2O emission rate was recorded for alfalfa+wheat (0.15±0.03 g N_2O-N ha^{-1} h^{-1}), and for 16 January 2018, when an increase in the soil N_2O emission fluxes occurred for continuous alfalfa (0.13±0.07 g N_2O-N ha^{-1} h^{-1}), as compared to a sharper decrease for alfalfa+wheat (0.02±0.07 g N_2O-N ha^{-1} h^{-1}). During this time the decrease in the soil N_2O emissions followed the reduction in the soil temperature compared to the previous period, while a general increase in soil water content was observed. From March to the end of April 2018, the soil N_2O emissions were comparable to the previous period, and both treatments showed similar fluxes. In this period an increase in soil temperatures occurred in conjunction with a reduction in soil moisture and a slow increase in soil N_2O emissions.

Between the beginning of May and early July 2018, continuous alfalfa was characterised by three relevant N_2O peaks that occurred at the end of May 2018 (0.34±0.11, 0.53±0.14, 0.42±0.17 g N_2O-N ha^{-1} h^{-1}, for 22, 24, 29 May 2018, respectively), soon after rainfall. Compared to the previous period, the alfalfa+wheat

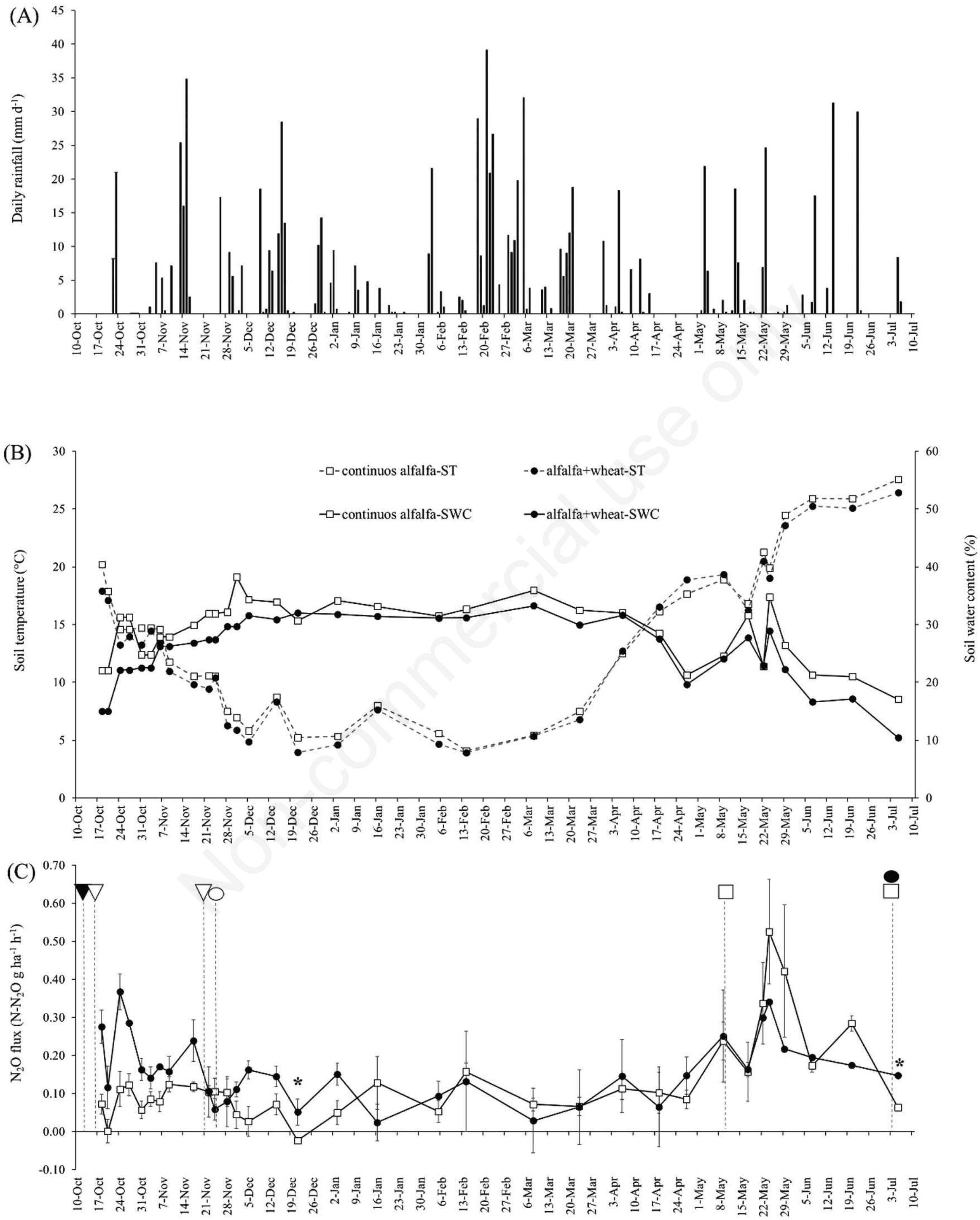

Figure 2. A) Monitored daily rainfall during the study period. B) Seasonal variations of the soil water content (SWC) and the soil temperature (ST), as indicated. C) Soil nitrous oxide fluxes for 'continuous alfalfa' (open squares) and 'alfalfa+wheat' (closed circles). Filled upside-down triangle, date of spading for alfalfa+wheat; empty upside-down triangles, dates of harrowing for alfalfa+wheat; open circle, date of wheat sowing for alfalfa+wheat; open squares, date of the mowing for continuous alfalfa; black circle, date of wheat harvesting for alfalfa+wheat. Data are means ± standard errors (n=3). *P<0.05, continuous alfalfa *versus* alfalfa+wheat.

treatment showed an increase in the soil N_2O emissions fluxes in line with the increasing soil temperature and decreasing soil water content, except in the second half of May, when rainfall and N_2O pulses occurred. In conclusion, compared to continuous alfalfa, alfalfa+wheat showed similar soil N_2O emission trends, except for the last three sampling dates when it showed a constant decrease.

Cumulative weekly and seasonal soil nitrous oxide emissions

The cumulative weekly soil N_2O emissions increased over the study period for both treatments, as 0.00±0.00 to 0.77±0.09 N-N_2O kg ha^{-1} for continuous alfalfa, and 0.01±0.00 to 0.85±0.18 N-N_2O kg ha^{-1} for alfalfa+wheat. The cumulative weekly soil N_2O emissions showed higher values for alfalfa+wheat compared to continuous alfalfa throughout the monitored period (Figure 3). No significant time×treatment interactions were seen, while the treatment had a significant effect on the cumulative weekly soil N_2O emissions over the monitored period.

Immediately after alfalfa termination for alfalfa+wheat there were higher soil N_2O emissions compared to continuous alfalfa. Indeed, from October 2017 to the first week of February 2018, the cumulative weekly soil N_2O emissions were almost always significantly greater for alfalfa+wheat, compared to continuous alfalfa (*i.e.*, from 25 October to 29 November 2017; from 27 December 2017 to 7 February 2018).

From the second decade of February 2018 until the beginning of May 2018, no significant differences between the treatments emerged and a slight increasing trend was observed for both treatments. Then from early May 2018 until the end of the study period, the absence of significant differences in soil N_2O emissions between treatments was confirmed, although a more pronounced increasing trend was recorded compared to the previous period.

In general, the seasonal cumulative soil N_2O emissions for continuous alfalfa and alfalfa+wheat (*i.e.*, last cumulative weekly point of Figure 3) did not differ significantly between the two treatments over the monitored period: 0.77±0.09, 0.85±0.18 kg N-N_2O ha^{-1}, respectively.

Discussion

Soil N_2O emissions are related to pedo-climatic conditions (*e.g.*, rainfall, soil temperature) and management factors (*e.g.*, crop residue type and incorporation depth, tillage type and timing), which thus represent the main drivers of soil variations in N_2O emissions (Estavillo *et al.*, 2002; Ball *et al.*, 2007; Butterbach-Bahl *et al.*, 2013; Luo *et al.*, 2013; Krauss *et al.*, 2017). In addition to these factors, some others might also affect soil N_2O emissions. These include crop type and behaviour, in relation to the phenological stages, and overall, the cropping system used (Liu *et al.*, 2015). The dynamics of the soil N_2O emission were therefore examined over the study period in relation to these main factors.

Unlike some studies that were carried out under similar climatic conditions (Cayuela *et al.*, 2017; Volpi *et al.*, 2018), in the present study, no relationships were found between soil N_2O emissions, soil temperature and soil water content (data not shown). Although the soil water content is one of the major drivers of soil N_2O emissions and regulation of oxygen availability (Butterbach-Bahl *et al.*, 2013), in the present field study the soil N_2O fluxes varied markedly throughout the study period following the dynamics of the soil temperature, which is another important climatic factor that induces variations in soil N_2O fluxes (Luo *et al.*, 2013). Soil N_2O emission pulses are typical of Mediterranean climates, and these usually occur after rainfall and rewetting after dry periods (Aguilera *et al.*, 2013). As also seen by Aguilera *et al.* (2013), in the present study the soil N_2O emissions increased sharply after rainfall, and the consequent increase in soil water content.

As expected, an increase in soil N_2O emissions occurred soon after alfalfa termination under alfalfa+wheat, compared to continuous alfalfa, which was probably due to soil aeration by the tillage and the quality of the crop residues incorporated (low C:N ratio) (Lin *et al.*, 2013; Basche *et al.*, 2014). Both of these factors might have promoted easier mineralisation of the residues (Toma and Hatano, 2007), to increase the substrate for the nitrification and denitrification processes (Estavillo *et al.*, 2002). Nitrification and denitrification, which are particularly active for the 0-0.1 m soil

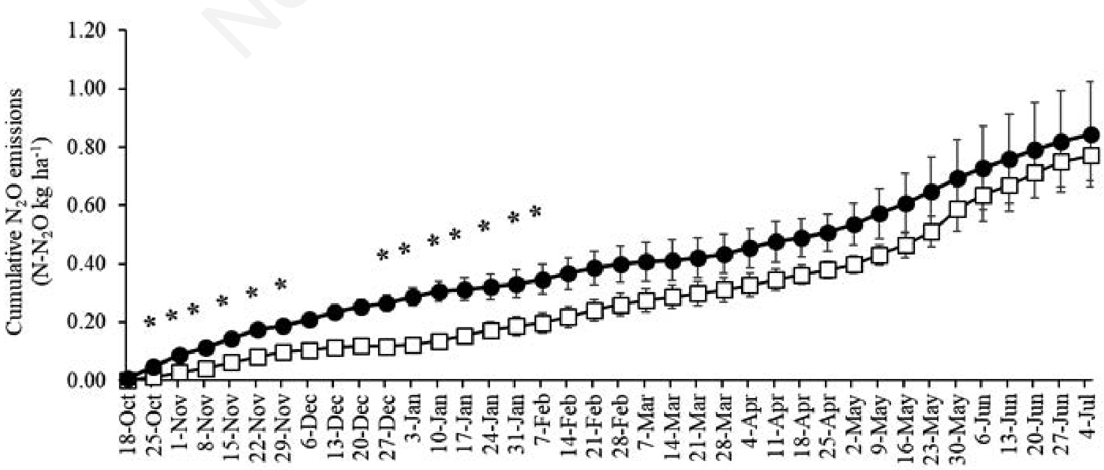

Figure 3. Cumulative weekly soil N_2O emissions over the study period for continuous alfalfa (open squares) and alfalfa+wheat (closed circles). Data are means ± standard error (n=3). *P<0.05, continuous alfalfa *versus* alfalfa+wheat. The last cumulative weekly point represents the cumulative seasonal soil N_2O emission.

layer, were identified as the processes that contribute most to soil N_2O emissions over the short term after termination of permanent grassland by tillage (Estavillo *et al*., 2002). In the present study, the soil conditions were mainly favourable to nitrification processes, especially soon after the alfalfa termination, although denitrification processes might also have occurred. Indeed, a study by Huang *et al.* (2004) with conditions favourable to nitrification showed a negative correlation between soil N_2O emission and residue C:N ratio. This was attributed to both ease of mineralisation of this type of crop residue, with the consequent increase in N availability (Basche *et al.*, 2014), and stimulation of microbial activity, which promoted oxygen consumption (Lesschen *et al*., 2011), and which might have created temporary anaerobic microsites that would then have enhanced N_2O production via denitrification processes (Huang *et al.*, 2004; Mutegi *et al.*, 2010; Jensen *et al.*, 2012). Similarly, in the present study, the soil tillage might have promoted oxygen diffusion into the soil, with the consequent mineralisation and nitrification processes, which might have also caused a temporary anoxic environment through increased microbial activity and respiration; this would have favoured denitrification processes. Furthermore, as indicated by Álvaro-Fuentes *et al*. (2008), soil tillage for alfalfa+wheat might have led to break-up of the soil aggregates, with the consequent release of N_2O from their core, which is under anoxic conditions (Borer *et al*., 2018), and which would promote denitrification processes.

The data obtained in the present study are in line with Abalos *et al*. (2016), who reported an important increase in soil N_2O emission soon after termination of a perennial grass–legume mixture in September, with ploughing to a depth of 0.20 m. The emissions recorded in the present study soon after alfalfa termination were probably lower than would be expected after summer alfalfa termination (Krauss *et al*., 2017), as also observed by Ball *et al.* (2007), who carried out second-year grass–clover termination by ploughing (depth, 0.25 m) and recorded soil N_2O emissions that were almost double after the summer tillage (*i.e*., in July), compared to autumn tillage (*i.e*., in October). Indeed, although soil moisture has the greatest effect on soil N_2O emission, denitrification is particularly sensitive to increased and increasing temperatures. These conditions increase oxygen consumption by microorganism respiration and the consequent soil anaerobiosis, with this anaerobiosis is a precursor and major driver of soil N_2O emissions (Butterbach-Bahl *et al.*, 2013). However, in addition to the paucity of information on the effects of perennial legume termination in terms of soil N_2O emissions (Jensen *et al*., 2012), the correct interpretation of the data available is uncertain, as they are mainly context dependent. Indeed, some studies have reported that high soil N_2O emissions after alfalfa termination by ploughing in late summer is mainly due to the particular environmental conditions, such as high autumn soil moisture and spring thawing of the soil, as for a glacio-lacustrine clay floodplain (Westphal *et al*., 2018; Tenuta *et al*., 2019). Alternatively, in a field experiment in Grey Luvisol with loam texture, another study reported that the timing of alfalfa termination (*i.e*., spring, summer, late summer) and the method used (*i.e*., tillage, herbicide, both) had no influence on soil N_2O emissions for 7-year-old alfalfa (Malhi *et al.*, 2010).

In the present study, crop type and rotation also had fundamental roles in the regulation of the magnitude of the soil N_2O emissions. From October 2017 to the end of December 2017, during the wheat seedling stage, the alfalfa+wheat soil N_2O emissions accounted for 32.6% of the total emissions, which was double that for continuous alfalfa (15.5%). Indeed, during the initial wheat stage, when the wheat N uptake is expected to be low compared to the later stages (Delogu *et al.*, 1998; Li *et al.*, 2012), high N levels from the mineralised legume residues after the tillage should be available in the soil for alfalfa+wheat. On this basis, the N released into the soil after alfalfa termination (on 11 October 2017) cannot be used immediately by the wheat that was sown about a month and a half later (on 23 November 2017), and this therefore might have increased the nitrification and denitrification substrate for N_2O production. Between January and the end of February 2018, during the wheat tillering-double ridge stage, the alfalfa+wheat soil N_2O emissions accounted for 14.4% of its total emissions, with similar amounts for continuous alfalfa (18.3%). In this second period, the soil N_2O emissions were reduced for alfalfa+wheat, which was probably due to the greater use of N by the wheat in the sowing-greening stages, in line with that reported by Liu *et al*. (2015) under a wheat crop cycle. From March to the end of April 2018, during the wheat double ridge-jointing stage, the alfalfa+wheat soil N_2O emissions were similar to the previous period (15.0%) and similar to continuous alfalfa (16.9%). As in the previous wheat stage, during this period the low level of soil N_2O emission might have been due to nitrate subtraction in potential nitrification and denitrification processes, due to the high N uptake by the wheat crop at this stage (Delogu *et al.*, 1998). Between the beginning of May and early July 2018, during the wheat booting-maturity stage, the alfalfa+wheat soil N_2O emissions accounted for 37.7% of its total emissions, which was lower than for continuous alfalfa (49.4%). During this last monitoring period a general increase in soil N_2O emission might also have been due to the increase in soil temperature and rainfall that occurred in May (Aguilera *et al.*, 2013). For continuous alfalfa, the increase in the soil N_2O emission fluxes might also have been due to the mowing that was performed in early May 2018, which might have reduced the N uptake from the root system (Erice *et al.*, 2011). This might be related to the removal of the photosynthetic tissues, with the consequent change in the alfalfa N metabolism. In particular, herbage cutting might have led to reduction in the uptake of the mineral N forms from the soil, and to a general decrease in nodule activity (Erice *et al*., 2011). For alfalfa+wheat, the decreasing trend of the last sampling period can be explained by the higher N uptake that occurred from the heading to the maturity stage (Delogu *et al.*, 1998; Li *et al.*, 2012). Despite the variations in the soil N_2O emissions highlighted through the study period, especially for the alfalfa+wheat treatment, the cumulative seasonal emissions did not show significant differences. This might be linked to the autumn alfalfa termination which might have shortened the time window between N release and N uptake, which will have reduced the substrate for nitrification and denitrification processes, except soon after the incorporation of the crop residues. The cumulative seasonal soil N_2O emissions in this study are consistent with those reported in other studies under similar climatic conditions. For example, Volpi *et al.* (2018) reported a cumulative soil N_2O emission of 0.87 kg N-N_2O ha^{-1} for durum wheat seeded after clover, which included minimum tillage (*i.e*., disk harrow, at the beginning of September; depth, 0.10 m) without N fertilisation, in a Mediterranean environment. These data contribute to the definition of mitigation strategies for GHG emissions (Purwanto and Alam, 2020) that can be used for this crop rotation in a Mediterranean environment. Moreover, to increase the impact and effectiveness of the mitigation practices, these should be included in site-specific agro-environmental climate measures at the landscape scale (Toderi *et al*., 2017).

Conclusions

This study helps to fill an important knowledge gap concerning the effects on soil N_2O emissions relating to perennial legume termination in Mediterranean crop rotation systems. To identify GHG mitigation options, the present study analysed soil N_2O emissions with termination of the perennial legume in an alfalfa-wheat rotation in a Mediterranean environment.

Perennial legume termination in early autumn appears to have provided less favourable conditions for the mineralisation process compared to hypothetical termination in summer, with higher temperatures. The initial higher soil N_2O emissions for alfalfa+wheat that emerged from the cumulative weekly analysis appeared to be due to the alfalfa mineralisation process after the tillage, and to the unavoidable asynchrony between the N released following alfalfa termination and the low N uptake by the following wheat. Reducing time-window between alfalfa termination and wheat N uptake can contain the N_2O emissions**,** except after an initial inevitable increase in the soil N_2O emissions. However, this initial higher soil N_2O emission for alfalfa+wheat did not affect the seasonal cumulative soil N_2O emissions, compared to continuous alfalfa.

In conclusion, under the rotation system analysed here, the mitigation effects of the perennial legume on the soil N_2O emissions were not lost after its termination by tillage.

In this production context, further studies are needed to confirm these effects of perennial legume termination by tillage on soil N_2O emissions, including the need to compare this to other alfalfa termination methods (*e.g.*, using a desiccant and subsequent cereal sod-seeding). To contribute further to the identification of mitigation strategies for GHG emissions in crop rotation systems under Mediterranean conditions, studies are also needed that focus on N_2O and other important GHGs (*e.g.*, CO_2, CH_4) in this and other relevant production contexts. These should include: i) soils where the main tillage performed in autumn is more difficult due to the rainfall regime and the soil requirements; and ii) other cereal-based crop rotation systems with short-lived perennial legumes, such as sulla (*Hedysarum coronarium* L.) and common sainfoin (*Onobrychis viciifolia* Scop.).

Highlights

- Soil N_2O emissions peak after alfalfa termination and rainfall.
- Soil N_2O emissions increase after spring alfalfa mowing.
- Seasonal cumulative soil N_2O emissions are similar for alfalfa and alfalfa followed by wheat.
- Mitigation effects of perennial legume on soil N_2O emissions are not lost after termination by tillage under alfalfa-wheat rotation.

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