Nitrous oxide emissions from clover in the Mediterranean environment

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Abstract

Introducing nitrogen N_2-fixing crops into cereal-based crop rotations reduces N-fertiliser use and may mitigate soil emissions of nitrous oxide (N_2O). However, the effect of the cultivation of N_2-fixing crops on N_2O emissions is still not well understood. N_2O from N_2-fixing crops can be emitted in two ways: during biological N_2 fixation itself and when legume residues are returned to the soil. A field trial was carried out on clover (*Trifolium squarrosum* Savi) to test the role of leguminous crops on N_2O emissions in the Mediterranean environment. Monitoring was performed from December 2013 to September 2014. Cumulated N-N_2O fluxes were calculated for the growing season (Phase 1) and the post-harvest period (Phase 2) in order to assess the importance of each phase. Our results did not show statistically significant differences between the two phases in term of contribution to the total cumulative N-N_2O emissions, in fact Phase 1 and Phase 2 accounted respectively for 43 and 57% of the total.

Introduction

Nitrous oxide (N_2O) is an ozone depleting and long-lived (about 121 years) greenhouse gas with a global warming potential about 298 times higher respect to CO_2 (IPCC, 2013). Evaluating the magnitude of N_2O emissions from agriculture and the possible mitigation strategies has a scientific and practical relevance, in fact emissions from agricultural and natural soils account for 56-70% of all the global sources (Syakila and Kroeze, 2011). N_2O from soils is produced by microbial processes of nitrification and denitrification and its release is mainly related to nitrogen supply management (Rees *et al.*, 2013; Snyder *et al.*, 2014). The magnitude of N_2O emissions is affected also by several site-specific aspects such as soil characteristics, climatic conditions and crop management (*e.g.*, soil tillage, residue management, irrigation level *etc.*). The optimization of legumes management can offer an opportunity to reduce greenhouse gases (GHG) emissions from the agricultural sector (Smith *et al.*, 2008). In fact, introducing N_2-fixing legumes into cereal-based crop rotations reduces synthetic N fertilizer use and may mitigate direct N_2O emissions from soil and reduces the use of fossil energy to produce N fertilizer (Jensen *et al.*, 2012). N_2O from legume crops can be produced both during nitrogen fixation and when crop residues decompose in the soil. The effect of the cultivation of legumes on N_2O emissions is not well understood (Tellez-Rio *et al.*, 2015). Recent studies observed a high variability between different sites and suggested that legume-derived N can be also a source of N_2O emissions (Rochette and Janzen, 2005). There are weak evidences that the N fixation itself contributes significantly to the total emissions (Zhong *et al.*, 2009; Jensen *et al.*, 2012). Rochette *et al.* (2004) observed lower N_2O emissions in legumes than in N-fertilized crops. On the contrary, peaks of N_2O emissions were recorded after clover harvest when residues decomposition occurred. Post-harvest peaks in N_2O flux are mainly due to asynchrony between the N release and the demand of the following crop (Crews and Peolples, 2005). Legume residues decomposition is often fast as the carbon/nitrogen (C/N) ratio is low and the main sources of N_2O from N_2-fixing crops is the decomposition of the residues (Van Der Weerden *et al.*, 1999; Baggs *et al.*, 2000). Root nodules C/N ratio is lower than the ratio of the other type of plants residues, thus in the soil decomposition of nodules is very fast and can be the major source of N_2O during the postharvest period (Uchida and Akiyama, 2013). An experimental field was cultivated with clover (*Trifolium squarrosum L.*) with the aim to assess the magnitude of N_2O daily flux and cumulative emissions in the Mediterranean environment. GHG monitoring and analysis of data were done both for the crop growth and the fallow period, in order to evaluate the contribution of the two phases to the overall cumulative emissions.

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Materials and methods

Site description and experimental set up

The experimental field was located in the Centre for Agro-Environmental Research *E. Avanzi* (CIRAA), in San Piero a Grado, in the Pisa (central Italy) coastal plain. Climate is Mediterranean with a mean annual precipitation of 907 mm and a mean annual temperature of 15°C (long term average 1986-2013). Clover (*Trifolium squarrosum*

L.) was sown on Dec 2013 in a fertile sandy-loam soil. Tillage was conducted by ploughing (30 cm depth) followed by rotary harrowing immediately before sowing. Weeds and pest control were never necessary during the trial. Harvest was performed at the end of May 2014. The experimental set up was constituted by four sampling areas (5×6 m), each one provided with a polyvinyl chloride (PVC) collar for emissions monitoring. At harvest aboveground biomass was sampled in two areas of 0.5 m^2 per plot and fresh weight was measured. Then, subsamples were dried at 60°C until constant weight to determine the dry biomass yield.

Monitoring protocol and prototype characteristics

The monitoring campaign started on 10 Dec 2013 and ended on 16 Sept 2014. Monitoring was carried out with a bimonthly frequency. Monitoring was performed from crop sowing to harvest (Phase 1) and during 4 months of the fallow period (Phase 2). Due to unfavourable environmental condition cultural operations were delayed and it was not possible to maintain the field trial until the tillage for the following crop, so this study does not include N_2O monitoring after the incorporation of crop residues with tillage.

N_2O was monitored directly in the field with the LIFE+IPNOA mobile prototype described by Bosco *et al.* (2015) through the non-steady state chamber technique. GHG concentration within the chamber was measured at a time step of 1 second (ppb s^{-1}), the increase in the headspace was checked for linearity over a period of 2-3 min. Data were recorded by a palm top computer connected *via* Bluetooth®. Each PVC collar had a 15 cm radius, was 15 cm high and was inserted for 5 cm into the soil.

In order to follow crop growth three PVC stackable extensions were installed on the collars when needed. Soil temperature and volumetric water content were recorded beside each collar at a depth of 5 cm by a dielectric probe (Decagon Devices GS3; Decagon Devices, Inc., Pullman, WA, USA) linked to the prototype *via* Bluetooth®. Water filled pore space (%) (WFPS) was calculated from total porosity using bulk density considering the particle density as 2.65 g cm^{-3}.

Mineral nitrogen determination in soil

Soil samples for mineral nitrogen determination were collected once a month in the 0-20 cm layer during a monitoring day. A sample from each sampling area was composed of three soil cores. Samples were frozen at –20°C. A 12 g subsample of moist soil was extracted with 1 M KCl using a 1:10 soil: extractant ratio and 30 min shaking time. Concentrations of N-NO_3 were determined with ultraviolet spectrophotometric technique (Goldman and Jacobs, 1961). N-NH_4 concentrations were determined photometrically after using the Spectroquant® kit (Merck Millipore Corp., Billerica, MA, USA). Soil mineral N content was calculated from N sample concentration considering soil dry weight.

Statistical analysis

Statistical analysis was performed with the R software (R Core Team 2015; R Foundation for Statistical Computing, Vienna, Austria). Level of significance was α=0.05. Nitrous oxide flux was calculated by linear regression of N_2O concentration increase during chamber closure time taking into account air temperature, atmospheric pressure and headspace volume. Cumulative N_2O emissions over the period were calculated by linear interpolation of two following sampling dates and the numerical integration over time. Differences between Phase 1 and Phase 2 for cumulative N_2O emissions were tested with a one-way analysis of variance (ANOVA). Relationships between N_2O daily flux and soil characteristics were tested after log transformation by stepwise multiple linear regression analysis.

Results

Meteorological pattern, nitrous oxide fluxes, soil water filled pore space and temperature, soil NO_3^- and NH_4^+

In winter 2014, heavy rains occurred from January to March (>500 mm) causing a data gap from 9 Jan 2014 to 10 Mar 2014 in the N_2O flux measurements (Figure 1A).

During the whole monitoring period the total rainfall was 1033 mm, 12% highest than the long-term yearly average. The highest recorded mean daily temperature was 36.0°C on 9 Jun 2014, while the minimum daily temperature was –1°C on 17 Dec 2013. The average daily temperature along the monitoring period was 15.5°C. The dry yield at harvest was about 10 Mg ha^{-1}.

The highest N_2O daily flux was 0.67±0.04 mg N_2O m^{-2} day^{-1} on 10 Dec 2013, the lowest was –0.11±0.07 mg N_2O m^{-2} day^{-1} on 10 Mar 2014 and the mean was 0.32±0.06 mg N_2O m^{-2} day^{-1} (Figure 1B).

N_2O flux decreased from December until the end of March, afterwards the magnitude of the flux increased and reached a peak in April 22 with 0.61±0.12 mg N_2O m^{-2} day^{-1}. The mean N_2O flux during Phase 1 was 0.29±0.03 mg N_2O m^{-2} day^{-1}. In Phase 2 the N_2O flux magnitude was stable around the average of 0.37±0.02 mg N_2O m^{-2} day^{-1}. Statistical analysis did not show differences between the mean daily fluxes of the two phases. Daily mean, minimum and maximum values of N-NO_3 and N-NH_4 soil concentration, soil WFPS and temperature during Phase 1 and 2 were reported in Table 1.

Peaks in N-NO_3 concentration (19.2 ppm) on 22 Apr 2014 and 24 July 2014 (15.3 ppm) (Figure 1C) corresponded to peaks in N_2O flux. Relationship between the log transformed N_2O, N-NO_3 and N-NH_4 soil concentration and soil temperature during Phase 1 was described by a multiple linear regression ($P<0.01$, adjusted r^2=0.4).

Cumulative N- Nitrous oxide emissions

Total cumulative emissions over the monitoring period (280 days) was 482 g N-N_2O ha^{-1}. Phase 1 (163 days) and Phase 2 (117 days) accounted respectively for 43 and 57% of the total. Cumulative N-N_2O emissions in the two phases did not show significant differences.

Table 1. Mean, minimum and maximum values of N-NO_3 and N-NH_4 concentration in soil, soil water filled pore space and temperature during Phase 1 and 2. Soil water filled pore space and soil temperature were recorded at 5 cm depth. With Phase 1 is identified the growing period, while Phase 2 is the period after the crop harvest.

Phase	N-NO_3 (ppm)			N-NH_4 (ppm)			WFPS (%)			Soil temperature (°C)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
1	8.6	0.0	19.2	2.8	1.5	3.7	29.7	9.1	62.3	17.2	10.0	24.8
2	9.1	3.8	15.3	3.8	2.5	6.2	29.1	5.7	63.5	32.7	29.9	35.5

WFPS, water filled pore space.

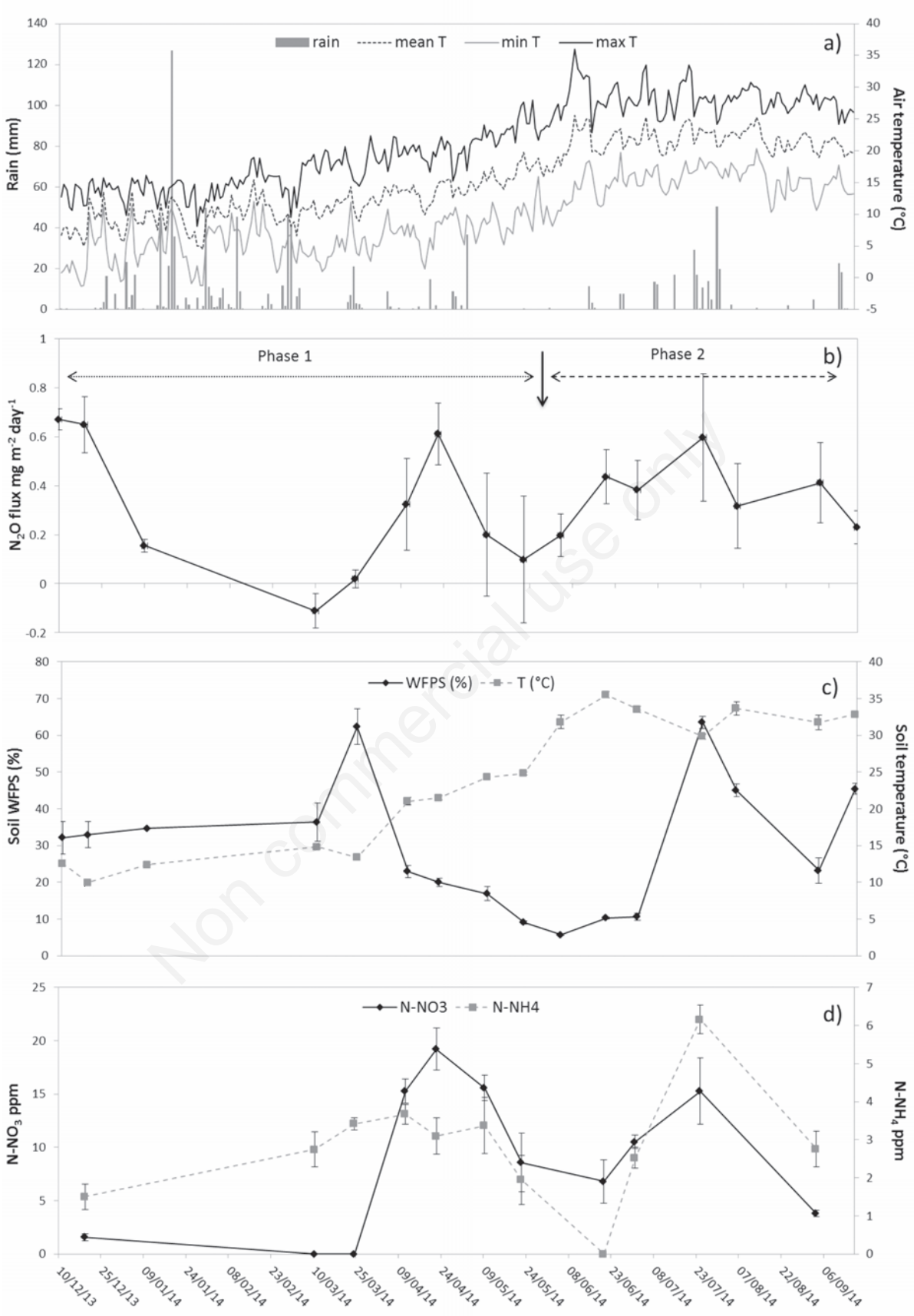

Figure 1. Pattern of meteorological data (A), daily soil N_2O flux (B), soil water filled pore space (WFPS) and temperature (C), N-NO_3 and N-NH_4 (D) soil concentration during the monitoring campaign.

Discussion

Mean daily N_2O flux in Phase 2 showed a very limited range of variation, while during the presence of the crop (Phase 1) peaks were detectable in few dates, at the beginning of the monitoring campaign and in April. The high N_2O flux at the sowing was possibly due to the incorporation of the previous crop residues with tillage (Baggs *et al.*, 2000). From January to the beginning of April N_2O flux was low, concurrently with a very low $N\text{-}NO_3$ concentration in soil (around 0 ppm). From April to the beginning of May high N_2O fluxes corresponded to a high $N\text{-}NO_3$ availability, probably promoted by the N-fixation activity of the plants. This can be explained by the significant multiple linear regression found for Phase 1 between N_2O, $N\text{-}NO_3$, $N\text{-}NH_4$ and soil temperature, where the $N\text{-}NO_3$ is the variable with the highest predictive capability ($P<0.001$). During Phase 2 the N_2O flux magnitude was stable, likely due to the constant degradation rate of the roots nodules (Uchida and Akiyama, 2013). Cumulative $N\text{-}N_2O$ emissions reported by Barton *et al.* (2011) from a semiarid climate were more than three times lower that our value (127 g $N\text{-}N_2O$ ha^{-1} for 350 days). Our cumulative emissions were slightly lower than mean data from papers on Mediterranean environment reported in the review of Aguilera *et al.* (2013) on annual basis (700 g $N\text{-}N_2O$ ha^{-1} y^{-1}). Flessa *et al.* (2002) in a temperate climate reported cumulative $N\text{-}N_2O$ higher than our value, around 2 kg $N\text{-}N_2O$ ha^{-1} y^{-1} from lupine. The same author observed that cumulative emissions from lupine were lower than emissions from fertilized crops in the same area but highlighted that the ploughing of a clover field after the harvest had a negative effect on the growing period of the subsequent crop enhancing emissions (7-12 kg $N\text{-}N_2O$ ha^{-1} y^{-1}), probably due to the mineralization of biologically N fixed residue and the increased microbial respiration. Our results confirmed that $N\text{-}N_2O$ cumulative emissions from clover in both during crop growth and in fallow period were smaller compared with the cumulative $N\text{-}N_2O$ of a fertilized durum wheat (1831 g $N\text{-}N_2O$ ha^{-1}) cultivated at the same study site in the 2013-2014 growing season (Bosco *et al.*, 2015).

Conclusions

Our results highlighted that $N\text{-}N_2O$ emissions from clover, both during the crop growth and in fallow period, were lower than emissions from a fertilized crop in the same environment. Between the two periods no significant differences were found, so the two phases contribute equally to the total cumulative $N\text{-}N_2O$ emissions. Anyway our study did not include N_2O flux monitoring after incorporation of clover residues with tillage, which seems to represent a high source of N_2O emissions.

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