Effect of soil texture and wheat plants on N₂O fluxes: A lysimeter study

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Keywords: Nitrous oxide, soil texture, irrigation, mitigation, ¹⁵N, denitrification

Abstract

Agricultural soils are a major source of nitrous oxide (N₂O) emissions and an understanding of factors regulating such emissions across contrasting soil types is critical for improved estimation through modelling and mitigation of N₂O. In this study we investigated the role of soil texture and its interaction with plants in regulating the N₂O fluxes in agricultural systems. A measurement system that combined weighing lysimeters with automated chambers was used to directly compare continuously measured surface N₂O fluxes, leaching losses of water and nitrogen and evapotranspiration in three contrasting soils types of the Riverine Plain, NSW, Australia. The soils comprised a deep sand, a loam and a clay loam with and without the presence of wheat plants. All soils were under the same fertilizer management and irrigation was applied according to plant water requirements. In fallow soils, texture significantly affected N₂O emissions in the order clay loam > loam > sand. However, when planted, the difference in N₂O emissions among the three soils types became less pronounced. Nitrous oxide emissions were 6.2 and 2.4 times higher from fallow clay loam and loam cores, respectively, compared with cores planted with wheat. This is considered to be due to plant uptake of water and nitrogen which resulted in reduced amounts of soil water and available nitrogen, and therefore less favourable soil conditions for denitrification. The effect of plants on N₂O emissions was not apparent in the coarse textured sandy soil probably because of aerobic soil conditions, likely caused by low water holding capacity and rapid drainage irrespective of plant presence resulting in reduced denitrification activity. More than 90% of N₂O emissions were derived from denitrification in the fine-textured clay loam - determined for a two week period using K¹⁵NO₃ fertilizer. The proportion of N₂O that was not derived from K¹⁵NO₃ was higher in the coarse-textured sand and loam, which may have been derived from soil N through nitrification or denitrification of mineralized N. Water filled pore space was a poorer predictor of N₂O emissions compared with volumetric water content because of variable bulk density among soil types. The data

may better inform the calibration of greenhouse gas prediction models as soil texture is one of the primary factors that explain spatial variation in N₂O emissions by regulating soil oxygen. Defining the significance of N₂O emissions between planted and fallow soils may enable improved yield scaled N₂O emission assessment, water and nitrogen scheduling in the pre-watering phase during early crop establishment and within rotations of irrigated arable cropping systems.

Introduction

Nitrous oxide (N₂O) has a global warming potential 298 times that of carbon dioxide (Forster et al., 2007) and is a major ozone depleting substance (Ravishankara et al., 2009). Agricultural soils are a major source of direct and indirect N₂O emissions (Mosier et al., 1998) accounting for 60% of anthropogenic N₂O emissions (Syakila and Kroeze, 2011). Nitrification and denitrification are the main biological processes of N₂O emissions (Firestone et al., 1989) although several other processes may also be responsible for N₂O emissions (Butterbach-Bahl et al., 2013). Nitrification is the biological oxidation of ammonium (NH₄⁺) to nitrite (NO₂⁻) or nitrate (NO₃⁻) under aerobic conditions, and denitrification is the reduction of NO₃⁻ to NO₂⁻, nitric oxide (NO), N₂O and molecular dinitrogen (N₂) under anaerobic conditions (Bouwman, 1998).

In agricultural soils, the total N₂O emissions in a season are heavily contributed to by the episodes of large N₂O pulses observed after irrigation and rainfall events which are primarily derived from denitrification (Barton et al., 2013; Scheer et al., 2008; Trost et al., 2013). One

of the main factors regulating the production of N₂O by the denitrification process is soil is redox potential (Husson, 2013; Li, 2007) which is defined as the availability of electrons in soil and determined by the balance of the concentrations of reductants (e.g. organic matter) and oxidants (e.g. O₂, NO₃, SO₄) present. Oxygen is used as the preferred electron acceptor until the redox potential drops to <+300 mV below which nitrate, manganese, iron, sulphate and carbon dioxide are used as electron acceptors (Patrick Jr and Reddy, 1978). Factors that control the availability of O₂ or the aeration of soil, namely O₂ partial pressure in the gas phase, matric potential, soil water content (Bollmann and Conrad, 1998) and the interaction of these with the type and amount of organic matter, concentration of mineral N, temperature and pH, controls the availability of electrons in soils (Bremner and Shaw, 1958; Skiba and Ball, 2002) and hence, the process of denitrification and N₂O emissions. The soil proxy that represents an integration of these parameters is soil texture (Corre et al., 1996). Through differences in air filled porosity (aeration), it is generally considered that fine textured soils provide conditions more favourable for denitrification and N₂O emission at lower soil moisture contents than coarser textured soils which favour nitrification (Bollmann and Conrad, 1998; Parton et al., 1996b). Fine textured soils have small pores that become more easily anaerobic and remain that way for longer duration than coarser textured soils (Bollmann and Conrad, 1998; Groffman and Tiedje, 1991). It is important to recognize that soil aeration is not simply related to soil water content, but determined by transport and consumption rates of oxygen (Cook and Knight, 2003) which can be affected by environmental variables such as temperature and soil water content (Cook et al., 2013).

Plants are an integral component in agricultural systems and may also affect N₂O emissions to the atmosphere. However, to date there is apparently no consensus as to whether plants promote or suppress N₂O emissions. Plant roots may affect nitrification and denitrification

processes which produce N_2O in soil, by changing soil aeration, labile organic matter, water content and mineral N (Philippot et al., 2009). Plants are also known to directly produce N_2O in their tissues (Hakata et al., 2003) and act as a conduit for the transport of N_2O produced in soil to the atmosphere (Pihlatie et al., 2005). In a recent summary of published data for upland crops, planted soil was found to emit twice as much N_2O as unplanted soil in upland crops (Hayashi et al., 2014). This stimulatory effect of plants is likely to be caused by increased denitrification rates which have often been observed to increase in the presence of plants (Prade and Trollidenier, 1988). Plant effects on denitrification are thought to be due to increasing O_2 demand in the root zone and supplying labile organic matter from the fine roots and root exudates allowing denitrification to occur at lower water content (Cook et al., 2013; Smith and Tiedje, 1979). The $\text{N}_2:\text{N}_2\text{O}$ ratios during denitrification may also be affected by the presence of plants caused by biogeochemical conditions created by the rhizosphere and phenology (Henry et al., 2008; Vinther, 1984). Conversely, other authors have considered upland crops may reduce N_2O emissions by lowering soil N concentration and soil water content (Bouwman, 1996).

Most of the previous studies that have compared N_2O emissions between different soil textures have been conducted in laboratory conditions (Balaine et al., 2013; Castellano et al., 2010; van der Weerden et al., 2012) due to the difficulty encountered in conducting comparative studies in the field caused by variation in weather, topography and crop responses at spatially diverse sites, even if sites can be chosen at the sub-kilometre scale (Gu et al., 2013). Lysimeters provide the ability to study a range of soil textures simultaneously under natural climatic conditions. They also enable the effect of plants to be considered when explaining the N_2O emission response of soils that hold and drain water differently. Therefore, lysimeters provide a valid system for potentially identifying improved indicators that predict agricultural N_2O emissions in soils of different textures and under different

management. The objectives of this study were to investigate the effect of soil texture and wheat plant growth on total N₂O emissions, denitrification-driven N₂O emissions, leaching losses and evapotranspiration. To our knowledge, this is the first study in which surface fluxes of N₂O, leaching losses of mineral N, water losses through leaching and evapotranspiration and plant N uptake were all measured simultaneously from three different soil types.

2. Materials and Methods

2.1. Lysimeter soil core collection and instrumentation

This study was conducted using a large experimental weighing soil lysimeter facility with underground access, located at Griffith, NSW, details of which have been described in earlier work (Jamali et al., 2015). The lysimeter was installed with 18 intact soil cores (diameter 0.7 m x height 1.2 m) comprising six cores from each of three soil types with a range of soil textures (Table 1). All soils were sampled from within the Murrumbidgee Irrigation Area (MIA), southern NSW, Australia (see section 2.3 below). Lysimeter cores were collected in 6 mm thick, mild steel cylinders with a cutting edge welded to the bottom which allowed the cylinders to be pushed into the ground using a crane-operated pneumatic hammer and extracted intact, through an excavated side trench. An H-shaped PVC manifold was installed at the bottom of each core that was fitted with an outlet allowing drainage discharge to a container for leachate collection. The space around the manifold was filled with fine sand and a steel plate was welded to seal the base of each core. Twelve of the cores, four from each soil type, were installed with EnviroPro[®] probes, (MEA, Magill, SA, Australia) for measuring volumetric water content (θ) at 0.1m intervals throughout the soil profile. The 12 cores were located within the lysimeter housing upon weighing load cells for the direct measurement of evapotranspiration by mass loss.

2.2. Automated greenhouse gas measurements

The 12 instrumented cores were fitted with automated chambers for measuring near-continuous fluxes of N₂O from the soil surface (Figure 1). The chambers were built from 8 mm thick Perspex® (B&M Plastics Pty Ltd, Yennora, NSW). The opening and closing mechanism was controlled by electric linear actuators ensuring a soft closure with minimum disturbance to the weighing lysimeter load cells. These chambers were connected to a measurement system (Scheer et al., 2014) which consists of a sampling unit and a gas chromatograph (GC, SRI8610, Torrance, CA, USA), fitted with a ⁶³Ni electron capture detector (ECD) for N₂O analysis, a flame ionization detector (FID) for CH₄ analysis and an infra-red gas analyser (LI820, LI-COR®, St Joseph, MI, USA) for CO₂ analysis. The 12 chambers are divided into three sets of four chambers. Each set closed for 60 minutes during a measurement cycle and each chamber sampled sequentially for three minutes followed by a calibration gas (0.5 ppm N₂O, 4 ppm CH₄ and 500 ppm CO₂). This process was repeated after 15 minutes thus collecting four gas samples per chamber per hour. Thus in a 3-hour measurement cycle, chambers remained closed for one hour and open for two hours allowing chambers to restore ambient conditions before the start of next cycle. Nitrous oxide fluxes are calculated using the slope of linear change in concentration of four gas samples and corrected for chamber temperature and pressure using the ideal gas law (Barton et al., 2008).

2.3. Soil characterisation

The chemical and physical properties of the 0-10 cm layer of the three soil types are presented in Table 1. Bulk density was determined from five cores (0.1 m diameter x 1 m deep) collected adjacent to the large core collection sites using a mechanical corer. Another

three cores per site were collected for the determination of particle size density and chemical analyses. Samples were composited for the particle size density according to soil depth interval while individual samples were used for chemical analyses. Total carbon (TC), total organic carbon (TOC) and total nitrogen (TN) were determined using a Leco CNS analyser and standard methods (Rayment and Lyons, 2011). Soil mineral N (NH_4^+ and NO_3^-) was measured using soil extracts with 2M potassium chloride (1 soil:10 KCl). Extracts were frozen prior to analysis using a segmented flow LACHAT QuickChem[®] 8500 flow injection analyser. Dissolved organic carbon (DOC) was measured using 0.5M potassium sulphate (K_2SO_4) soil extracts (1soil:10 K_2SO_4) (Rayment and Lyons, 2011).

<Insert Table 1>

Mundiwa Clay Loams: Classified as a *Chromosol* (Isbell, 2002), these soils are locally known as transitional red-brown earths, between grey and red clays and the red-brown earths without sub-plasticity, and located at the lower part of the Riverine Plain near Yanco, NSW (Lat. -34.603242, Long 146.382000). These soils are commonly used for irrigated arable cropping throughout the southern NSW irrigation districts. Typically they comprise ~ 50-60% sand and silt to 30 cm and then transition to > 60% clay at 30 cm (data not shown). Bulk densities increase from 1.27 g cm^{-3} at the surface (Table 1) to 1.6 g cm^{-3} at 1 m (data not shown).

Hanwood Loams: Locally described as red-brown earths and classified as *Chromosols* (Isbell, 2002) these soils are located in Hanwood (Lat. -34.323675, Long. 146.068674). They are well drained soils, with sub-plasticity and are mainly used for highly productive

horticulture around Griffith, NSW, Australia. Sands generally decrease from > 60% at the surface (Table 1) to ~ 40% at 40 cm (data not shown). This soil had the highest bulk density of 1.49 g cm⁻³ at top layer among three soil types (Table 1), which dropped to ~1.3 g cm⁻³ between 0.3-0.7 m layers but increased to 1.56 g cm⁻³ at 1m depth (data not shown).

Banna Sands: Located in Hanwood (Lat.-34.319910, Long 146.078422) this deep sand soil was 60-70% sand down to 0.8 m and a transition to 40-50% clays below 0.8m (Table 1).. Bulk densities increase from 1.28 g cm⁻³ at the surface (Table 1) to 1.74 g cm⁻³ at 1 m (data not shown).

The three soils described above showed clear contrast in the proportion of sand, silt and clays and have also been described elsewhere (Hornbuckle and Christen, 1999). Bulk density tends to follow the trend Hanwood Loam > Banna Sand ≥ Mundiwa Clay loam (Table 1). Soil pH ranged from 8.4 – 9.0, with all types being mildly alkaline at the surface (Table 1) and increasing in alkalinity with depth. All of the soils were low in organic C, total N and mineral-N (Table 1).

2.4. Experimental treatments and crop management.

Six cores from each soil type were installed into the lysimeter facility in May, 2013. Four of the cores per soil type were fully instrumented to measure GHG fluxes, soil moisture, soil temperature, leaching and evapotranspiration (ET). The remaining two cores from each soil type were used to enable soil sampling for the determination of temporal changes in mineral N and DOC over the course of the experiment. Half of the cores were planted with wheat on 24th June, 2013 and half left unplanted; the two treatments are referred to as “Wheat” and

“Fallow” hereafter. All cores received the same amount of fertilizer (Table 2) which included 20 kg N ha⁻¹ as diammonium phosphate (DAP) at sowing and 80 kg N ha⁻¹ as ¹⁵N-labelled (60% enrichment) potassium nitrate (KNO₃) mixed with three litres of water (6.8 mm) on the 12th August, 2013. The crop reached flowering in early October and was harvested on the 18th November, 2013.

Irrigation management was kept the same within a soil type but varied between soil types depending on plant water requirements. For example, the rapidly draining sand was watered more frequently in order to maintain plant growth, compared with the clay loam which, with frequent irrigations, was prone to waterlogging. Irrigation was applied to all cores of a soil type when soil water content in any of the cores within that soil type dropped below 60% of plant-available-water as monitored by soil moisture probes. As such, different soil types received different amounts of water as shown in Table 3.

2.5. Soil sampling for mineral-N and DOC seasonal dynamics

Temporal changes in soil chemical properties were monitored in two cores per soil type, one planted with wheat and the other left fallow. Three smaller cores (2.5 cm diameter x 10 cm depth) were collected from the 0-10 cm layer by using a steel corer fortnightly or more frequently at strategic timings such as after fertilizer and/or irrigation applications. These cores were composited and analysed for NO₃⁻, NH₄⁺, DOC and pH.

2.6. ¹⁵N₂O measurements and analysis

A two-week long experiment was conducted to investigate the contribution of denitrification in N₂O emissions from the three soil types while using the same 12 lysimeter cores used for automated N₂O measurements. A few days prior to ¹⁵N-labelled KNO₃ application on 12th August, 2013, lysimeter cores were gradually watered to achieve saturated soil conditions suitable for denitrification at the time of fertilizer addition. It was considered that applying irrigation after fertilizer addition would have created variable NO₃⁻ levels due to leaching losses that may differ among soil types. The sampling for ¹⁵N-N₂O was conducted between the 12th and 26th August, 2013. The chambers were closed for seven hours and 20 ml gas samples were collected manually using syringes and immediately transferred to pre-evacuated 12 ml vials. The lysimeter rain out shelter was used during the 7-hour manual gas sampling to provide some shade and reduce excessive heating inside the chambers. Gas samples were collected on days 0, 1, 2, 3, 4, 6, 8, 10 and 12 days after ¹⁵N-labelled KNO₃ application. These gas samples were analysed using an isotope ratio mass spectrometer (IRMS) (Sercon, 20-20, UK) linked to a Sercon Cryprep trace gas preparation system at the Queensland University of Technology, Brisbane, Australia.

Nitrous oxide emissions derived from ¹⁵N-labelled KNO₃ were calculated as below:

$$^{15}\text{N-N}_2\text{O flux} = \frac{(\text{atom}\% \text{ } ^{15}\text{N-N}_2\text{O gas sample} - \text{atom}\% \text{ } ^{15}\text{N air})}{(\text{atom}\% \text{ } ^{15}\text{N fertilizer} - \text{atom}\% \text{ } ^{15}\text{N air})} \times \text{N}_2\text{O flux}$$

where N₂O flux is that measured by the automated chamber system on the same day.

Cumulative ¹⁵N-N₂O emissions over the period between 12th and 26th August, 2014 were calculated by integrating daily ¹⁵N₂O fluxes and using mean fluxes for the days when ¹⁵N₂O flux was not measured.

2.7. ¹⁵N recovery in plants

At the end of season, wheat plants were harvested from all chambers, dried at 60°C for 48 hours and separated into grain and straw. Ground grain and straw plant samples were analysed for total N using Shimadzu® TOC-L analyser and for ¹⁵N using the method described by Teece and Fogel (2004).

The recovery of ¹⁵N in wheat plant samples was calculated using standard methods (IAEA, 2001) as below:

$$^{15}\text{N Recovery} = \frac{(\% \text{Ndff} \times \% \text{N in plants} \times \text{mass of grain or straw})}{(\text{Rate of } ^{15}\text{N fertilizer applied})}$$

Where %Ndff is the fraction of N derived from fertilizer and is calculated as:

$$\% \text{Ndff} = \frac{(\text{atom\% } ^{15}\text{N plant sample} - \text{atom\% } ^{15}\text{N at natural abundance})}{(\text{atom\% } ^{15}\text{N fertilizer} - \text{atom\% } ^{15}\text{N at natural abundance})}$$

The recovery of ¹⁵N was calculated separately for grain and straw, and were integrated to calculate the total ¹⁵N recovery in aboveground part of wheat plants.

2.8. Drainage water leaching measurements

Leached water was collected at a minimum of once per week in 10 litre plastic containers connected to drainage outlets at the base of the cores. Leachate volume was measured by dipping a ruler that was calibrated to convert depth to volume. The leachate collection containers were quickly replaced with another set of clean containers. At each leachate measurement occasion, a 50 ml sub-sample was collected, filtered through a Whatman® 42

and frozen for later analysis for mineral N using a LACHAT QuickChem[®] 8500 flow injection analyser.

2.8 Calculations and statistical analyses

Daily N₂O fluxes measured by the automated GHG measurement system were integrated to calculate the total N₂O emissions over the entire wheat season. The daily N₂O flux data was transformed using $\log_{10} (\text{N}_2\text{O flux} + 50)$ to improve the normality of data. The overall significance of differences ($\alpha = 0.05$) in N₂O emissions of different soil types and treatments was tested using Analyses of variance (ANOVA). In case of a significant result in ANOVA, Tukey's post hoc test was used to further test the differences among individual soil types and treatments.

2.9 Climate

The experimental site experiences a Mediterranean type climate with long-term (1962-2014) annual average rainfall of 409 mm, and average minimum and maximum temperatures of 9.6 °C and 25.1 °C, respectively. During this study (June – November, 2013), the lysimeter site received 153 mm as rainfall, with minimum and maximum average daily temperatures of 5.1°C and 28.2°C, respectively (CRCIF and CSIRO, 2015).

3. Results

3.1. Seasonal N₂O fluxes

In fallow cores, greater N₂O emissions occurred in the fine textured soil compared with the freer draining soils in the order clay loam > loam > sand (Table 2). The differences in N₂O emissions from planted cores were less pronounced and not significant among three soil types (Table 2). Nitrous oxide emissions were diminished in the clay loam and loam soils, planted to wheat compared with fallow soils but the effect was negligible in the sandy soil. (Fig. 2). The temporal dynamics of N₂O emissions in three soils types are explained below:

Mundiwa Clay Loam (MCL): Mean daily N₂O flux in MCL ranged from -0.3 ± 0.2 to 156 ± 83 g N₂O-N ha⁻¹ day⁻¹ in the “bare soil” treatment and -1 ± 2 to 67 ± 54 g N₂O-N ha⁻¹ day⁻¹ in “wheat” treatment (Fig. 2A). Total emissions of N₂O over the entire wheat season (Table 2) were significantly ($p < 0.001$) higher in “bare soil” treatment (3.65 ± 0.97 kg N₂O-N ha⁻¹ season⁻¹) compared with “wheat” treatment (0.59 ± 0.31 kg N₂O-N ha⁻¹ season⁻¹).

High N₂O pulses were observed from MCL at much lower irrigation and rainfall events compared with other soil types, especially when mineral N was not limiting in the soil. For example, addition of irrigation water in the first week of August resulting in WFPS of up to 60% did not stimulate N₂O fluxes. However, when KNO₃ was applied on the 12th of August, high N₂O pulses of up to 131 ± 42 g N₂O ha⁻¹ day⁻¹ (fallow) and 66 ± 54 g N₂O ha⁻¹ day⁻¹ (wheat) occurred. These emission events lasted several days before decreasing to pre-fertilizer levels (Fig. 2A). The soil water content at 0-10 cm depth was significantly ($p < 0.001$) lower in “wheat” treatment compared with “fallow” treatment. The difference further increased as the season progressed because of increasing water uptake by plant growth which also affected the N₂O fluxes (Fig. 2A). In the “fallow” treatment, irrigation and rain water was predominantly held in the top 10 cm soil layer and also ponded on top (Fig. 3A). Conversely, in the “wheat” treatment, water infiltrated into deeper layers

immediately after irrigation/rainfall events resulting in more aerated conditions at 0-10 cm depth compared with ‘fallow’ cores (Fig. 3B). By the 10th of October, the WFPS had dropped to 34% and 21% in the ‘fallow’ and ‘wheat’ treatments, respectively, with N₂O fluxes <5 g N₂O-N ha⁻¹ day⁻¹ in both the treatments (Fig. 2A). Irrigation water application of 50 mm on the 10th October increased WFPS to 61% in “fallow” treatment, resulting in N₂O fluxes of up to 128 g N₂O-N ha⁻¹ day⁻¹. However, the same irrigation application in the ‘wheat’ treatment increased WFPS to only 31%, which resulted in a negligible effect on N₂O emissions (Fig. 2A). Higher water contents did not always result in immediate increase in N₂O emissions even when mineral N was not limiting. For example, an irrigation application of 50 mm on the 31st October increased WFPS to 65% in ‘fallow’ treatment and also resulted in surface ponding. However, the N₂O emissions only started increasing after three days when WFPS dropped to ≤62% with the highest N₂O pulse of 157 g N₂O-N ha⁻¹ day⁻¹ recorded at a corresponding 56% WFPS (Fig. 2A).

<Insert Table 2>

Hanwood Loam (HL): The average daily N₂O fluxes in HL ranged between -2 ± 4 and 49 ± 20 g N₂O ha⁻¹ day⁻¹ in “wheat” treatment and between -5 ± 0 and 277 ± 184 g N₂O ha⁻¹ day⁻¹ in “bare soil” treatment (Fig. 2B). The total N₂O emitted over the entire wheat season in HL was twice as high in the “fallow” treatment (1.1 ± 0.6 kg N₂O-N ha⁻¹ season⁻¹) compared with “wheat” treatment (0.46 ± 0.1 kg N₂O-N ha⁻¹ season⁻¹); although the differences were not significant (Table 2).

Irrigation applications in early (1-12th) August increased WFPS to 56% (VWC = 25%). However, KNO₃ application on the 12th August resulted in a negligible increase in N₂O emissions despite WFPS remaining >50% for seven days after fertilizer addition in both the treatments (Fig. 2B). Large N₂O emission events from HL were only observed after a minimum irrigation of 50 mm was applied in October which resulted in WFPS > 60%. This water content was apparently the lower threshold at which a significant N₂O emission pulse was generated in this soil type (Fig. 4A). A 50 mm irrigation application on the 10th October resulted in WFPS of up to 66% in fallow soil generating N₂O pulses of up to 277 ± 183 g N₂O ha⁻¹ day⁻¹ while the corresponding WFPS and N₂O flux in wheat treatment were 63% and 49 ± 20 g N₂O-N ha⁻¹ day⁻¹, respectively (Fig. 2B). In HL, the highest WFPS of 71% (VWC = 31%) was reached in “bare soil” treatment after a 50 mm irrigation on 20th October which resulted in average daily N₂O flux of 220 ± 212 g N₂O-N ha⁻¹ day⁻¹ (Fig. 2B). The size of N₂O pulses in response to 50 mm irrigation events were always smaller in “wheat” treatment compared with “bare soil” possibly because of plant uptake resulting in significantly lower soil water content ($p < 0.001$) and mineral N content (Fig. 2B and 3C, D). The N₂O pulses generated following 50 mm irrigation events were short-lived (Fig. 2B) which is consistent with a rapid decline in soil water contents due to high infiltration rates and rapid transfer of water from shallower to deeper layers in the profile of this soil type (Fig. 3).

Banna Sand (BS): The N₂O fluxes in BS were similar between “bare soil” and “wheat” treatments, and the lowest among the three soil types, ranging from -1 ± 1 to 13 ± 4 g N₂O ha⁻¹ day⁻¹ (Fig. 2C). The cumulative N₂O emissions over the entire wheat season were 0.43 ± 0.13 kg N₂O-N ha⁻¹ season⁻¹ in “bare soil” and 0.41 ± 0.1 kg N₂O-N ha⁻¹ season⁻¹ in

“wheat” treatment (Table 2). The difference in the means of two treatments was not significant.

Irrigation applications between the 1st-12th August resulted in WFPS of 32% in “bare soil” and 27% in “wheat” treatment on the day (12th August) of fertilizer (KNO₃) application. Nitrous oxide fluxes increased from pre-fertilizer level of <1 g N₂O ha⁻¹ day⁻¹ to a maximum of 6.1 ± 0.1 g N₂O ha⁻¹ day⁻¹ in “bare soil” treatment but negligible change was observed in “wheat” treatment. Several subsequent irrigations of up to 50 mm resulted in N₂O fluxes of up to 12 ± 2 g N₂O ha⁻¹ day⁻¹ (WFPS 34%) and 13 ± 4 g N₂O ha⁻¹ day⁻¹ (WFPS 31%) in “bare soil” and “wheat” treatments, respectively. The high N₂O pulses observed in the clay loam and loam did not occur in BS. The irrigation and rain water quickly drained to deeper soil layers in BS compared with the other soil types (Fig. 3) which resulted in lower water contents in 0-10 cm layer.

3.2. N₂O emissions derived from ¹⁵N-labelled KNO₃ fertilizer

Fluxes of ¹⁵N-N₂O were measured between 12th and 26th August, 2014, after adding 80 kg N ha⁻¹ fertilizer as ¹⁵N-labelled (60% enriched) KNO₃. All results presented in this section are for the above stated 15-day period. The mean daily N₂O emissions as measured by the automated system were at least an order of a magnitude greater in MCL compared with the other two soil types (Fig. 5).

Nitrous oxide fluxes from MCL in “bare soil” treatment ranged from 31.4 ± 3.5 g N₂O-N ha⁻¹ day⁻¹ to 124.3 ± 26.0 g N₂O-N ha⁻¹ day⁻¹, of which 79% to 94% was derived from ¹⁵N-labelled KNO₃ fertilizer on different days of measurement (Fig. 5). In “wheat” treatment of

MCL, the N₂O fluxes were comparatively lower ranging from 3.8 ± 2.2 g N₂O-N ha⁻¹ day⁻¹ to 66.5 ± 53.8 g N₂O-N ha⁻¹ day⁻¹, with ¹⁵N- derived N₂O constituting 55% to 92% (Fig. 5). Nitrous oxide emissions from HL and BS were significantly lower (<7 g N₂O-N ha⁻¹ day⁻¹) than those emitted from MCL over the duration of the two week monitoring period. However, the ¹⁵N-derived N₂O emissions (i.e. those derived from fertilizer and generated through the process of denitrification), still contributed significantly to total N₂O emissions (Fig. 5). Total N₂O emissions as well as the ¹⁵N-contribution generally decreased with time. This temporal decrease in N₂O and ¹⁵N-N₂O was more pronounced in “wheat” treatments because of plant uptake of water (and N) as reflected by a significant linear relationship between VWC and ¹⁵N-fraction of N₂O emission in all soil types (Fig. 6).

3.3. Leached water losses, evapotranspiration and plant N uptake

Soil texture and the presence of plants clearly controlled water and nitrogen losses through leaching which were greatest in the fallow treatments of freer draining BS and HL. Leaching did not occur in the fine textured MCL (Table 3). Mineral N lost through leaching was mainly in the form of NO₃⁻ with $\leq 2\%$ in NH₄⁺ form. Fallow treatment of BS lost the greatest amount of water (142 ± 2 mm) and mineral N (26.9 ± 0.2 kg N ha⁻¹) through leaching while no losses occurred in the “wheat” treatment of BS (Table 2 and 3). From HL, 99 ± 13 mm (water) and 5.3 ± 2.7 kg N ha⁻¹ (mineral N) was lost through leaching in fallow treatment with smaller losses also recorded from “wheat” treatment (Table 2 and 3).

Surface water losses through evaporation or evapotranspiration (referred to as ET) were highest in “wheat” treatments, because of plant transpiration, losing 96% (MCL), 92% (HL) and 97% (BS) of total water received by cores as irrigation and rainfall during the crop

duration (Table 3). In “bare soil” treatment, MCL lost the highest amount of water through evaporation (79%), clearly because of poor drainage resulting in observed water ponding on the surface and/or being restricted to the 0-10 cm soil layer for a longer period of time (Fig. 3).

Plant N uptake and plant ¹⁵N recovery was similar among three soil types (Table 2). Yield was in the order MCL > HL > BS (Table 2) but was lower than what might be expected for a commercial crop in the region mainly caused by chamber effects (elevated temperatures) that resulted in pinched grain in most chambers.

<Insert Table 3>

The seasonal changes in soil chemical properties including mineral N, pH and DOC are explained in supplementary material including figures S1, S2 and S3.

4. Discussion

4.1. Regulation of N₂O fluxes in soils with variable texture

Soil texture predominantly determines the hydraulic conductivity, water-holding capacity, porosity and aeration of a particular soil type (Bollmann and Conrad, 1998; Saxton et al., 1986) which in turn regulate the soil redox potential and N₂O emissions. The capillary pores in clay soils hold more water against gravity than sand and silt resulting in higher soil moisture content at field capacity (Saxton et al., 1986). This is confirmed by our data showing significant differences ($p < 0.001$) in soil moisture which followed the order MCL > HL > BS (Fig. 2 and 3). The differences in soil moisture among soil types correspond

with the pattern of N₂O emissions (Table 2). The soil water content plays an indirect role in regulating N₂O emissions mainly by affecting the oxygen supply and demand in soil which control the processes of nitrification and denitrification, and by regulating the diffusion of N₂O out of soil (Clough et al., 2005; Cook and Knight, 2003).

The greatest and most prolonged response of N₂O emission to irrigation and rainfall events was observed in clay loam which corresponded with soil water content dynamics (Fig. 2). The ¹⁵N₂O emissions were an order of a magnitude higher in the clay loam compared with loam and sand (Fig. 5), indicating that in the analysed time span emissions from the clay loam soil were mainly derived from denitrification, favoured by anaerobic conditions in the biologically active upper soil layer. This is similar to previous observations of fine textured soils which become anoxic with lower amounts of water application and remain so for longer duration because of smaller pores and clay particles holding more tightly onto moisture (Bollmann and Conrad, 1998; Groffman and Tiedje, 1991). Sand showed minimum N₂O emission response to rainfall/irrigation while in loam, large but short-lived N₂O peaks only occurred at WFPS of $\geq 60\%$ observed after irrigation applications of 50 mm later in the season. The absence of N₂O peaks in sand and short duration of such emission in loam is considered to occur due to the low water holding capacity and free draining nature of these soils. Thus, the anaerobic conditions suitable for denitrification in the biologically more active upper soil layer persisted for a short period of time in coarse-textured soils. This is in contrast to the shape of the large, broad, emission peaks observed in clay loam (Fig. 2) where high N₂O emissions were observed more continuously which corresponded with high soil water contents persisting for a longer period of time after irrigations compared with coarse-textured soils (Fig. 2). The prolonged response of emissions from clay loam (Fig. 2A) compared with the sharp short lived emission response

from loam (Fig. 2B) is likely related to the soil oxygen levels and the rate at which N₂O is able to escape by diffusion across the water-air interface in the surface interval which in turn are regulated by the differences in the pore size distribution and volumetric fractions of air and water of these two soils.

Following an irrigation application in fallow clay loam soil on 31st October which resulted in water ponding at surface and high soil water contents, the high N₂O emissions occurred after a delay of three days when WFPS dropped from 65% to ≤62%. This delay in high N₂O emissions can have several explanations: 1) a diffusion barrier was created by the ponded water and high soil water contents which prevented N₂O escaping to the atmosphere; 2) the time taken by microbes to consume pre-existing oxygen in the surface soil or in a dissolved state thus delaying denitrification and resulting N₂O emissions; 3) complete denitrification to N₂ during low oxygen levels (high water contents) on the initial three days (Bouwman, 1998; Clough et al., 2006; Cook et al., 2013). A further factor which may control the shape of the N₂O emission peak and affect the timing of N₂O emission events in response to water and nitrogen application is the depth in the soil profile at which N₂O is being generated (Balaine et al., 2013; Clough et al., 2005). Many of the above mentioned processes occur simultaneously thus making it difficult to isolate the processes responsible for N₂O production, consumption and transport in field conditions (Butterbach-Bahl et al., 2013; Clough et al., 2006; Clough et al., 2005).

In the sand and loam, although N₂O emissions were low in August after ¹⁵N-labelled KNO₃ application, a significant part of these emissions was derived from denitrification despite soil water contents considered sub-optimal for denitrification (Fig. 5). These low denitrification-driven N₂O emissions may originate from anaerobic microsites present

within largely aerobic soil conditions (Parkin, 1987). This process is not entirely consistent with the findings of (Groffman and Tiedje, 1991), who reported that denitrification occurrence in micro sites at relatively high air filled porosity was limited to fine-textured soils only, and that anaerobiosis in coarse-textured soils was controlled by the absolute amount of water present, rather than the formation of microsites. It must be emphasized here that anaerobic conditions do not refer to high water contents per se but the supply and demand of oxygen which is affected by environmental variables such as available carbon, soil water content and soil temperature (Bartholomeus et al., 2008; Cook and Knight, 2003; Cook et al., 2013). Another explanation of ^{15}N - N_2O emissions from sand and loam could be the possible denitrification activity in subsoil (Balaine et al., 2013; Clough et al., 2005) which had higher water contents (Fig. 3) and possibly higher NO_3^- levels because of rapid drainage, both favouring denitrification. Although, nitrification was not measured in this study, the data suggests that a greater proportion of N_2O may be produced from nitrification in the freer draining soils and that the N supply is derived from inorganic or organic soil pools rather than from fertilizer.

4.2. Appropriate variable for N_2O prediction

Water filled pore space (WFPS), the fraction of soil pore space that is filled with water, has usually been correlated with N_2O emissions to obtain understanding of the critical thresholds for soil nitrification and denitrification processes and the calibration of predictive computer models (Dobbie and Smith, 2001; Linn and Doran, 1984). However, Parton et al. (1996a) suggested that the response of denitrification to WFPS is impacted by soil texture and available carbon levels. Farquharson and Baldock (2008) criticized that the use of WFPS in describing N_2O processes is problematic when used across soils with different bulk densities. These observations are supported by our work as the episodes of

high N₂O emissions following irrigation events were measured at $\geq 45\%$ WFPS in clay loam; however, such large N₂O emissions in loam were measured at $\geq 60\%$ WFPS (Fig. 4). Accordingly, Weitz et al. (2001) suggested that the threshold value for N₂O production in porous soils may have to be shifted towards higher WFPS than earlier suggested (Davidson et al., 1991) which is consistent with our results. Farquharson and Baldock (2008) observed that soils with different bulk densities will have different volumes of air and water at a given WFPS thus confounding any relationship with N₂O emissions, as endorsed elsewhere (Cook et al., 2013). In our study, clay loam and sand had similar bulk densities resulting in a similar slope for the relationship between WFPS and VWC; however, this slope was different for the loam because of its higher bulk density (Fig. 7). Consequently, in the period between 12th and 26th August when soil N was not limiting because of recent fertilizer addition, the relationship between VWC and N₂O emissions was much stronger than the relationship between WFPS and N₂O emissions using an exponential growth function (Fig. 8). We did not attempt to derive these relationships for the whole wheat season because of seasonal changes in mineral N and plants being interactive variables. Our results endorse the suggestion that VWC may be a better descriptor when N₂O emissions are primarily derived from denitrification, e.g. in this study (Farquharson and Baldock, 2008; van der Weerden et al., 2012).

Matric potential (MP) and relative diffusivity (RD) have been suggested as stronger predictors of N₂O emissions than WFPS and VWC when dealing with soils of variable texture (Andersen and Petersen, 2009; Balaine et al., 2013; Castellano et al., 2010; van der Weerden et al., 2012). However, these studies were conducted in laboratory conditions using smaller repacked or intact cores and that the methods may be difficult to apply in field conditions. Van der Weerden et al. (2012) maintain that VWC may remain the most

reliable indicator of N₂O emissions compared with MP and RD due to easier acquisition of field data and it being simpler to relate with other components of the water balance.

4.2. Effect of plants on surface N₂O emissions

The effect of plants on N₂O emissions varied according to soil texture and was least in coarser-textured soils. We observed 6.2 and 2.4 times higher N₂O emissions in fallow cores of clay loam and loam soil types, respectively, compared with planted cores, and no effect in sand (Table 2), which is in contrast with the general understanding that plants stimulate N₂O emissions (Hayashi et al., 2015). The field studies that reported stimulatory effects of maize plants in upland China also reported similar soil moisture conditions in the top 10 cm soil layer in planted and fallow treatments because of high summer rainfall (Ding et al., 2007; Ni et al., 2012; Song and Zhang, 2009). Similarly, Verma et al. (2006) found 1.4 times higher N₂O emissions from irrigated wheat compared with fallow soils on a sandy well-drained soil in the central plains of India and also reported similar soil water contents between planted and fallow soils. Conversely, we observed significantly ($p < 0.001$) lower soil water contents in the 0-10 cm layer of planted cores compared with fallow cores of clay loam and loam.

In clay loam, most of the irrigation water remained in or ponded above the 0-10 cm soil layer in the fallow cores compared with the planted cores where water drained to deeper soil layers rapidly (Fig. 3). The roots might have aided water percolation to deeper layers by creating preferential flow pathways and/or creating a higher moisture gradient by taking up water. Lower water contents created by plant water uptake generally result in higher oxygen diffusion into soil (depending on soil texture) which does not favour denitrification – the main process responsible for N₂O emissions in our study especially in loam and clay

loam soils. Thus, although denitrification potential is considered greater in the rhizosphere because of higher available carbon, the actual rate of denitrification in the rhizosphere may still be limited by other regulating factors such as NO_3^- (Philippot et al., 2009; Smith and Tiedje, 1979) and soil oxygen levels (Prade and Trolldenier, 1988; Wollersheim et al., 1987). For example, we found lower total and denitrification-driven N_2O emissions in planted cores compared with fallow cores in clay loam soil despite high NO_3^- levels after KNO_3 application on 12th August (Fig. 3A). The relationship between soil water content and denitrification-driven N_2O emissions (Fig. 6) shows that plant uptake of water would have created aerated soil conditions not suitable for denitrification. It has been shown that rhizosphere effect on denitrification is limited to the soil oxygen levels being near the threshold required for denitrification as the smaller amounts of oxygen can then be consumed by root and microbial respiration thus creating anaerobic conditions (Prade and Trolldenier, 1988; Wollersheim et al., 1987). Oxygen consumption by root and microbial respiration creates anaerobic conditions at lower water contents thus shifting the denitrification threshold to lower water contents in the planted soils compared with fallow soils (Cook et al., 2013). The effect of plants on denitrification also depends on temperature which regulates the plant and soil activity as shown for soils with and without pasture plants (Uchida et al., 2011).

Our N_2O results are in agreement with the winter part of Ding et al. (2007) who reported higher N_2O emissions in fallow soils compared with soils planted with wheat, and attributed the difference to increased nitrification rates caused by higher temperatures in fallow soil due to absence of plant shade. Nitrification, although not measured, is not likely to have played a key role in our study because of the type of fertilizer used (i.e. KNO_3) and the fact that high N_2O emissions were only observed after applying large irrigations, both of

which favour denitrification. The differences in temperature between fallow and planted cores were only significant in loam and were more pronounced from September (spring) onwards (Fig. S4) which might have aided the denitrification-driven high N₂O pulses observed in fallow cores of loam in October.

The minimal impact of plants in coarse-textured soils is likely caused by soil oxygen levels being unsuitable for denitrification regardless of plant presence due to rapid drainage and drying that occurs in sandier soils. This is evident in low N₂O emissions in loam and sand in mid-August despite high NO₃⁻ levels after fertilizer addition. As discussed above, the rhizosphere effect on denitrification is limited to soil oxygen levels being near the threshold required for denitrification (Prade and Trolldenier, 1988; Wollersheim et al., 1987) which was not true for sand because of rapid drainage. Conversely, fine-textured soils (e.g. clay loam) have poorer drainage and plant transpiration has time to make a significant effect on soil water contents especially in the biologically most active top soil layer. This argument is further supported by the differences in soil water content of planted and fallow cores being significant ($p < 0.001$) and larger for clay loam and loam but not significant for sand which corresponds with the pattern of N₂O emissions (Table 2).

Nitrogen uptake by plants, as evident in generally lower mineral N contents in 0-10 cm soil layer (Fig. S1) of cores planted with wheat compared with fallow cores, although only significant for NO₃⁻ in loam, may also have affected N₂O emissions by reducing mineral N availability (Bouwman, 1996). The effect of plant N uptake on N₂O emissions was most evident in loam in October when large N₂O emissions were only observed from fallow cores despite similar soil water contents following 50 mm irrigations. Similarly, the N₂O peaks in planted cores of clay loam were also smaller compared with fallow cores following

50 mm irrigations in October, although lower water contents (as proxy for oxygen levels) in planted cores could have been partly responsible for the observed difference in N₂O emissions.

Conclusions

Knowing that organic carbon, pH and mineral-N are major drivers of N₂O production through denitrification and often related to texture, the three contrasting soils used in this study were chosen for their similarity in these parameters. Soil texture played a key role in driving N₂O emissions. The soil water dynamics used as a proxy for soil oxygen levels and governed by plant growth mediated ET and soil texture were the primary drivers of denitrification-driven N₂O emissions in our study when N was not limiting. Higher N₂O emissions in the fine-textured clay loam primarily derived from higher denitrification activity because of anaerobic soil conditions created by higher water holding capacity and slower drainage compared with coarse-textured loam and sand. Plant presence resulted in reduced N₂O emissions. However, this effect was less pronounced in coarser-textured soils thus highlighting the importance of soil texture-plant interaction in regulating N₂O emissions. Our results suggest that the timing of fertilizer application is critical in the period before and immediately after sowing of wheat crops, when the water and nitrogen uptake by plants is minimal, for the mitigation of direct N₂O emissions. The results indicated that in irrigated wheat crops, where the timing of water application can be controlled, the timing of fertilizer application should also be carefully considered to match plant demand when possible.

Acknowledgments

This research was funded by the Australian Department of Agriculture through Filling the Research Gap (FtRG) funding and CSIRO's Office of the Chief Executive (OCE). This study would not have been successful without the excellent technical support provided by Roy Zandona and Alison Fattore – technical officers at CSIRO, Griffith. We are grateful to Drs Ryan Farquharson and Henrike Mielenz, CSIRO, for their valuable feedback which helped improve the manuscript. We are also thankful to Bruce McCorkell from the NSW Department of Primary Industries for advice on statistical analyses.

References

- Andersen, A.J. and Petersen, S.O., 2009. Effects of C and N availability and soil-water potential interactions on N₂O evolution and PLFA composition. *Soil Biology and Biochemistry*, 41(8): 1726-1733.
- Balaine, N. et al., 2013. Changes in relative gas diffusivity explain soil nitrous oxide flux dynamics. *Soil Science Society of America Journal*, 77(5): 1496-1505.
- Bartholomeus, R.P., Witte, J.-P.M., van Bodegom, P.M., van Dam, J.C. and Aerts, R., 2008. Critical soil conditions for oxygen stress to plant roots: Substituting the Feddes-function by a process-based model. *Journal of Hydrology*, 360(1): 147-165.
- Barton, L. et al., 2008. Nitrous oxide emissions from a cropped soil in a semi-arid climate. *Global Change Biology*, 14(1): 177-192.
- Barton, L., Murphy, D.V. and Butterbach-Bahl, K., 2013. Influence of crop rotation and liming on greenhouse gas emissions from a semi-arid soil. *Agriculture, Ecosystems & Environment*, 167(0): 23-32.

669 Bollmann, A. and Conrad, R., 1998. Influence of O₂ availability on NO and N₂O release by
670 nitrification and denitrification in soils. *Global Change Biology*, 4(4): 387-396.

671 Bouwman, A.F., 1996. Direct emission of nitrous oxide from agricultural soils. *Nutrient*
672 *Cycling in Agroecosystems*, 46(1): 53-70.

673 Bouwman, A.F., 1998. Environmental science: Nitrogen oxides and tropical agriculture.
674 *Nature*, 392(6679): 866-867.

675 Bremner, J.M. and Shaw, K., 1958. Denitrification in soil. II. Factors affecting denitrification.
676 *The Journal of Agricultural Science*, 51(01): 40-52.

677 Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R. and Zechmeister-Boltenstern,
678 S., 2013. Nitrous oxide emissions from soils: how well do we understand the
679 processes and their controls? *Philosophical Transactions of the Royal Society B:*
680 *Biological Sciences*, 368(1621).

681 Castellano, M.J. et al., 2010. Hydrological and biogeochemical controls on the timing and
682 magnitude of nitrous oxide flux across an agricultural landscape. *Global Change*
683 *Biology*, 16(10): 2711-2720.

684 Clough, T., Kelliher, F., Wang, Y. and Sherlock, R., 2006. Diffusion of ¹⁵N-labelled N₂O
685 into soil columns: a promising method to examine the fate of N₂O in subsoils. *Soil*
686 *Biology and Biochemistry*, 38(6): 1462-1468.

687 Clough, T.J., Sherlock, R.R. and Rolston, D.E., 2005. A Review of the Movement and Fate
688 of N₂O in the Subsoil. *Nutrient Cycling in Agroecosystems*, 72(1): 3-11.

689 Cook, F.J. and Knight, J.H., 2003. Oxygen Transport to Plant Roots. *Soil Science Society of*
690 *America Journal*, 67(1): 20-31.

691 Cook, F.J., Knight, J.H. and Kelliher, F.M., 2013. Modelling oxygen transport in soil with
692 plant root and microbial oxygen consumption: depth of oxygen penetration. *Soil*
693 *Research*, 51(6): 539-553.

694 Corre, M.D., vanKessel, C. and Pennock, D.J., 1996. Landscape and seasonal patterns of
695 nitrous oxide emissions in a semiarid region. *Soil Science Society of America Journal*,
696 60(6): 1806-1815.

697 CRCIF and CSIRO, 2015. Irrigateway.

698 Davidson, E.A., Rogers, J. and Whitman, W., 1991. Fluxes of nitrous oxide and nitric oxide
699 from terrestrial ecosystems. *Microbial production and consumption of greenhouse*
700 *gases: methane, nitrogen oxides, and halomethanes.*: 219-235.

701 Ding, W., Cai, Y., Cai, Z., Yagi, K. and Zheng, X., 2007. Nitrous oxide emissions from an
702 intensively cultivated maize–wheat rotation soil in the North China Plain. *Science of*
703 *the Total Environment*, 373(2): 501-511.

704 Dobbie, K. and Smith, K., 2001. The effects of temperature, water-filled pore space and land
705 use on N₂O emissions from an imperfectly drained gleysol. *European Journal of Soil*
706 *Science*, 52(4): 667-673.

707 Farquharson, R. and Baldock, J., 2008. Concepts in modelling N₂O emissions from land use.
708 *Plant Soil*, 309(1-2): 147-167.

709 Firestone, M.K., Davidson, E.A., Andreae, M. and Schimel, D., 1989. Microbiological basis
710 of NO and N₂O production and consumption in soil. *Exchange of trace gases between*
711 *terrestrial ecosystems and the atmosphere.*: 7-21.

712 Forster, P. et al., 2007. Changes in Atmospheric Constituents and in Radiative Forcing. In: S.
713 Solomon et al. (Editors), *Climate Change 2007: The Physical Science Basis.*
714 *Contribution of Working Group I to the Fourth Assessment Report of the*
715 *Intergovernmental Panel on Climate Change.* Cambridge University Press,
716 Cambridge, United Kingdom and New York, NY, USA, pp. 129-234.

717 Groffman, P.M. and Tiedje, J.M., 1991. Relationships between denitrification, CO₂
718 production and air-filled porosity in soils of different texture and drainage. *Soil*
719 *Biology and Biochemistry*, 23(3): 299-302.

720 Gu, J.X. et al., 2013. A regional experiment suggests that soil texture is a major control of
721 N₂O emissions from tile-drained winter wheat fields during the fertilization period.
722 *Soil Biology & Biochemistry*, 60: 134-141.

723 Hakata, M., Takahashi, M., Zumft, W., Sakamoto, A. and Morikawa, H., 2003. Conversion of
724 the Nitrate Nitrogen and Nitrogen Dioxide to Nitrous Oxides in Plants. *Acta*
725 *Biotechnologica*, 23(2-3): 249-257.

726 Hayashi, K. et al., 2014. Trace gas and particle emissions from open burning of three cereal
727 crop residues: Increase in residue moistness enhances emissions of carbon monoxide,
728 methane, and particulate organic carbon. *Atmospheric Environment*, 95: 36-44.

729 Hayashi, K., Tokida, T., Kajiura, M., Yanai, Y. and Yano, M., 2015. Cropland soil–plant
730 systems control production and consumption of methane and nitrous oxide and their
731 emissions to the atmosphere. *Soil Science and Plant Nutrition*, 61(1): 2-33.

732 Henry, S. et al., 2008. Disentangling the rhizosphere effect on nitrate reducers and
733 denitrifiers: insight into the role of root exudates. *Environmental Microbiology*,
734 10(11): 3082-3092.

735 Hornbuckle, J.W. and Christen, E., 1999. Physical properties of soils in the Murrumbidgee
736 and Coleambally irrigation areas. *CSIRO Land and Water*.

737 Husson, O., 2013. Redox potential (Eh) and pH as drivers of soil/plant/microorganism
738 systems: a transdisciplinary overview pointing to integrative opportunities for
739 agronomy. *Plant and Soil*, 362(1-2): 389-417.

740 IAEA, 2001. Use of isotope and radiation methods in soil and water management and crop
741 nutrition, International Atomic Energy Agency/FAO, Vienna.

742 Isbell, R., 2002. The Australian soil classification, 4. CSIRO publishing.

743 Jamali, H., Quayle, W.C. and Baldock, J., 2015. Reducing nitrous oxide emissions and
 744 nitrogen leaching losses from irrigated arable cropping in Australia through optimized
 745 irrigation scheduling. *Agricultural and Forest Meteorology*, 208(0): 32-39.

746 Li, C., 2007. Quantifying greenhouse gas emissions from soils: Scientific basis and modelling
 747 approach. *Soil Sci Plant Nutr*, 53: 344-352.

748 Linn, D. and Doran, J., 1984. Effect of water-filled pore space on carbon dioxide and nitrous
 749 oxide production in tilled and nontilled soils. *Soil Science Society of America
 750 Journal*, 48(6): 1267-1272.

751 Mosier, A. et al., 1998. Closing the global N₂O budget: nitrous oxide emissions through the
 752 agricultural nitrogen cycle. *Nutrient Cycling in Agroecosystems*, 52(2-3): 225-248.

753 Ni, K. et al., 2012. Nitrous oxide emissions from a rainfed-cultivated black soil in Northeast
 754 China: effect of fertilization and maize crop. *Biol Fertil Soils*, 48(8): 973-979.

755 Parkin, T.B., 1987. Soil Microsites as a Source of Denitrification Variability¹. *Soil Sci. Soc.
 756 Am. J.*, 51(5): 1194-1199.

757 Parton, W. et al., 1996a. Generalized model for N₂ and N₂O production from nitrification
 758 and denitrification. *Global biogeochemical cycles*, 10(3): 401-412.

759 Parton, W.J. et al., 1996b. Generalized model for N₂ and N₂O production from nitrification
 760 and denitrification. *Global Biogeochemical Cycles*, 10(3): 401-412.

761 Patrick Jr, W. and Reddy, C., 1978. Chemical changes in rice soils. *Soils & rice*.

762 Philippot, L., Hallin, S., Börjesson, G. and Baggs, E., 2009. Biochemical cycling in the
 763 rhizosphere having an impact on global change. *Plant Soil*, 321(1-2): 61-81.

764 Pihlatie, M., Ambus, P., Rinne, J., Pilegaard, K. and Vesala, T., 2005. Plant-mediated nitrous
 765 oxide emissions from beech (*Fagus sylvatica*) leaves. *New Phytologist*, 168(1): 93-98.

766 Prade, K. and Trolldenier, G., 1988. Effect of wheat roots on denitrification at varying soil
767 air-filled porosity and organic-carbon content. *Biol Fertil Soils*, 7(1): 1-6.

768 Ravishankara, A.R., Daniel, J.S. and Portmann, R.W., 2009. Nitrous Oxide (N₂O): The
769 Dominant Ozone-Depleting Substance Emitted in the 21st Century. *Science*,
770 326(5949): 123-125.

771 Rayment, G.E. and Lyons, D.J., 2011. *Soil Chemical Methods: Australasia*. CSIRO
772 Publishing.

773 Saxton, K.E., Rawls, W.J., Romberger, J.S. and Papendick, R.I., 1986. Estimating
774 Generalized Soil-water Characteristics from Texture¹. *Soil Sci. Soc. Am. J.*, 50(4):
775 1031-1036.

776 Scheer, C. et al., 2014. Impact of nitrification inhibitor (DMPP) on soil nitrous oxide
777 emissions from an intensive broccoli production system in sub-tropical Australia. *Soil*
778 *Biology and Biochemistry*, 77: 243-251.

779 Scheer, C., Wassmann, R., Kienzler, K., Ibragimov, N. and Eschanov, R., 2008. Nitrous
780 oxide emissions from fertilized, irrigated cotton (*Gossypium hirsutum* L.) in the Aral
781 Sea Basin, Uzbekistan: Influence of nitrogen applications and irrigation practices. *Soil*
782 *Biology and Biochemistry*, 40(2): 290-301.

783 Skiba, U. and Ball, B., 2002. The effect of soil texture and soil drainage on emissions of
784 nitric oxide and nitrous oxide. *Soil Use and Management*, 18(1): 56-60.

785 Smith, M.S. and Tiedje, J.M., 1979. The Effect of Roots on Soil Denitrification. *Soil Science*
786 *Society of America Journal*, 43(5): 951-955.

787 Song, C. and Zhang, J., 2009. Effects of soil moisture, temperature, and nitrogen fertilization
788 on soil respiration and nitrous oxide emission during maize growth period in northeast
789 China. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 59(2): 97-
790 106.

- Syakila, A. and Kroeze, C., 2011. The global nitrous oxide budget revisited. *Greenhouse Gas Measurement and Management*, 1(1): 17-26.
- Teece, M.A. and Fogel, M.L., 2004. Preparation of ecological and biochemical samples for isotope analysis. *Handbook of stable isotope analytical techniques*. Elsevier: 177-202.
- Trost, B. et al., 2013. Irrigation, soil organic carbon and N₂O emissions. A review. *Agron. Sustain. Dev.*, 33(4): 733-749.
- Uchida, Y., Clough, T.J., Kelliher, F.M., Hunt, J.E. and Sherlock, R.R., 2011. Effects of bovine urine, plants and temperature on N₂O and CO₂ emissions from a sub-tropical soil. *Plant Soil*, 345(1-2): 171-186.
- van der Weerden, T.J., Kelliher, F.M. and de Klein, C.A.M., 2012. Influence of pore size distribution and soil water content on nitrous oxide emissions. *Soil Research*, 50(2): 125-135.
- Verma, A., Tyagi, L., Yadav, S. and Singh, S.N., 2006. Temporal changes in N₂O efflux from cropped and fallow agricultural fields. *Agriculture, Ecosystems & Environment*, 116(3-4): 209-215.
- Vinther, F.P., 1984. Total denitrification and the ratio between N₂O and N₂ during the growth of spring barley. *Plant Soil*, 76(1-3): 227-232.
- Weitz, A., Linder, E., Frohking, S., Crill, P. and Keller, M., 2001. N₂O emissions from humid tropical agricultural soils: effects of soil moisture, texture and nitrogen availability. *Soil Biology and Biochemistry*, 33(7): 1077-1093.
- Wollersheim, R., Trolldenier, G. and Beringer, H., 1987. Effect of bulk density and soil water tension on denitrification in the rhizosphere of spring wheat (*Triticum vulgare*. *Biol Fertil Soils*, 5(3): 181-187.

Figure captions

Figure 1: Wheat growing in automated chambers installed at lysimeters at CSIRO Griffith; first set of four chambers is closed for sampling while remaining eight chambers are open.

Figure 2: Seasonal N₂O fluxes, water-filled pore space (WFPS) at 0-10 cm depth and “irrigation + rainfall” received by lysimeter cores; error bars represent standard error of the mean (n=2); note the change in y-scale (N₂O) of bottom panel (c); arrows on x-axis show the timing of fertilizer application.

Figure 3: Changes in soil volumetric water content (VWC) at 10 cm intervals to a depth of 50 cm in response to irrigation applications in October 2014; note the change of scale in y-axis.

Figure 4: Scatterplot showing the distribution of average daily N₂O fluxes at different water-filled pore space (WFPS %) at 0-10 cm depth during the complete season according to: A) Soil type, and B) Treatment

Figure 5: Average daily N₂O emissions and fraction of N₂O derived from ¹⁵N-labelled KNO₃; error bars are standard error of the mean (note the change of scale on y-axis on left hand side)

Figure 6: Relationship between soil water content at 0-10 cm depth and the fraction of N₂O derived from K¹⁵NO₃ fertilizer during 12th-26th August, 2013.

Figure 7: Relationship between: a) Water filled pore space (WFPS) and volumetric water content (VWC) at 0-10 cm depth during the complete wheat season

Figure 8: Exponential relationship: A) between volumetric water content at 0-10 cm depth and N₂O emissions, and B) between WFPS at 0-10 cm depth and N₂O emissions in the first two weeks following KNO₃ fertilizer application; .

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871 Tables

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873 Table 1: Physical and chemical properties of 0-10 cm layer of three soil types used in this study; bulk density and chemical properties are mean
874 of five and three samples, respectively, with standard errors of the mean in parenthesis.

Soil property	Mundiwa Clay Loam	Hanwood Loam	Banna Sand
Bulk Density (g cm-3)	1.27 (0.02)	1.49 (0.04)	1.28 (0.01)
Sand (%)	34.7	69.8	74.2
Silt (%)	24.7	6.4	7.8
Clay (%)	40.5	23.8	18.0
TOC (mg C kg-dry-soil ⁻¹)	1.17 (0.13)	1.23 (0.13)	0.93 (0.13)
TN (mg N kg-dry-soil ⁻¹)	0.11 (0.01)	0.12 (0.01)	0.08 (0.01)
C:N ratio	10.5 (0.33)	9.9 (0.18)	9.8 (1.72)
NO ₃ ⁻ (mg N kg-dry-soil ⁻¹)	0.29 (0.10)	1.68 (0.59)	6.03 (2.14)
NH ₄ ⁺ (mg N kg-dry-soil ⁻¹)	0.66 (0.50)	0.17 (0.79)	0.50 (0.50)
pH	8.4 (0.2)	9.0 (0.1)	8.5 (0.2)

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Table 2: Diammonium phosphate (DAP) and urea fertilizer inputs, total N₂O emissions, plant biomass, grain yield, N uptake by plants, recovery of ¹⁵N in aboveground parts of plant, and leaching losses of water and mineral N; all values are mean of two replicate cores with standard errors in parenthesis.

Soil type	Treatment	DAP fertilizer (kg N ha ⁻¹)	Urea fertilizer (kg N ha ⁻¹)	Total fertilizer (kg N ha ⁻¹)	Total N ₂ O emissions (kg N ha ⁻¹)	Biomass (Mg ha ⁻¹)	Yield (Mg ha ⁻¹)	Plant N uptake (kg N ha ⁻¹)	Plant ¹⁵ N recovery (%)	Leaching (Kg N ha ⁻¹)
Mundiwa Clay Loam	Fallow	20	80	100	3.65 (0.97) a	-	-	-	-	0
	Wheat	20	80	100	0.59 (0.31) b	5.8 (0.6)	1.8 (0.2)	72 (10)	33.0 (3.8)	0
Hanwood Loam	Fallow	20	80	100	1.10 (0.65) b	-	-	-	-	5.32 (2.70)
	Wheat	20	80	100	0.46 (0.14) b	7.2 (1.0)	1.5 (0.7)	75 (16)	32.0 (5.9)	0.41 (0.36)
Banna Sand	Fallow	20	80	100	0.43 (0.13) b	-	-	-	-	26.90 (0.21)
	Wheat	20	80	100	0.41 (0.10) b	7.3 (0.3)	0.8 (0.3)	72 (11)	33.1 (5.5)	0

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895 Table 3: Water inputs (irrigation and rainfall) and losses (ET and leaching) in different soil types during wheat season; values in parentheses are
896 standard errors of the mean (n=2)

Soil type	Treatment	Irrigation (mm)	Rainfall (mm)	Total water (mm)	ET (mm)	Leaching (mm)
Mundiwa Clay Loam	Fallow	245	153	398	314 (5)	0
	Wheat	245	153	398	382 (21)	0
Hanwood Loam	Fallow	259	153	412	230 ¹	99 (13)
	Wheat	259	153	412	380 (14)	9 (5)
Banna Sand	Fallow	365	153	518	236 (8)	142 (2)
	Wheat	365	153	518	500 (35)	0

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898 ¹data could only be retrieved from one core

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Figure 1

Figure 1

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Figure 8