

# LIFE+IPNOA mobile prototype for the monitoring of soil N<sub>2</sub>O emissions from arable crops: first-year results on durum wheat

Simona Bosco,<sup>1</sup> Iride Volpi,<sup>1</sup> Nicoletta Nasso,<sup>1</sup> Di Nasso,<sup>1</sup> Federico Triana,<sup>1</sup> Neri Roncucci,<sup>1</sup> Cristiano Tozzini,<sup>1</sup> Ricardo Villani,<sup>1</sup> Patricia Laville,<sup>2</sup> Simone Neri,<sup>3</sup> Federica Mattei,<sup>3</sup> Giorgio Virgili,<sup>3</sup> Stefania Nuvoli,<sup>4</sup> Luigi Fabbrini,<sup>4</sup> Enrico Bonari<sup>1</sup>

<sup>1</sup>Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy; <sup>2</sup>INRA UMR Environnement et Grandes Cultures, Thiverval Grignon, France; <sup>3</sup>West Systems S.r.l., Firenze, Italy; <sup>4</sup>Department of Competitiveness and Development, Tuscany Region, Firenze, Italy

## Abstract

Agricultural activities are co-responsible for the emission of the most important greenhouse gases: carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O). Development of methodologies to improve monitoring techniques for N<sub>2</sub>O are still needful. The LIFE+IPNOA project aims to improve the emissions monitoring of nitrous oxide from agricultural soils and to identify the agricultural practices that can limit N<sub>2</sub>O production. In order to achieve this objective, both a mobile and a stationary instrument were developed and validated. Several experimental field trials were set up in two different

sites investigating the most representative crops of Tuscany (Central Italy), namely durum wheat, maize, sunflower, tomato and faba bean. The field trials were realized in order to test the effect on N<sub>2</sub>O emissions of key factors: tillage intensity, nitrogen fertiliser rate and irrigation. The field trial on durum wheat was set up in 2013 to test the effect of tillage intensity (minimum and conventional tillage) and nitrogen fertilisation rate (0, 110, 170 kg N ha<sup>-1</sup>) on soil N<sub>2</sub>O flux. Monitoring was carried out using the IPNOA mobile prototype. Preliminary results on N<sub>2</sub>O emissions for the durum wheat growing season showed that mean daily N<sub>2</sub>O fluxes ranged from -0.13 to 6.43 mg m<sup>-2</sup> day<sup>-1</sup> and cumulative N<sub>2</sub>O-N emissions over the period ranged from 827 to 2340 g N<sub>2</sub>O-N ha<sup>-1</sup>. Tillage did not affect N<sub>2</sub>O flux while increasing nitrogen fertilisation rate resulted to significantly increase N<sub>2</sub>O emissions. The IPNOA mobile prototype performed well during this first year of monitoring, allowing to catch both very low fluxes and peaks on N<sub>2</sub>O emissions after nitrogen supply, showing a good suitability to the field conditions.

Correspondence: Simona Bosco, Institute of Life Sciences, Scuola Superiore Sant'Anna, P.zza Martiri della Libertà 33, 56127 Pisa, Italy.  
Tel.: +39.050.883512 - Fax: +39.050.883526.  
E-mail: s.bosco@sssup.it

Key words: Nitrous oxide flux; chamber method; greenhouse gas mitigation; minimum tillage; ploughing; nitrogen rate.

Funding: this research was carried out with the contribution of the LIFE financial Instrument of the European Union, within the framework of the project LIFE+IPNOA *Improved flux Prototypes for N<sub>2</sub>O emission from Agriculture* (LIFE/11 ENV/IT/302, [www.ipnoa.eu](http://www.ipnoa.eu)). The views expressed in this work are the sole responsibility of the authors and do not necessarily reflect the views of the European Commission.

Acknowledgements: a special thanks is also due to the technical staff at the Institute of Life Sciences, particularly to Alessio Barbaferi, Fabio Taccini, Sergio Cattani and Giampaolo Pala.

Conference presentation: SIA XLIII Congress, Pisa, 2014.

Received for publication: 25 February 2015.  
Revision received: 28 April 2015.  
Accepted for publication: 13 May 2015.

© Copyright S. Bosco et al., 2015  
Licensee PAGEPress, Italy  
Italian Journal of Agronomy 2015; 10:669  
doi:10.4081/ija.2015.669

This article is distributed under the terms of the Creative Commons Attribution Noncommercial License (by-nc 3.0) which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

## Introduction

Atmospheric greenhouse gas (GHG) concentration has increased continuously since pre-industrial era to nowadays. The 5<sup>th</sup> Assessment report of the Intergovernmental Panel on Climate Change (IPCC) underlined the necessity of severe actions of mitigation and adaptation to avoid the risk of irreversible effects on global climate (IPCC, 2013). Therefore, the European Commission on 23 October 2014 undersigned new targets of GHG emissions reduction by at least 40% below the 1990 level by 2030 (European Commission, 2014).

Beside carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O) is one of the main GHG contributing to global warming and atmosphere ozone depletion. Atmospheric nitrous oxide (N<sub>2</sub>O) concentration has increased by 19% since 1750 to an average global value of 324 ppb in 2011 (IPCC, 2013). The interest in understanding N<sub>2</sub>O emission processes and in investigating the most effective mitigation techniques is also due to the long lasting persistence of this gas in atmosphere (about 121 years) and its global warming potential, which is about 298 times higher respect to CO<sub>2</sub> (IPCC, 2013).

Agriculture activities are co-responsible of the release in atmosphere of significant amount of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O (Smith *et al.*, 2008). In particular, nitrous oxide is the main GHG from agriculture and contributes from 30% to 45% of global anthropogenic N<sub>2</sub>O emissions (Fowler *et al.*, 2009). Around 70% of the global annual flux of N<sub>2</sub>O derives both from managed and natural soils, mainly as an intermediate product of the two microbiological processes of nitrogen (N) trans-

formation in soil, nitrification and denitrification (Mosier *et al.*, 1998).

Concerning arable lands, previous studies highlighted that the magnitude of N<sub>2</sub>O emissions depends mainly on agricultural management practices such as tillage intensity, irrigation and most of all on the supply of N fertilisers. Their importance varies in space and time due to influences of site-specific factors such as climate conditions (*e.g.*, air temperature, rainfall) and soil conditions (*e.g.*, texture, soil organic carbon, pH, water filled pore space, soil temperature, *etc.*) (Davidson *et al.*, 1991; Mosier *et al.*, 1998; Bouwman *et al.*, 2002). All these factors regulate soil microbial activity, which has a direct effect on N<sub>2</sub>O emissions (Butterbach-Bahl *et al.*, 2013).

Tillage practices affect soil physic characteristics and soil carbon dynamics, thus influencing GHG emissions, although results from several studies in different climate conditions have shown contrasting results (Venterea *et al.*, 2005; Abdalla *et al.*, 2013; van Kessel *et al.*, 2013). Through its influence on soil N availability, N supply is perhaps the key parameter for N<sub>2</sub>O production in soil (Rochette *et al.*, 2008; Rees *et al.*, 2013). Furthermore, N input exceeding crop requirements and an asynchronous timing of N supply in relation to crop needs have been identified as great contributors to N<sub>2</sub>O emissions from arable land (Snyder *et al.*, 2014). Therefore, a major challenge for agriculture is how to improve crop N use efficiency, reducing N<sub>2</sub>O emissions while also achieving greater N effectiveness in crop yield (Venterea *et al.*, 2012). The uncertainty concerning N<sub>2</sub>O mitigation strategies is especially relevant for Mediterranean-type cropping systems because of the scarcity of studies on this specific climate (Aguilera *et al.*, 2013).

Opportunities for mitigating N<sub>2</sub>O emissions at the field level can arise from a clearer understanding of the system complexity leading to emissions. However, the monitoring of N<sub>2</sub>O emissions presents some difficulties due to the wide temporal (Laville *et al.*, 2011; Flessa *et al.*, 2002) and spatial variability (Jahangir *et al.*, 2011). Moreover, different methodologies are used for chamber measurements. Chamber based measurement approaches are currently the only way to compare the effect of treatments in field experiments. Chambers are an intrusive gas flux measuring method, in fact their deployment often modifies the flux being measured, consequently several precautions need to be taken to avoid biased flux estimates (Rochette and Eriksen-Hamel, 2008; Heinemeyer and McNamara, 2011). Most soil flux N<sub>2</sub>O measurement are made through the use of small non-flow-through non-steady-state (NFT-NSS) chambers (Chadwick *et al.*, 2014) because of its simplicity and low cost. From NFT-NSS chambers headspace samples are taken while the chamber is closed for an incubation period of 30-60 min (Cowan *et al.*, 2014). Bias in flux estimation due to changes in soil temperature, air temperature and humidity, and gas leakage inside the chamber increase with deployment time. Indeed, long deployment time (>30 min) alters considerably the diffusion gradient between soil and atmosphere (Rochette and Eriksen-Hamel, 2008). Generally, gas samples are returned to the laboratory in sealed vials or syringes for N<sub>2</sub>O analysis by gas chromatography (GC). Therefore, storage of air samples is a problematic issue because of gas leaking from containers and contamination risk. The flux is inferred from the increment of gas concentration in the chamber headspace. Because of the limits imposed by the logistics of sample collection and subsequent laboratory analysis, the typical samples number range from two to four per chamber closure, consequently fluxes calculated by any regression model are poorly constrained (Pedersen *et al.*, 2010). In addition, the resolution of GC is usually poor (>10 nmol mol<sup>-1</sup> for N<sub>2</sub>O), thus detecting small changes of N<sub>2</sub>O concentration is difficult, and in many cases the analysis of gas concentration has been the largest source of error in soil N<sub>2</sub>O flux estimation (Cowan *et al.*, 2014).

Recently, N<sub>2</sub>O laser instrumentation has become more accessible, and advances in infrared laser technology have produced fast-response (>10 Hz) measurement capabilities with improved sensibility (<5

nmol mol<sup>-1</sup>) (Cowan *et al.*, 2014). In automatic systems used in the field, this technology is currently associated with the use of flow-through non-steady-state chambers. In the latter, flux is calculated from the concentration difference between the air flowing at a known rate through the chamber inlet and outlet after the chamber headspace concentration has reached an equilibrium (Livingston and Hutchinson, 1995). This technology permits an immediate visualization of the gas concentration increment inside the chamber. Moreover, the chamber deployment time can be shorter than the closure time requested by the NFT-NSS technology, thus improving the flux estimation (Heinemeyer and McNamara, 2011).

The LIFE+ *Improved flux Prototypes for N<sub>2</sub>O emission from Agriculture* (IPNOA) project (2012-2016, LIFE/11 ENV/IT/302, www.ipnoa.eu) is coordinated by West Systems S.r.l. (Pontedera, Italy) with the partnership of the Institute of Life Sciences of Scuola Superiore Sant'Anna, the French National Institute For Agricultural Research (INRA) UMR *Environnement et Grandes Cultures* and the Tuscany Region. The main objectives of the IPNOA project are: i) to develop and validate two prototypes (mobile and stationary) for measuring the soil N<sub>2</sub>O fluxes directly in the field, thus improving the monitoring of these emissions from agricultural soils; ii) to implement several experimental trials concerning the main arable crops cultivated in Tuscany, in order to identify the best management practices (BMPs) help in reducing N<sub>2</sub>O emissions; iii) to calibrate and to validate a model to estimate the N<sub>2</sub>O annual budget and scale up the results and the so-identified BMPs to regional scale.

Specifically, the aim of this paper is to present the innovative mobile prototype developed in the LIFE+IPNOA and to show the N<sub>2</sub>O flux results for the 2013-2014 growing season on durum wheat, cultivated under different tillage intensities and N fertilisation rates.

## Materials and methods

### IPNOA mobile prototype description and N<sub>2</sub>O monitoring protocol

A mobile prototype was developed by West Systems S.r.l. in order to evaluate N<sub>2</sub>O emissions at field scale using a fast chamber technique (Figure 1). While the stationary station, equipped with six automated chambers, allows a better estimation of the temporal variability of fluxes from soil, the mobile prototype responds better to the necessity to investigate spatial variability and, in agricultural trials, to test different replicated treatments. The main challenge was to develop a mobile system capable of moving on various field surfaces, equipped with very reliable N<sub>2</sub>O gas analyser, electrically autonomous and enough robust to face up field conditions.

The prototype was equipped with an LRG N<sub>2</sub>O/CO detector for N<sub>2</sub>O and with an LGR ultraportable greenhouse gas analyser (CH<sub>4</sub>, CO<sub>2</sub>, H<sub>2</sub>O) [Los Gatos Research (LRG), Inc., Mountain View, CA, USA]. Both detectors were installed on a light tracked vehicle appropriate to access agricultural fields (total dimensions: 1.49 m height, 1.16 m width and 1.46 m depth; total weight: around 600 kg). The instrument was connected to a chamber through a 20 m long tube of 4 mm diameter. The chamber (flow-through non-steady state steel chamber) had a height of 10 cm and a diameter of 30 cm; the headspace volume was 6868 cm<sup>3</sup> (West Systems S.r.l.). The pumping flow was about 240 scm<sup>3</sup> min<sup>-1</sup>. A PVC collar with the same diameter as the chamber (30 cm) was placed in each plot and inserted in the soil to a depth of about 5 cm. To guarantee a tight seal with the collars, the chamber was provided with a rubber ring that fits into the collar lip. An internal fan maintained the homogeneity of the air mixture within the chamber during the meas-

urements.  $N_2O$  concentration within the chamber was measured at a time step of 1 s ( $ppb\ s^{-1}$ ), and the increase in the headspace was checked for linearity for a period of 2-3 min. Data were recorded by a palmtop connected via Bluetooth®.

$N_2O$  flux was measured in all the experimental fields bimonthly, and samplings were intensified immediately after N fertilisation events and after residue incorporation with tillage, when measurements were carried out twice a week for two/three consecutive weeks. Depending on the row spacing, the collar was placed in the interrow space or the crop was left uncut within the collar. At every measurement soil temperature and volumetric water content were recorded in the proximity of each collar by a dielectric probe (GS3; Decagon Devices, Inc., Pullman, WA, USA) inserted into the soil at a depth of 5 cm and linked to the prototype by a Bluetooth® connection. Water filled pore space (WFPS) was calculated from total porosity using bulk density, measured by the soil core method and considering a particle density of  $2.65\ g\ cm^{-3}$ .

The mobile prototype was validated before the beginning of the monitoring campaign through three experiments. The first was conducted in Scotland (Edinburgh) during the *Easter Bush* international campaign organised by the *Integrated non-CO<sub>2</sub> Greenhouse-gas Observing System* (InGOS) project in June 2013. The Easter Bush site is located 10 km south of Edinburgh, Scotland UK ( $3^{\circ}12' W$ ,  $55^{\circ}52' N$ , 190 m a.s.l.). The area is situated between two intensively-managed grassland fields. The test compared the mapping of  $N_2O$  emissions obtained using the mobile prototype with measurements of  $N_2O$  fluxes using the Eddy

covariance methodology. Measurements with IPNOA mobile prototype were made on a large grid of 1 ha with more than 30 sampling points on 24, 25 and 26 June 2013. IPNOA chamber measurements were compared with eddy covariance measurements during the period from 24 to 26 June. Overall, identical magnitudes of fluxes were observed between the two methodologies (Laville *et al.*, 2015).

On July 2013, a second experiment was conducted at Grignon (France) on barley crop. The cross validation was conducted in a field of 1 hectare close the EGC INRA building ( $48^{\circ}50' N$   $1^{\circ}56' E$ , 127 m a.s.l.). In this case, tests were conducted to compare performances of different gas analysers, different chambers (auto or manual chambers) and a test to detect IPNOA chamber response time (Laville *et al.*, 2015).

Performances of IPNOA LGR  $N_2O$  spectrometer were compared with other three INRA gas analysers, such as a filter correlation spectrometer (Thermo 46C; Thermo Environmental Instruments, Inc., Franklin, MA, USA), a quantum cascade laser tunable infrared laser differential absorption spectroscopy (QCL-TILDAS; Aerodyne Research Inc., Billerica, MA, USA) and with a gas chromatograph (GC; Varian CP-3000; Varian, Inc., Santa Clara, CA, USA). Calibrated gas cylinders were used to estimate the response time.

The responses of the prototype were very satisfactory in the comparisons with other devices (auto or manual chambers) and gas analysers (Thermo 46C, GC, QCL-TILDAS). The response time is short allowing limiting deployment time of the chamber, in fact 90 s were enough to start gas accumulation phase and 5 min of chamber deployment were

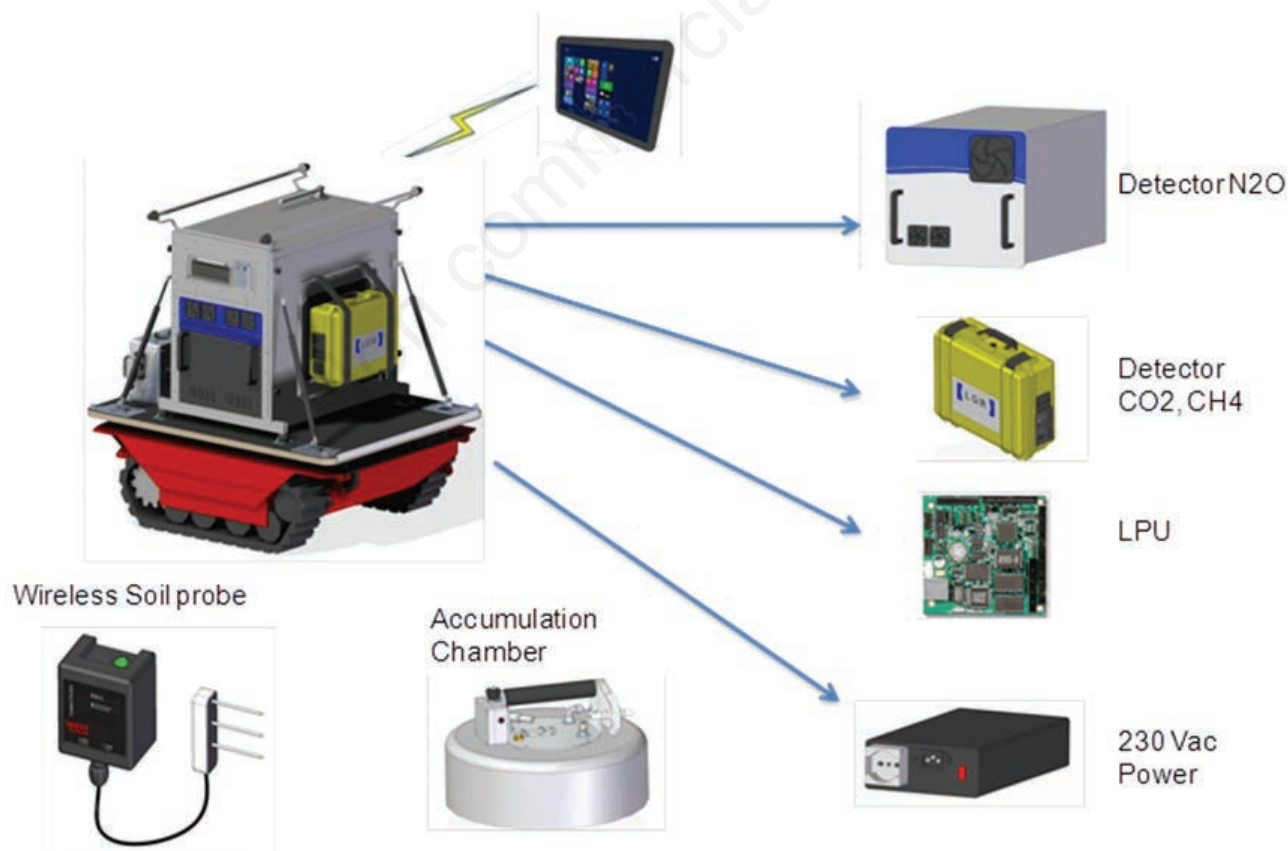


Figure 1. IPNOA mobile prototype arrangement for  $N_2O$ ,  $CO_2$  and  $CH_4$  emissions monitoring.

enough to estimate fluxes with a high resolution (Laville *et al.*, 2015). Finally, IPNOA chamber was validated through the participation to the *INGOS N<sub>2</sub>O chamber inter-comparison campaign 2014* at Hyytiälä Forestry Field Station (Finland), where 22 chambers of different sizes, shapes and attributes (fan, vent-tube, sampling, seals) from different research groups were tested against a known reference flux of N<sub>2</sub>O. The IPNOA chamber performed well showing a good fitting of the measured flux with linear regression and a low leakage rate.

### Field trials description

The IPNOA experimental field trials are located in two representative sites within Tuscany region: i) the Centre for Agro-Environmental Research E. Avanzi (CIRAA), located in San Piero a Grado (Pisa); and ii) the Centre for Agricultural Technologies and Extension Services (CATES), located in Cesa (Arezzo). The GHG monitoring is conducted on different crops: durum wheat (*Triticum durum* Desf., var. Tirez), maize (*Zea mays* L., var. DKC4316), sunflower (*Helianthus annuus* L., var. Pacific), tomato (*Solanum lycopersicum* L., var. perfectpeel) and faba bean (*Vicia faba minor* L., var. vesuvio). Key factors for each crop were identified to design the experimental trials: tillage intensity and N rate for durum wheat and sunflower; irrigation and N rate for maize and tomato; tillage intensity for faba bean. This paper presents preliminary results on the durum wheat field trial at CIRAA.

### Durum wheat field trial at CIRAA

Durum wheat was cultivated from November 2013 at CIRAA, in the Pisa (central Italy) coastal plain, characterised by a Mediterranean climate. The soil is a silty clay loam derived from alluvial sediments (Soil

Survey Staff, 1975). A split-plot design with four replicates was used. The main plot was assigned to the tillage intensity factor, which consisted in conventional tillage (CT) (ploughing, 30 cm depth) and minimum tillage (MT) (10 cm depth). The sub-plot was assigned to the N fertilisation factor, which consisted in three N fertilisation rates: no fertilisation (N<sub>0</sub>), 110 kg N ha<sup>-1</sup> (N<sub>1</sub>) and 170 kg N ha<sup>-1</sup> (N<sub>2</sub>). Nitrogen fertiliser was distributed three times: i) after sowing (26 November); ii) during tillering (7 March); and iii) during stem elongation (8 April). Durum wheat was sown on 14 November 2013 and harvested on 30 June 2014 (Table 1). The previous crop was berseem clover (*Trifolium alexandrinum* L.).

### Data analysis

N<sub>2</sub>O flux was calculated by performing a linear regression on the logged N<sub>2</sub>O concentration data, which was corrected for atmospheric pressure and air temperature. A linear mixed-effects model was used to analyse N<sub>2</sub>O log transformed flux data using the R lme4 package (Bates *et al.*, 2014). To analyse the whole dataset, tillage, N fertilisation rate and date were considered as fixed variables, while collar and blocks were considered as random factors. Significance was determined using the R LMERConvenienceFunctions package (Tremblay and Ransijn, 2013). Significance was tested also analysing each sampling date separately. Cumulative N<sub>2</sub>O emissions over the period were calculated by linear interpolation of two neighbouring sampling dates and the numerical integration over time. A linear mixed effects model was used for cumulative values. Tillage intensity and nitrogen fertilisation rate were considered as fixed variables, while blocks were considered as a random factor. Tukey's honest significant difference *post hoc* test ( $\alpha=0.05$ ) was used to reveal significant differences among treatments.

**Table 1. Crop practices on durum wheat at CIRAA during the 2013-2014 growing season.**

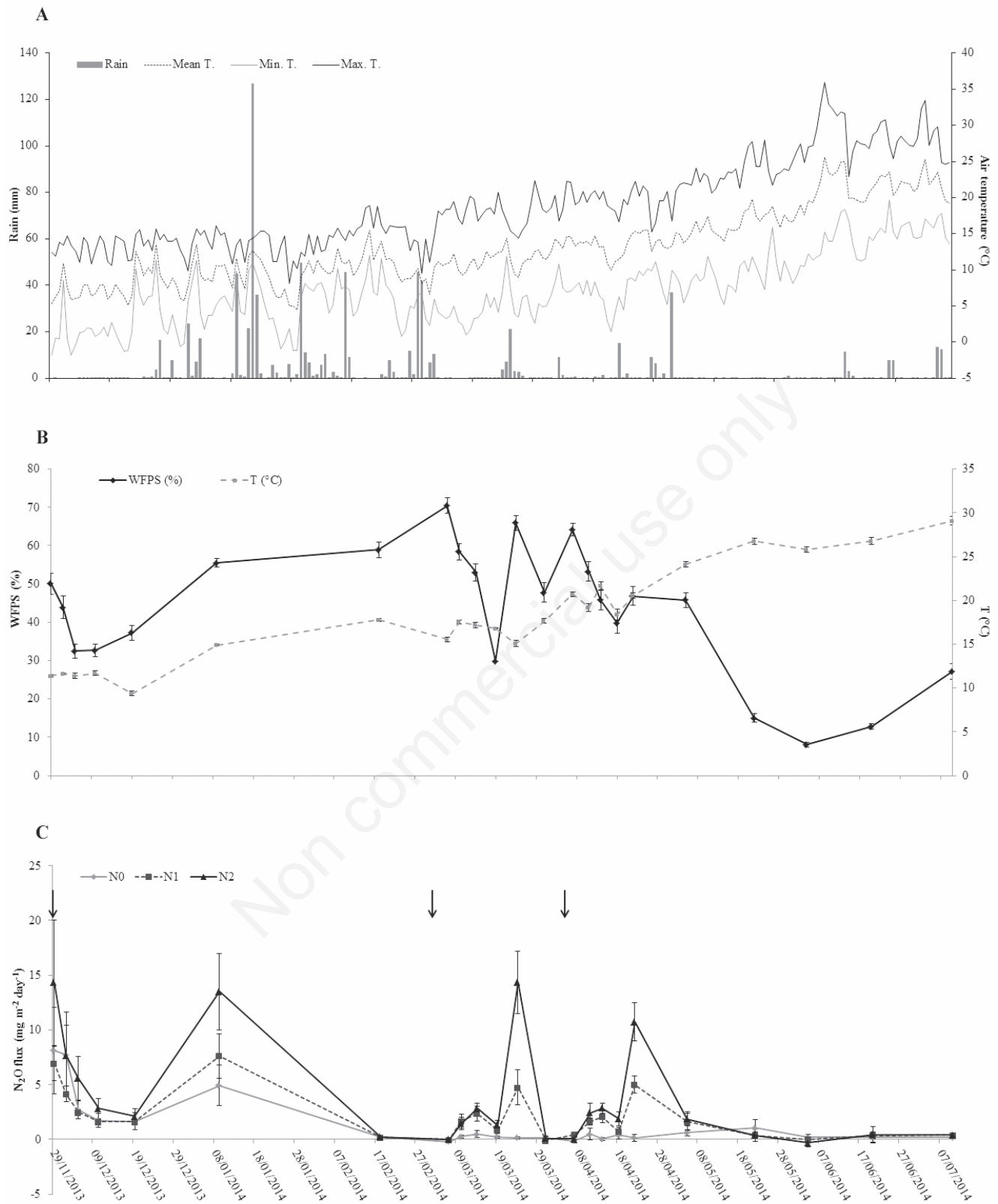
Tillage	Unit	CT Ploughing (30 cm depth)	MT Minimum tillage (10 cm depth)
N fertilisation level	(kg N ha <sup>-1</sup> )	N <sub>0</sub> =0, N <sub>1</sub> =110, N <sub>2</sub> =170	N <sub>0</sub> =0, N <sub>1</sub> =110, N <sub>2</sub> =170
After sowing	(kg urea ha <sup>-1</sup> )	N <sub>0</sub> =0, N <sub>1</sub> =78.5, N <sub>2</sub> =78.5	N <sub>0</sub> =0, N <sub>1</sub> =78.5, N <sub>2</sub> =78.5
At tillering	(kg ammonium nitrate ha <sup>-1</sup> )	N <sub>1</sub> =110; N <sub>2</sub> =200	N <sub>1</sub> =110; N <sub>2</sub> =200
At stem elongation	(kg urea ha <sup>-1</sup> )	N <sub>1</sub> =80.4; N <sub>2</sub> =145.7	N <sub>1</sub> =80.4; N <sub>2</sub> =145.7
P fertilisation	(kg triple super phosphate ha <sup>-1</sup> )	200	200
Pest control		Curative	Curative
Weed control		Post-emergence	Post-emergence
Residues		Removed	Removed

CT, conventional tillage; MT, minimum tillage; N, nitrogen; N<sub>0</sub>, 0 kg N ha<sup>-1</sup>; N<sub>1</sub>, 110 kg N ha<sup>-1</sup>; N<sub>2</sub>, 170 kg N ha<sup>-1</sup>; P, phosphorus.

**Table 2. Mean N<sub>2</sub>O flux for the three-fertilisation rates ± standard error (n=8) and *post hoc* test results.**

N level	Mean N <sub>2</sub> O flux (mg m <sup>-2</sup> day <sup>-1</sup> )	SE (mg m <sup>-2</sup> day <sup>-1</sup> )	P value
N <sub>0</sub>	0.61 <sup>b</sup>	±0.22	-
N <sub>1</sub>	0.91 <sup>a</sup>	±0.20	-
N <sub>2</sub>	1.69 <sup>a</sup>	±0.45	-
N <sub>0</sub> - N <sub>1</sub>	-	-	0.047*
N <sub>0</sub> - N <sub>2</sub>	-	-	0.001**
N <sub>1</sub> - N <sub>2</sub>	-	-	0.080

SE, standard error; N<sub>0</sub>, 0 kg N ha<sup>-1</sup>; N<sub>1</sub>, 110 kg N ha<sup>-1</sup>; N<sub>2</sub>, 170 kg N ha<sup>-1</sup>. <sup>ab</sup>Different letters represent significant differences in treatments; \*P<0.05; \*\*P<0.01.



**Figure 2.** Pattern from November 2013 to July 2014 of: A) air temperatures and rainfall; B) water filled pore space (WFPS) and soil temperature; C) N<sub>2</sub>O flux as average value among tillage levels (N<sub>0</sub>=0 kg N ha<sup>-1</sup>; N<sub>1</sub>=110 kg N ha<sup>-1</sup>; N<sub>2</sub>=170 kg N ha<sup>-1</sup>). Significant differences among N treatment in each sampling data is reported (\*P<0.05; \*\*P<0.01; \*\*\*P<0.001).

## Results and discussion

### Climate, soil conditions and N<sub>2</sub>O emissions patterns

The pattern of air temperature and rainfall from 29 November 2013 to 10 July 2014 are reported in Figure 2A. The mean air temperature varied from 4.5°C in November to 25.6°C in June, with an average mean temperature of 13°C over the period. Cumulated rainfall from December to June was equal to 810 mm, a value much higher than the long term average around 470 mm (1986-2013). The rainiest months were January (363 mm), February (158 mm) and March (101 mm).

The soil temperature and WFPS are presented in Figure 2B, as mean values of all the treatments. From November to July mean soil temperature varied from 9.4°C to 29°C, with a mean value over the period of 18.3°C. WFPS showed a mean value around 43%, with minimum mean value (8%) registered in June and the maximum values around 70% in March and April.

The N<sub>2</sub>O emissions through durum wheat growing season showed very low values (<0.5 mg m<sup>-2</sup> day<sup>-1</sup>) during most sampling days, with the exception of the period immediately after sowing (29 Nov - 19 Dec) and the three-fertilisation events (Figure 2C).

Tillage did not affect significantly N<sub>2</sub>O emissions over the whole monitoring period, while nitrogen rate, date and their interaction were highly significant (P<0.001). Overall, N<sub>2</sub>O emissions ranged from -0.13 to 6.43 mg N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup> (average value 1.07±0.19 mg N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup>). *Post hoc* test highlighted no significant differences between N<sub>1</sub> and N<sub>2</sub> (Table 2).

N<sub>2</sub>O flux at the beginning of the monitoring period presented a decreasing pattern with relatively high values with no significant differences among nitrogen treatments (P>0.05). A possible explanation of this trend could be related to the nitrogen mineralisation of clover residues, enhanced by tillage practices before sowing. In fact, the increase of N<sub>2</sub>O emissions after crop residues incorporation has been reported by many studies, in particular residues with low C:N ratios produced higher emissions (Baggs *et al.*, 2000; Lehtinen *et al.*, 2014).

High peaks observed after nitrogen fertilisation events were significant different among N levels, as reported in Figure 2C. N<sub>2</sub>O peaks after topdressing fertilisation was observed after about 14-16 days, with maximum values of N<sub>2</sub>O around 6.43 mg N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup> on 24 March and 4.81 mg N<sub>2</sub>O m<sup>-2</sup> day<sup>-1</sup> on 22 April. On the contrary, fertilisation at sowing produced N<sub>2</sub>O peak emissions after 21 days. In fact the period after fertilisation (from 26 November to 19 December) was characterised by low temperature (6.5°C) and low rain (3.6 mm), while a N<sub>2</sub>O peak was registered after a week with 50 mm of rain. The magnitude of N<sub>2</sub>O flux is strongly influenced by the amount and the distribution of rainfall, since maximum N<sub>2</sub>O emission rates from all treatments may occur after rewetting of dry soil (Ruser *et al.*, 2006; Tellez-Rio *et al.*, 2015).

### N<sub>2</sub>O cumulative emissions in the growing season

No significant differences were found between CT and MT on N<sub>2</sub>O-N cumulative emissions, while nitrogen rate resulted to significantly affect N<sub>2</sub>O-N cumulative emissions (P=0.003) and it explained 45% of the overall variability. *Post hoc* test underlined differences among nitrogen rate levels as reported in Table 3.

Cumulative N<sub>2</sub>O-N emissions over durum wheat growing season resulted to be higher than those reported in similar studies for winter wheat in temperate climate with values ranging from 410 to 1100 g N<sub>2</sub>O-N ha<sup>-1</sup> y<sup>-1</sup> (Drury *et al.*, 2008; Smith *et al.*, 1998). Laville *et al.* (2011) reported values of N<sub>2</sub>O-N cumulative emissions for a barley crop monitored with automatic chambers equal to 1700 g N<sub>2</sub>O-N ha<sup>-1</sup> y<sup>-1</sup>, a value higher than our cumulative flux (1322 g N<sub>2</sub>O-N ha<sup>-1</sup>) for N<sub>1</sub> (110 kg N ha<sup>-1</sup>), as barley was fertilised with a similar N rate (108 kg N ha<sup>-1</sup>).

Cumulative emissions in a Mediterranean environment on winter cereals from Aguilera *et al.* (2013) were about 300 g N<sub>2</sub>O-N ha<sup>-1</sup>, a value largely lower than the values obtained in our experiment. The high N<sub>2</sub>O-N cumulative emissions in the IPNOA experiment might have been enhanced by the abundant rainfalls occurred during the 2013-2014 growing season, which resulted in average high WFPS. Indeed, microbial processes producing N<sub>2</sub>O emissions increase as WFPS increases, as it regulates the oxygen availability to soil microbes (Butterbach-Bahl *et al.*, 2013). In the durum wheat field trial, WFPS resulted to be greater than 30% in about 80% of the monitoring days.

### IPNOA mobile prototype performance

During this first year of monitoring the IPNOA mobile prototype showed a good performance. A good sensitivity was registered in the entire range of emissions during the monitoring period. The precision of LGR N<sub>2</sub>O/CO analyser is in the range of 0.1 to 0.050 ppb, therefore the detection limit of the system was around 0.04 ng N m<sup>-2</sup> s<sup>-1</sup> (Laville *et al.*, 2015). These values are about 500 times higher than traditional techniques such as GC (Hensen *et al.*, 2013). Five minutes of chamber deployment were enough to estimate flux with a high resolution, with the possibility to perform a real time check of the linearity of the flux thanks to a scan rate of 1 s. The prototype showed a good resistance to the field environment, indeed no limiting conditions occurred in the range of air temperature from 0°C to 36°C or in windy conditions. On the other hand, access to the field was difficult in very wet periods, in fact the durum wheat field trial was unreachable due to heavy precipitation from 9 January to 18 February 2014 (around 400 mm), and it resulted in a reduced sampling frequency.

The operational capacities in terms of mobility and gas analyser stability were satisfactory. The prototype demonstrated also good supply autonomy with duration of around 8 h. The remote transmission for the operation of the commands and acquisition of the data with a palmtop

**Table 3. Cumulative N<sub>2</sub>O emissions for the three-fertilisation rates ± standard error (n=8) expressed as N<sub>2</sub>O-N and *post hoc* test results.**

N level	Cumulative N <sub>2</sub> O emissions (N <sub>2</sub> O-N g ha <sup>-1</sup> period <sup>-1</sup> )	SE (N <sub>2</sub> O-N g ha <sup>-1</sup> period <sup>-1</sup> )	P value
N <sub>0</sub>	827 <sup>b</sup>	±247	-
N <sub>1</sub>	1322 <sup>b</sup>	±180	-
N <sub>2</sub>	2340 <sup>a</sup>	±330	-
N <sub>0</sub> - N <sub>1</sub>	-	-	0.387
N <sub>0</sub> - N <sub>2</sub>	-	-	0.002**
N <sub>1</sub> - N <sub>2</sub>	-	-	0.034*

SE, standard error; N<sub>0</sub>, 0 kg N ha<sup>-1</sup>; N<sub>1</sub>, 110 kg N ha<sup>-1</sup>; N<sub>2</sub>, 170 kg N ha<sup>-1</sup>. <sup>a,b</sup>Different letters represent significant differences in treatments; \*P<0.05; \*\*P<0.01.

was very user-friendly. Moreover, data visualisation on the palmtop in real time allows evaluating the goodness of the measure.

Some considerations need to take into account concerning the IPNOA system, related to the prototype cost, due mainly to the value of the two detectors, and to the prototype dimension and weight. In fact, an adequate van is needed to transport the instrument close to the field.

## Conclusions

The IPNOA mobile prototype performed well in field conditions, allowing recording a wide range of N<sub>2</sub>O flux, including very low emissions. Preliminary results on durum wheat showed a decisive influence of nitrogen rate on N<sub>2</sub>O production, while conventional and minimum tillage did not produce differences in N<sub>2</sub>O flux. However, these first-year findings will be endorsed by data from the other site of study (CATES) and by further years of investigation.

Data on N<sub>2</sub>O flux will be used to calibrate and to validate a process-based model for the assessment of emissions at regional scale and to develop mitigation scenarios based on alternative crop managements. Finally, the results will contribute to the drafting of the BMP for the N<sub>2</sub>O emissions reduction in Tuscany.

## References

- Abdalla M, Osborne B, Lanigan G, Forristal, Williams M, Smith P, Jones B, 2013. Conservation tillage systems: a review of its consequences for greenhouse gas emissions. *Soil Use Manage.* 29:199-209.
- Aguilera E, Lassaletta L, Gattinger A, Gimeno BS, 2013. Managing soil carbon for climate change mitigation and adaptation in Mediterranean cropping systems: A meta-analysis. *Agric. Ecosyst. Environ.* 168:25-36.
- Baggs EM, Rees RM, Smith KA, Vinten AJA, 2000. Nitrous oxide emission from soils after incorporating crop residues. *Soil Use Manage.* 16:82-87.
- Bates D., Maechler M., Bolker B., Walker S., Christensen R.H.B., Singmann H., Dai B., 2014. Linear mixed-effects models using Eigen and S4. R package version 1.1-7. Available from: <http://CRAN.R-project.org/package=lme4>
- Bouwman AF, Boumans LJM, Batjes NH, 2002. Emissions of N<sub>2</sub>O and NO from fertilized fields: Summary of available measurement data. *Glob. Biogeochem. Cycles.* 16:6-1-6-13.
- Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S, 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 368:20130122.
- Chadwick DR, Cardenas L, Misselbrook TH, Smith KA, Rees RM, Watson CJ, McGeough KL, Williams JR, Cloy JM, Thorman RE, Dhanoa MS, 2014. Optimising chamber methods for measuring nitrous oxide emissions from plot-based agricultural experiments. *Eur. J. Soil Sci.* 65:295-307.
- Cowan NJ, Famulari D, Levy PE, Anderson M, Bell MJ, Rees RM, Reay DS, Skiba UM, 2014. An improved method for measuring soil N<sub>2</sub>O fluxes using a quantum cascade laser with a dynamic chamber. *Eur. J. Soil Sci.* 65:643-52.
- Davidson EA, Vitousek PM, Matson PA, 1991. Soil emissions of nitric oxide in a seasonally dry tropical forest of Mexico. *J. Geophys. Res. Atmos.* 96:15439-45.
- de Mendiburu F, 2014. Statistical procedure for agricultural research. Package agricolae v.1.2-1. Available from: [cran.r-project.org/web/packages/agricolae/agricolae.pdf](http://cran.r-project.org/web/packages/agricolae/agricolae.pdf)
- Drury CF, Yang XM, Reynolds WD, McLaughlin NB, 2008. Nitrous oxide and carbon dioxide emissions from monoculture and rotational cropping of corn, soybean and winter wheat. *Can. J. Soil Sci.* 88:163-174.
- European Commission, 2014. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions - A policy framework for climate and energy in the period from 2020 to 2030. /\* COM/2014/015 final \*/. Available from: <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52014DC0015>
- Flessa H, Ruser R, Schilling R, Lofftfield N, Munch JC, Kaiser EA, Beese F, 2002. N<sub>2</sub>O and CH<sub>4</sub> fluxes in potato fields: automated measurement, management effects and temporal variation. *Geoderma* 105:307-25.
- Fowler D, Pilegaard K, Sutton MA, Ambus P, Raivonen M, Duyzer J, Simpson D, Fagerli H, Fuzzi S, Schjoerring JK, Granier C, Nefel A, Isaksen ISA, Laj P, Maione M, Monks PS, Burkhardt J, Daemmgen U, Neiryck J, Personne E, Wichink-Kruit R, Butterbach-Bahl K, Flechard C, Tuovinen JP, Coyle M, Gerosa G, Loubet B, Altimir N, Gruenhage L, Ammann C, Cieslik S, Paoletti E, Mikkelsen TN, Røpoulsen H, Cellier P, Cape JN, Horvath L, Loreto F, Niinemets UE, Palmer P, Rinne J, Misztal P, Nemitz E, Nilsson D, Pryor S, Gallagher MW, Vesala T, Skiba U, Brüeggemann N, Zechmeister-Boltenstern S, Williams J, O'Dowd C, Facchini MC, de Leeuw G, Flossman A, Chaumerliac N, Erisman JW, 2009. Atmospheric composition change: ecosystems-atmosphere interactions. *Atmos. Environ.* 43:5193-267.
- Heinemeyer A, McNamara NP, 2011. Comparing the closed static versus the closed dynamic chamber flux methodology: implications for soil respiration studies. *Plant Soil.* 346:145-51.
- Hensen A, Skiba U, Famulari D, 2013. Low cost and state of the art methods to measure nitrous oxide emissions. *Environ. Res. Lett.* 8:025022.
- IPCC (Intergovernmental Panel on Climate Change), 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.
- Jahangir MMR, Roobroeck D, Van Cleemput O, Boeckx P, 2011. Spatial variability and biophysicochemical controls on N<sub>2</sub>O emissions from differently tilled arable soils. *Biol. Fertil. Soils.* 47:753-66.
- Laville P, Lehuger S, Loubet B, Chaumartin F, Cellier P, 2011. Effect of management, climate and soil conditions on N<sub>2</sub>O and NO emissions from an arable crop rotation using high temporal resolution measurements. *Agric. For. Meteorol.* 151:228-40.
- Laville P, Neri S, Continanza D, Ferrante Vero L, Bosco S, Virgili G, 2015. Cross-Validation of a mobile N<sub>2</sub>O flux prototype (IPNOA) using Micrometeorological and Chamber methods. *J. Energy Power Engin.* 9:375-85.
- Lehtinen T, Schlatter N, Baumgarten A, Bechini L, Krüger J, Zavattaro L, Costamagna C, Spiegel H, 2014. Effect of crop residue incorporation on soil organic carbon (SOC) and greenhouse gas (GHG) emissions in European agricultural soils. *Soil Use Manage.* 30:524-38.
- Livingston GP, Hutchinson GL, 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error. In: P.A. Matson, R.C. Harriss (Eds.), *Biogenic trace gases: measuring emissions from soil and water.* Blackwell Science, Cambridge, UK, pp 14-50.
- Mosier AR, Duxbury JM, Freney JR, Heinemeyer O, Minami K, 1998.

- Assessing and mitigating N<sub>2</sub>O emissions from agricultural soils. *Clim. Chang.* 40:7-38.
- Pedersen AR, Petersen SO, Schelde K, 2010. A comprehensive approach to soil-atmosphere trace-gas flux estimation with static chambers. *Eur. J. Soil Sci.* 61: 888-902.
- Rees RM, Augustin J, Alberti G, Ball BC, Boeckx P, Cantarel A, Castaldi S, Chirinda N, Chojnicki B, Giebels M, Gordon H, Grosz B, Horvath L, Juszczak R, Kasimir Klemedtsson Å, Klemedtsson L, Medinets S, Machon A, Mapanda F, Nyamangara J, Olesen JE, Reay DS, Sanchez L, Sanz Cobena A, Smith KA, Sowerby A, Sommer M, Soussana JF, Stenberg M, Topp CFE, Van Cleemput O, Vallejo A, Watson CA, Wuta M, 2013. Nitrous oxide emissions from European agriculture - an analysis of variability and drivers of emissions from field experiments. *Biogeosciences.* 10:2671-82.
- Rochette P, Eriksen-Hamel NS, 2008. Chamber measurements of soil nitrous oxide flux: are absolute values reliable? *Soil Sci. Soc. Am. J.* 72:331-42.
- Rochette P, Worth DE, Lemke RL, McConkey BG, Pennock DJ, Wagner-Riddle C, Desjardins RL, 2008. Estimation of N<sub>2</sub>O emissions from agricultural soils in Canada. I. Development of a country specific methodology. *Can. J. Soil Sci.* 88:641-54.
- Ruser R, Flessa H, Russow R, Schmidt G, Buegger F, Munch JC, 2006. Emission of N<sub>2</sub>O, N<sub>2</sub> and CO<sub>2</sub> from soil fertilized with nitrate: Effect of compaction, soil moisture and rewetting. *Soil Biol. Biochem.* 38:263-74.
- Smith KA, McTaggart IP, Dobbie KE, Conen F, 1998. Emissions of N<sub>2</sub>O from Scottish agricultural soils, as a function of fertilizer N. *Nutri. Cycl. Agroecosyst.* 52:123-30.
- Snyder CS, Davidson EA, Smith P, Venterea RT, 2014. Agriculture: Sustainable crop and animal production to help mitigate nitrous oxide emissions. *Curr. Opin. Environ. Sustain.* 9-10:46-54.
- Soil Survey Staff. 1975. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. USDA-SCS Agric. Handb. 436. U.S. Gov. Print. Office, Washington, DC, USA.
- Tellez-Rio A, García-Marco S, Navas M, López-Solanilla E, Luis Tenorio J, Vallejo A, 2015. N<sub>2</sub>O and CH<sub>4</sub> emissions from a fallow-wheat rotation with low N input in conservation and conventional tillage under a Mediterranean agroecosystem. *Sci. Total Environ.* 508:85-94.
- Tremblay A, Ransijn J, 2013. LMERConvenienceFunctions: a suite of functions to back-fit fixed effects and forward-fit random effects, as well as other miscellaneous functions. R package version 2.0. Available from: <http://CRAN.R-project.org/package=LMERConvenienceFunctions>
- van Kessel C, Venterea RT, Six J, Adviento-Borbe MA, Linquist B, van Groenigen KJ, 2013. Climate, duration, and N placement determine N<sub>2</sub>O emissions in reduced tillage systems: a meta-analysis. *Glob. Chang. Biol.* 19:33-44.
- Venterea RT, Burger M, Spokas KA, 2005. Nitrogen oxide and methane emissions under varying tillage and fertilizer management. *J. Environ. Qual.* 34:1467-77.
- Venterea RT, Halvorson AD, Kitchen N, Liebig MA, Cavigelli MA, Del Grosso SJ, Motavalli PP, Nelson KA, Spokas KA, Singh BP, Stewart CE, Ranaivoson A, Strock J, Collins H, 2012. Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. *Front. Ecol. Environ.* 10:562-70.