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Title: Greenhouse gas emissions from the energy crop oilseed rape (*Brassica napus*); the role of photosynthetically active radiation in diurnal N_2O flux variation.

Running title: Diel N_2O flux variation driven by PAR in OSR

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Abstract

Oilseed rape (OSR, *Brassica napus* L.) is an important feedstock for biodiesel, hence carbon dioxide (CO_2), methane (CH_4) and particularly fertiliser-derived nitrous oxide (N_2O) emissions during cultivation must be quantified to assess putative greenhouse gas (GHG) savings, thus creating an urgent and increasing need for such data.

Substrates of nitrification (ammonium (NH_4)) and denitrification (nitrate (NO_3)), the predominant N_2O production pathways, were supplied separately and in combination to OSR in a UK field trial aiming to: **i** produce an accurate GHG budget of fertiliser application; **ii** characterise short to medium-term variation in GHG fluxes; **iii** establish the processes driving N_2O emission. Three treatments were applied twice, one week apart: ammonium nitrate fertiliser (NH_4NO_3, 69 kg-N ha^{-1}) mimicking the farm management, ammonium chloride (NH_4Cl, 34.4 kg-N ha^{-1}) and sodium nitrate ($NaNO_3$, 34.6 kg-N ha^{-1}). We deployed SkyLine2D for the very first time, a novel automated chamber system to measure CO_2, CH_4 and N_2O fluxes at unprecedented high temporal and spatial resolution from OSR.

During three weeks following the fertiliser application, CH_4 fluxes were negligible, but all treatments were a net sink for CO_2 (*ca.* 100 g CO_2 m^{-2}). Cumulative N_2O emissions (*ca.* 120 g CO_2-eq m^{-2}) from NH_4NO_3 were significantly greater ($p < 0.04$) than from $NaNO_3$ (*ca.* 80 g CO_2-eq m^{-2}), but did not differ from NH_4Cl (*ca.* 100 g CO_2-eq m^{-2}), and reduced the carbon-sink of photosynthesis so that OSR was a net GHG source in the fertiliser treatment. Diurnal variation in N_2O emissions, peaking in the afternoon, was more strongly associated with photosynthetically active radiation (PAR) than temperature. This suggests that the supply of carbon (C) from photosynthate may have been the key driver of the observed diurnal pattern in N_2O emission and thus should be considered in future process-based models of GHG emissions.

Introduction

Carbon dioxide (CO_2) has risen from pre-industrial levels of 280 ppm (IPCC 2007) to around 410 ppm and is widely acknowledged to be driving anthropogenic climate change (IPCC, 2014; Carlton *et al.*, 2015). Other biogenic greenhouse gases (GHGs), nitrous oxide (N_2O) and methane (CH_4), having global warming potentials over 100 years (GWP) of 298 and 34 times that of CO_2 (Myrhe *et al.*, 2013) have also increased from pre-industrial levels by more than 50 and 250% respectively (Conrad, 2009; Myrhe *et al.*, 2013). As a consequence, some of the most sensible and emerging strategies for reducing national GHG burdens specifically tackle these more potent GHG gases. However, before mitigation strategies can be implemented, a concerted effort to reduce the huge uncertainty (± 37 %) in estimates of N_2O emissions (Committee on Climate Change, 2017) is needed.

Generally, during growth, crops in Europe sequester carbon (C) from the atmosphere (Schulze *et al.*, 2010), and European agricultural land is also a net sink for CH_4 (Ciais *et al.*, 2010). In contrast, one of the biggest global sources of N_2O is agriculture (Reay *et al.*, 2012) and, in 2013, agricultural N_2O contributed approximately 8% of the UK's annual net GHG emissions, more than half of the emissions from transport and all industrial emissions (DECC, 2015). Accounting for more than 30 Mt CO_2 equivalents per year, N_2O is the single biggest contributor to UK agricultural GHG emissions (DEFRA, 2014a), and arable farming, as a result of the application of fertilisers, is an especially large emitter of N_2O.

Oilseed rape (OSR, *Brassica napus* L.) was grown on 36 million ha in 2014 (FAO 2017), 6.5 million ha of which are found in continental Europe, a greater area than used for potatoes, sugar beet, pulses and even maize (http://ec.europa.eu). In the UK, 11% of available agricultural land (675,000 ha) was dedicated to its cultivation in 2013 (DEFRA, 2014b) and it is typically grown in rotation with wheat (*Triticum aestivum* L.) or barley (*Hordeum vulgare* L.). Whereas in the UK rapeseed oil is used mainly for food products, in Europe OSR is the most widely-used feedstock for biodiesel (de Vries *et al.*, 2014), where 6 Mt (*ca*. 60%) of rape oil is used for this purpose (AHDB 2017a). Since GHG mitigation

is a key aim of using OSR for energy production, it is essential that accurate accounting of all its associated GHG emissions is prepared to assess the putative GHG savings. This requirement will be particularly exigent when the EU's Renewable Energy Directive, setting a 50% GHG reduction target for biofuels compared to fossil fuels, comes into action in 2018 (EU, 2009), whilst the default GHG saving from OSR is just 38% (Gerasimchuk, 2013). This shortfall might be expected to reduce the demand for OSR diesel, but 2016 saw record volumes produced, and industry analysts predict that whilst the OSR biodiesel-fraction of total biofuel production must drop, the absolute volume required will remain unchanged since the total output of bioenergy production in the EU must increase to meet the 2020 target of 10% (AHDB 2017b).

Measurements from soil under OSR have shown considerable variation in the magnitude of N_2O fluxes, ranging from < 40 μg m^{-2} h^{-1} (Barton *et al.*, 2010) to over 2000 μg m^{-2} h^{-1} (Drewer *et al.*, 2012). Studies of GHG fluxes from OSR (Hellebrand *et al.*, 2003; Barton *et al.*, 2010; Drewer *et al.*, 2012; Asgedom *et al.*, 2014) have generally relied on manual chambers, deployed with sampling frequencies of once a month up to a maximum of five times a week, focussed around fertilisation events. Due to the size of OSR, chambers rarely include the vegetation, but where they do (Jeuffroy *et al.*, 2013), the use of opaque chambers dictates that reported CO_2 fluxes are ecosystem respiration and not net ecosystem exchange (NEE); with the exception of a single study in Germany (Kutsch *et al.*, 2010), there is an alarming scarcity of NEE data for this important crop. The scarcity and low temporal resolution of appropriate data hinders our understanding of the magnitude of GHG source-sink dynamics and the driving processes associated with OSR.

Knowledge of the controlling processes of GHG fluxes facilitates design of GHG mitigation strategies, and while the processes controlling ecosystem CO_2 (Reay & Grace, 2007) and CH_4 (Le Mer & Roger, 2001) fluxes are well understood, those controlling N_2O fluxes are less clear. Of several microbial N_2O production pathways, nitrification and denitrification are considered the most important in soils (Smith, 2017). The former is the aerobic oxidisation of ammonium (NH_4^+) to nitrate (NO_3^-), whilst the

latter is an anaerobic sequence of heterotrophic reactions through which NO_3^- is reduced to dinitrogen gas (N_2) via N_2O, and requires a carbon (C) source (Wrage *et al.*, 2001). Nitrogen fertiliser is applied in many forms; since soils differ in their capacity for nitrification or denitrification (Bateman & Baggs, 2005), fertiliser type can affect consequential N_2O fluxes (Dobbie & Smith, 2003a; Zhang *et al.*, 2014; Zhou *et al.*, 2014). Ultimately both nitrification and denitrification depend on nitrogen (N) substrate availability (Dalal *et al.*, 2003), but multiple pathways and other contributing factors, soil temperature, moisture, pH (nitrification) (Parton *et al.*, 1996), soil organic carbon availability (dentrification), oxygen (O_2) concentration, water-filled pore space (WFPS) (Davidson *et al.*, 1993) and soil respiration (Castaldi, 2000) (denitrification) ensure that N_2O fluxes are notoriously difficult to predict, especially at fine temporal resolution (Fitton *et al.*, 2014b).

Despite this lack of understanding of variation in N_2O emissions, rudimentary management guidelines already exist regarding the timing of fertiliser application (Environment Agency, 2015). These are designed to prevent N losses during rain through leaching and N_2O emissions but could benefit markedly from a fuller understanding of the processes governing N_2O fluxes to reduce future emissions (Rees *et al.*, 2013). Currently, IPCC tier 1 emissions factors (EF) guidance states that *ca.* 1% of applied N will be lost as N_2O over the course of the following year (De Klein *et al.*, 2006), but the accuracy of this method has been called into question, particularly for Europe (Gerber *et al.*, 2016).

Oilseed rape typically receives between 100 and 200 kg N ha^{-1} in fertiliser over the course of its cultivation (DEFRA, 2010), therefore understanding the response of OSR to N fertilisation, and developing the ability to reduce N_2O emissions from this crop would constitute a substantial saving in the UK's agricultural GHG footprint. In natural ecosystems, given the appropriate combination of conditions, as much as 20% of the total annual N_2O flux may be emitted in just 48 hours (Mummey *et al.*, 1997). In agricultural systems N_2O emissions have been seen to increase rapidly in the weeks following N fertiliser (Ambus *et al.*, 2010), sometimes by two or three orders of magnitude (e.g. Dobbie & Smith, 2003; Liu *et al.*, 2005), and emissions have also been shown to vary up to 200% on

a diurnal scale (Shurpali *et al.*, 2016). Since both sources and sinks of this trace gas fluxes can exist within a landscape, fluxes can be spatially and temporally heterogeneous (Chadwick *et al.*, 2014; Kravchenko & Robertson, 2015). Without continuous measurements of N_2O flux at an appropriate spatial resolution, the potential for failure in detecting significant emission events persists.

Eddy covariance (EC) can measure landscape scale GHG fluxes at high frequency, but cannot resolve measurements to the smaller plot-scale. This lack of fine spatial resolution severely hinders the ability of an investigator to conduct replicated manipulation experiments, which are vital for advancing understanding of the mechanistic controls of net GHG flux and validating mitigation strategies. In contrast, chambers are ideal for measuring at the small spatial scale, but the frequency of data produced using manual chambers is limited by the availability of personnel, with the associated laboratory analysis of gas samples being both time consuming and unsuitable for real-time monitoring. Automation, whilst expensive, increases the frequency of measurements but chambers are frequently opaque to prevent over-heating and are usually too small to accommodate any vegetation taller than a few centimetres. We deployed a novel automated system (SkyLine2D) incorporating a single, transparent, mobile chamber, suspended from an aerial rope transect, enabling reliable repeated near-continuous measurement of GHG fluxes from pre-designated measurement positions. By circulating the chamber headspace through a series of analysers, the system was capable of delivering a full GHG budget for CO_2, CH_4 and N_2O from an intact OSR crop at relatively low cost.

The objectives of this study were to provide an accurate GHG budget from OSR following fertiliser application, to characterise the short to medium-term variation in GHG fluxes and to establish the processes driving N_2O production from OSR following application of N fertiliser. Three mineral N treatments (ammonium nitrate (NH_4NO_3), ammonium chloride (NH_4Cl) and sodium nitrate ($NaNO_3$)) were applied to test the hypothesis that GHG fluxes would significantly differ depending upon the form of N applied to the crop.

Materials and methods

Study site

The study was conducted on a 7 ha field which was part of a working farm in the east of the United Kingdom. The field had been drilled with OSR in November 2013, and inorganic fertiliser was applied three times between 1st March and 1st April 2013. The field had been planted with barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) in rotation and the crop immediately preceding the OSR had been spring barley. The soil type was the Beccles 1 association (Drewer *et al.*, 2012) with fine silt over clay and the field was used to produce annual rotation arable crops. Bulk density at the site was measured as 1.33 ± 0.20 g cm^{-3} (0-10 cm depth) and 1.49 ± 0.14 g cm^{-3} (10-20 cm depth).

Experimental design

All measurements presented were made between 24th March and 14th April 2014 since this is the period of fastest crop growth and hence the time the farmer applied fertiliser. During the study the crop height increased from < 10 cm to nearly 1 m; the main flower buds were present but closed by 31st March (GS5.4), began to open by 2nd April (GS6.0) and the crop was in full flower by 13th April (GS6.5). Prior to this study, the first N fertiliser application to the crop (67.5 kg N ha^{-1}) occurred on 5th March, with two subsequent mineral N applications of 68.9 kg N ha^{-1} during the experiment on 24th March and 1st April. Background N_2O fluxes were measured on 18th March from the experimental transect and shown to be 144 ± 50 µg m^{-2} h^{-1}. The experimental applications mimicked the NH_4NO_3 fertiliser ('FER') treatment on five replicate plots (within 40 cm diameter collars), with additional ammonium-only ('NH_4') as NH_4Cl and nitrate-only ('NO_3'), as $NaNO_3$ treatments. The treatments were applied in pellet (NH_4NO_3) or powder form to each collar on a *pro rata* basis so that FER collars received the same N dose (68.9 kg-N ha^{-1}) as the rest of the field whilst the NH_4 and NO_3 treatments received the equivalent dose as the respective component parts of the fertiliser (i.e. NH_4: 34.6 kg-N ha^{-1}; NO_3: 34.4 kg-N ha^{-1}). Care was taken to ensure the treatments were applied evenly

within the area of the collars, to mimic the action of the spreader. Nitrogen additions were applied within one hour of the farmer's fertiliser application to the field, during which time the measurement collars were covered with plastic sheeting to avoid any stray inputs within the experimental collars

Greenhouse gas flux measurements

The SkyLine2D automated chamber system was developed in-house at the University of York. A single, cylindrical chamber was suspended from a motorised trolley, mounted on parallel horizontal ropes, 1 m apart and held above the crop by 2.5 m tall aluminium trellis arches (Fig. 1), placed 24 metres apart, allowing a trolley to repeatedly traverse a pre-selected transect across the crop. An indexing system identified designated 'stops' at which the chamber automatically lowered to conduct a measurement. Each landing base (collar) for the chamber consisted of a flat, horizontal circular flange of expanded polyvinyl chloride (PVC) with an inner diameter of 38 cm (**Error! Reference source not found.**) with a perpendicular PVC collar which was inserted *ca.* 2 cm below the soil surface in order to achieve a seal. Upon completion of the programmed measurement period at a collar, the chamber automatically lifted and the trolley moved to the next 'stop'. The sequence in which collars were sampled was programmable, allowing for randomisation or exclusion of specific collars, if required. In addition to automated operation, the system could be controlled manually, allowing an operator to move the trolley between points, and drop and raise the chamber, as necessary.

The SkyLine2D chamber was cylindrical and made of clear Perspex and a size (internal diameter = 40.74 cm, height = 62 cm, volume = 80,820 cm^3, Fig. 2) designed to completely accommodate the mature OSR crop over which the GHG flux measurements were made. Attention had to be given to ensuring that the growing crop was cleanly enclosed within the dropping chamber as the crop heightened, and this was achieved using loose stringing of the crop within the footprint of the base ring as it grew. The chamber was designed as a non-steady state dynamic chamber, with headspace

gas being circulated from the chamber through analytical equipment and returned through an umbilical via polyethylene tubing (Bev-A-Line IV, Cole-Parmer, London UK; internal diameter 3 mm, length 7 m). The aperture for the sampling tube was situated 10 cm from the top of the chamber (approximately 60 cm above the soil surface) and the gas return tube entered 5 cm above the bottom lip of the chamber (**Error! Reference source not found.**), avoiding sampling from directly above the soil surface, yet assisting in the mixing of the headspace gas. The base of the chamber was fitted with an ethylene propylene diene monomer (EPDM) rubber seal (Top Bubble Gasket, part no. 490750, Essentra Components, Milton Keynes UK) which formed a gas-tight closure when dropped on the flange of the landing base (**Error! Reference source not found.**), with a pressure sensor inside the seal being activated when the chamber was fully closed. Guides around the chamber bases ensured the chamber landed accurately, and to minimise pressure differences associated with closing a chamber over the soil, a vent was incorporated into the design of the chamber, after Xu *et al.* (2006). The system included a safety feature which would halt operation at high wind speeds; this threshold could be adjusted and was determined empirically through observation of the system's performance.

Greenhouse gas flux analysis

A Licor infrared gas analyser (IRGA: LI-8100, Licor, Lincoln NE USA) was housed in the motorised trolley to measure CO_2 concentrations and also to control the SkyLine2D chamber, acting in place of a Licor long-term automated chamber (LI-8100-101, Licor, Lincoln NE USA). The Licor software was used to calculate linear CO_2 fluxes, adjusted for temperature, chamber volume and enclosed soil area, following Healy *et al.* (1996). In order to also measure the fluxes of N_2O and CH_4, the exhaust from the IRGA was intercepted through T-pieces and fed via an additional 49.8 m of Bev-A-Line tubing to separate cavity ring-down (CRD) laser analysers for N_2O and CH_4 flux measurements (LGR isotopic N_2O analyser and LGR fast greenhouse gas analyser, Los Gatos Research, CA USA) housed in an enclosed shed at one end of the SkyLine2D apparatus (**Error! Reference source not found.**). The

gas for analysis was circulated in series, the stronger flow rate of the internal pump of the CH_4 analyser dictated that it was placed first in the sequence and a shunt for any over-pressure was used to compensate for different flow rates, before returning to the chamber. Both CRD analysers measured at 1 Hz, and fluxes were calculated as the change in concentration over time using linear regression, with a correction for volume, temperature and soil area. Chamber closures of 10 minutes were programmed for the flux measurements, with a gap of 5 minutes between chamber closures to allow refreshing of the chamber with ambient air. For each closure a 60 second 'dead band' was allowed for headspace mixing, then a two minute period was used for the regression to calculate CO_2 flux and a four minute period used for N_2O and CH_4 fluxes. Following this protocol, each cycle (the term used to designate a full series of measurements across the transect) was 270 minutes long, allowing for approximately six measurements at each of the 18 sampling points per day. The attenuation of light by the chamber was calculated by linear regression from concurrent measurements of photosynthetically active radiation (PAR) inside and outside of the chamber using two matched PAR sensors (SKP 215, Skye Instruments, Powys, Wales, UK) attached to a data logger (GP1, Delta-T Instruments, Cambridge UK), measuring at 1 Hz over the 21 days of the study period; this revealed a reduction of 29% in PAR inside the chamber. After determining the extent of light-interception, CO_2 flux measurements were further adjusted during hours of daylight (defined as periods where external PAR > 0 µmol m^{-2} s^{-1}) by using the equation from a light response curve, as described by Heinemeyer *et al.* (2013).

Ancillary measurements

High frequency (1 minute, averaged over 15 minutes) measurements of soil moisture and temperature at 5 cm depth were made in the centre of each landing base using temperature (UA-001-64 Hoboware, Onset Corporation, MA USA) and moisture probes (S-SMDM005 Decagon Devices Inc, WA USA).

Statistical analyses

All statistical analyses were conducted using SAS (SAS, 9.4, SAS Institute, NC USA). Quality control of flux calculations was initially performed by discarding faulty chamber closures and then using the output statistics from the linear regression of each chamber closure: if the R^2 value of the CO_2 flux was below 0.9, fluxes were discarded; for N_2O and CH_4 fluxes, non-significant ($p > 0.05$) regressions were then counted as zero fluxes. Cumulative fluxes were calculated by trapezoidal integration, but due to a series of power failures, after April 6th flux measurements tended to be intermittent so the cumulative fluxes of all three GHGs are calculated here only up to that date.

Where GHG flux data were not normally distributed, N_2O flux rates were log transformed and the reciprocal of the CO_2 fluxes were used. For repeated measures analysis, a mixed effects model was used to study the effects of time and N treatment on GHG fluxes (collar and block as random factors), pairwise comparisons were made using least squares, accounting for multiple comparisons using Tukey's range test. Two way analysis of variance was carried out on cumulative N_2O fluxes to test for effect of N treatment and sampling hour, and treatment effect was also tested on cumulative GHG balance using analysis of variance; post hoc testing was undertaken using Duncan's multiple range test. Due to the large variation in absolute fluxes over the study, in order to investigate diurnal patterns, fluxes of both CO_2 and N_2O were normalised, achieved by using the highest daily value of flux to constrain the data (forcing all normalised flux values to fall between 0 and 1). The total N_2O-N emitted over the study was calculated as a percentage of the total mineral N applied in the two experimental applications (24th March and 1st April) to give an estimate of the emission factor.

Results

The SkyLine2D system performed well producing ca. 4,000 flux measurements of the three major biogenic GHGs; CO_2, N_2O and CH_4 over the study. The equipment worked equally well both day and night, and air temperatures within the chamber never differed from ambient by more than 5˚C over a full ten minute chamber closure; 95% of measurements were within 3˚C of ambient and by using only the first three minutes of the closure for net ecosystem exchange (NEE) measurements the effect of any temperature increases were minimised.

GHG response to nitrogen fertiliser treatment

All flux measurements of N_2O showed a net emission from the soil to the atmosphere (by convention referred to here as a positive flux). Initial fluxes (24th -30th March), three weeks after the initial pre-experimental fertiliser application, were very low and did not exceed 250 µg m^{-2} h^{-1} during this period (Fig. 3a). Four days after the first NH_4NO_3 ('FER'), NH_4 only ('NH_4') and NO_3 only ('NO_3') fertiliser additions on 27th March, fluxes began to increase and, during the afternoon of March 29th N_2O emissions from all treatments were close to 500 µg m^{-2} h^{-1}, a rate which was maintained until the second N addition on April 1st. By the second N addition, fluxes were approaching 1000 µg m^{-2} h^{-1} (Fig. 3a) with distinct peaks in N_2O emission during the afternoons of March 31st to April 6th. These peaks increased steadily from *ca.* 500 µg m^{-2} h^{-1} on the 31st March to a maximum of 3131 µg m^{-2} h^{-1} on the 6th April and the highest mean flux (4266 µg m^{-2} h^{-1}) was recorded from the NH_4 collars on April 6th, with a further peak in N_2O emissions from all treatments seen on April 12th.

There was a significant effect of the N treatments on N_2O emissions, $F_{[2,356]}$= 9.76, p<0.0001, and there was a significant interaction between treatment and time over the study, $F_{[122,356]}$= 1.35, p< 0.02; during the 16 hours following the first application of the three N treatments, emissions from the NO_3 collars were significantly higher than from either the NH_4 or FER plots (p< 0.05). During the period 4-11 days after the N applications (between 28th March and 5th April), fluxes were greatest

from the FER treatment; over several cycles N_2O fluxes were significantly higher ($p< 0.04$) than at least one of either the NO_3 or the NH_4 treatments and for three cycles were higher than both the other treatments. No further statistically significant pairwise treatment effects were observed after this point, although the NH_4 plots tended to be highest during the peak following the second N addition.

Net ecosystem exchange of CO_2 (NEE) was characterised by positive fluxes (net emission) during hours of darkness, when respiration was the dominant process, and negative fluxes (net uptake) during the daytime when the OSR was photosynthesising. The amplitude of the oscillation between positive and negative fluxes increased through the study period as the crop grew and flowered which coincided with a rise in soil and air temperatures. Highest CO_2 emissions (ecosystem respiration) were seen overnight on March 30-31st (700 mg m^{-2} h^{-1}) and April 5-6th (898 mg m^{-2} h^{-1}) (Fig. 3b) and these peaks followed the two dates that showed the greatest net uptake in CO_2 (maxima of -1953 mg m^{-2} h^{-1} and -1765 mg m^{-2} h^{-1} respectively). N treatments did not have a significant effect on NEE throughout the study, $F_{[2,574]}= 1.38$, $p> 0.29$.

There was also no significant effect of the N treatments on CH_4 fluxes ($F_{[2,398]}= 0.15$, $p> 0.86$) (Fig. 3c) and while fluxes were often negative, indicating the soil was a net sink for CH_4, all net fluxes were close to zero, with a mean, maximum and minimum of 3, 150 and -140 µg m^{-2} h^{-1}.

Diurnal GHG flux patterns

In addition to the a diurnal pattern of NEE, throughout the study a clear and repeating diurnal trend in N_2O emissions was also observed, with peaks in the afternoon and lows throughout the night (Fig. 4). Analysis of this diurnal variation in N_2O fluxes (and to a lesser extent NEE) was confounded during periods where dramatic changes in flux rates occurred (two orders of magnitude in as little as three days for N_2O). Normalising the flux data showed that the maximum N_2O emission consistently occurred during the afternoon, peaking around 13:00 for the FER treatment, 14:00 for NH_4 and

around 12:00 for the NO_3 treatment (Fig. 5a) which coincided with maximum net ecosystem production (NEP) (greatest net uptake of CO_2) for all three nitrogen treatments (Fig. 5b). This characteristic is further reinforced by the strong positive relationship between the normalised fluxes of N_2O and CO_2 for each N treatment ($p<0.0001$; Fig. 6).

Environmental controls on GHG fluxes

When the absolute fluxes (non-normalised) were analysed across all dates, the strongest correlation between N_2O fluxes for the FER and NH_4 treatments was with soil temperature (Fig. 7a) whilst PAR also correlated with N_2O fluxes in the NO_3 treatment (Fig. 7b), though none explained more than 35% of the variance of these fluxes. These analyses did not explain the key driver of the diurnal variation in N_2O flux and when the normalised fluxes were correlated with the measured environmental variables, it was clear that PAR had the strongest relationship with both NEE, in a typical light-response relationship similar across all three N treatments (Fig. 7c) and strikingly with N_2O emissions as well, again across all three N treatments ($R^2 > 0.62$; Fig. 7d).

Cumulative fluxes and GHG balance

The strong diurnal pattern in N_2O flux raises concerns about the choice of sampling time used to estimate cumulative fluxes for N_2O. Since not every collar was measured hourly on each day, fluxes were binned into six 4-hour sub periods revealing a strong significant effect of sampling time on the cumulative N_2O flux ($F_{[5,72]}= 8.05$, $p< 0.0001$); measurements taken between 12:00-16:00 yielding a greater total emission estimate than at any other time of day (Fig. 8). The cumulative flux was significantly lower from NO_3 collars than from the FER treatment ($F_{[2,72]}= 3.62$, $p< 0.04$, Fig. 8) and whilst there was no significant interaction of sampling time and treatment ($F_{[2,72]}= 0.64$, $p> 0.77$) the difference between estimates based on 09:00-12:00 and 12:00-16:00 were less pronounced for the NO_3 treatment than for the other two treatments. These fluxes represented a total loss over 14 days

of FER 1.06 (± 0.23), NH_4 0.86 (± 0.23) and NO_3 0.64 (± 0.21) kg N_2O-N ha^{-1} which equated to 0.77, 1.25 and 0.92% respectively of the total N applied during the study period.

The OSR field was a net sink for CO_2 from 24th March to 6th April, accumulating FER 107.5 (± 23.5), NH_4 170.4 (± 16.94) and NO_3 115.1 (± 16.0) g CO_2 m^{-2}, with no significant effect of N treatment ($F_{[2,12]}$= 2.24, p< 0.15, Fig. 9). The contribution of CH_4 to the overall balance was negligible at < 0.3 % of the total GHG balance across all treatments but due to the magnitude of N_2O emissions the GHG sink was greatly reduced in the NO_3 and NH_4 treatments and the FER treatment was identified as a net weak source of GHGs (Fig. 9). The overall GHG balance did not significantly differ between N treatments ($F_{[2,12]}$= 2.85, p< 0.1).

Discussion

In contrast to the clear response of N_2O flux to fertiliser, no effect was apparent in NEE, and CH_4 fluxes were so small their contribution to the GHG balance was negligible. The increase in NEE between 28th and 30th March coincided with an increase in both PAR and air temperature and the similarity of NEE and biomass between nitrogen (N) treatments (unpublished data), despite FER receiving twice the N of the other treatments, indicated growth was not N limited. Maximum NEE reported here was similar to a controlled environment study of OSR (Paul *et al.*, 1990), but below that of a field trial conducted under higher light and temperature conditions (Muller *et al.*, 2005). N_2O fluxes were similar to the short-term response to N fertiliser Drewer *et al.* (2012) reported, but were between three (Hellebrand *et al.*, 2003; Kavdir *et al.*, 2008; Asgedom *et al.*, 2014) and ten times greater than reported elsewhere (Beaudette *et al.*, 2010) for similar rates of mineral N application to OSR. With the exception of Drewer et al. (2012), who measured N_2O flux in the hours immediately following fertilisation, these studies employed a weekly to monthly measurement regime, suggesting that low temporal resolution is a major factor in the lower fluxes reported therein.

Cumulative N_2O flux (equivalent to 0.77- 1.25% of applied N across the three treatments) counteracted most, and in the FER treatment all, of the sink effect of photosynthesis over the study. These values are not strictly emission factors, since an untreated control was not required to test our hypotheses, and this should be considered when interpreting these cumulative emissions. Despite this, the amount of N emitted as N_2O over just 14 days of our study fell within the IPCC inventory annual estimates of fertiliser emissions (De Klein *et al.*, 2006), thus the final total may be above those guidelines. Since OSR is the principal feedstock for biodiesel in Europe (de Vries *et al.*, 2014) it is essential that accurate measurements of N_2O fluxes are included in any lifecycle analysis (LCA), especially as a net GHG source was seen in the FER treatment (NH_4NO_3) reflecting the regimen employed by the farmer. The magnitude of GHG emissions due to high N input further supports existing scepticism (Smeets *et al.*, 2009; Del Grosso *et al.*, 2014, Walter *et al.*, 2015) regarding the effectiveness of OSR as an energy crop.

Not all field studies measuring agricultural N_2O fluxes at an appropriate temporal frequency report diurnal patterns (e.g. Barton *et al.*, 2008; Lognoul *et al.*, 2017), but several have shown N_2O emissions peaking during the afternoon (e.g. Ryden *et al.*, 1978; Blackmer *et al.*, 1982; Christensen, 1983; Livesley *et al.*, 2008; Simek *et al.*, 2010; Alves *et al.*, 2012; Das *et al.*, 2012, Marsden *et al.*, 2017), attributing this to soil temperature patterns (Blackmer *et al.*, 1982; Livesley *et al.*, 2008; Alves *et al.*, 2012). The daytime peak may be as much as 200% of night time emissions (Shurpali *et al.*, 2016) which isotopologue data indicated to be due to increased denitrification (Ostrom *et al.*, 2010). Dissolved CO_2 in tree xylem can contribute to measured NEE (Levy *et al.*, 1999) and N_2O has also been measured from tree leaves (Pihlatie *et al.*, 2005). Calculations based upon maximum measured transpiration in OSR, *ca.* 8 g m^{-2} h^{-1} (Pivec *et al.*, 2011), and the solubility of N_2O at 15˚C (5.95 10^{-4} mol mol^{-1}), suggest that whilst a transpiration-mediated flux of *ca.* 10,000 µg N_2O m^{-2} h^{-1} is theoretically possible, an ancillary experiment conducted during this study (data not shown) using short-term shading of the OSR vegetation to induce stomatal closure, revealed no difference

between fluxes of N_2O from shaded and unshaded vegetation, suggesting this was not a significant contributing factor.

We found strong evidence to suggest that PAR, rather than soil temperature drove diurnal N_2O flux variation. Christensen (1983) suggested that PAR influenced N_2O flux and Das *et al.* (2012) specifically investigated its role on N_2O flux, but concluded its influence was limited to warming the soil. In our study the relationship strengthened with increasing applied proportion of NO_3-N, the substrate for denitrification. Since C availability drives denitrification both directly (Firestone & Davidson, 1989) and indirectly as increased microbial respiration depletes O_2 (Farquharson & Baldock, 2008), it is logical that by mediating exuded photosynthate PAR strongly influences N_2O emission when vegetation is present. In a mesocosm experiment measuring GHG fluxes from bare agricultural soil, Ineson *et al.* (unpublished data) unequivocally demonstrated that without labile C, N_2O fluxes were negligible even under high rates of mineral N addition. However, we have not found any explanatory models of measured N_2O fluxes which use PAR, while soil organic carbon (SOC) or dissolved organic carbon (DOC) has only occasionally been used to explain N_2O fluxes from soils (e.g. Ambus & Christensen, 1993; Kaiser *et al.*, 1996; Lemke *et al.*, 1998; Harrison & Matson, 2003; Petersen *et al.*, 2008).

N_2O fluxes are notoriously difficult to model, especially at fine temporal resolution (Fitton *et al.*, 2014b) and although the models, DNDC (Abdalla *et al.*, 2009), DailyDayCent (Fitton *et al.*, 2014a) and ECOSSE (Dondini *et al.*, 2016), include various estimates of SOC, they also do not use PAR as a driving input. Furthermore, model validation often uses intermittent, daily flux measurements (e.g. von Arnold *et al.*, 2005; Perdomo *et al.*, 2009; Johnson *et al.*, 2010; Gauder *et al.*, 2012; Jeuffroy *et al.*, 2013), which rarely acknowledge the importance of selecting the appropriate time of day for sampling, despite this being essential to accurate GHG budgeting (Keane & Ineson 2017). The interdiel and diel flux variation reported here underlines how systematic errors may occur when sub-daily measurements are used to extrapolate long-term cumulative fluxes.

The diurnal variation in N_2O fluxes here was clearly linked to PAR, but PAR (NO_3 treatment) and soil temperature (FER and NH_4) were important drivers over the entire study. We suggest that most N_2O was produced by denitrification, thus driven by organic C in NO_3 collars, but denitrification in the FER and NH_4 treatments was partly coupled to nitrification hence the association with soil temperature (Fig. 7(a)). It is noteworthy that there was no significant relationship between N_2O fluxes and soil moisture, which is often cited as one of the key drivers of N_2O production (Skiba *et al.*, 1998; Skiba & Smith, 2000; Dobbie & Smith, 2003b). A possible explanation is that soil moisture, ranging between 50-75% water filled pore space (WFPS) throughout the study was variously favourable to both nitrification and denitrification, processes which have different WFPS optima (Bateman & Baggs, 2005).

The pronounced variation in N_2O fluxes presented here was captured due to the high temporal resolution of SkyLine2D. The automated system measured CO_2, CH_4 and N_2O from OSR for 21 days, providing nearly 4,000 flux measurements and the clear chamber ensured that fluxes included sinks and sources from soil and vegetation. Such data from tall vegetation is rare without using eddy covariance (EC) equipment, which currently cannot measure at the spatial resolution required to test hypotheses in replicated, manipulation experiments. Furthermore, SkyLine2D overcomes the shortcomings of previously described automated systems, such as low ($n < 10$) replication (e.g. Breuer *et al.*, 2000, Nishimura *et al.*, 2005, Barton *et al.*, 2008; Morris *et al.*, 2013), long chamber closures (e.g. Breuer *et al.*, 2000: 45-60 minutes) or storage of samples for subsequent laboratory analysis (Ambus *et al.*, 2010; Juszczak & Augustin, 2013).

The high N_2O emissions across all treatments, even at 50% of the management applied N rate, demonstrate how important this gas is for crop GHG balance. Nitrogen uptake efficiency is a problem in OSR, where it is as low as 50% (Bouchet *et al.*, 2016) and our findings underline this inefficiency. We suggest that since the management fertiliser rate, which received double the N of the NH_4 treatment, neither increased crop biomass, N content (unpublished data) or N_2O emissions,

that fertiliser is lost through immobilisation or leaching, as outlined in Bouchet *et al.* (2016). We have shown that PAR, probably by supplying labile C to facilitate denitrification, is a strong driver of N_2O emissions and its inclusion in GHG flux models should improve model accuracy, a vital tool to mitigate climate change. We would like to see work carried to manipulate diurnal fluctuation in DOC to directly investigate its effect on N_2O fluxes. Additionally, the pronounced diurnal pattern in N_2O flux demonstrated here underlines the critical importance of high frequency, high spatial resolution measurements. If automation is not possible, based on our data the appropriate sampling for OSR at this site would be around 08:00 or 16:00, to coincide with the daily mean flux. However, since diurnal patterns of N_2O flux differ between locations and crops (Alves *et al.,*. 2012), we stress the importance of characterising any diurnal pattern before selecting the appropriate sampling time, if single daily measurements are to be used in flux studies. Finally, the large GHG emission from the OSR suggest there are more suitable feedstocks which should be used for biofuel production.

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References

Abdalla M, Jones M, Smith P, Williams M (2009) Nitrous oxide fluxes and denitrification sensitivity to temperature in Irish pasture soils. Soil Use and Management, **25**, 376-388.

AHDB (2017a) https://cereals.ahdb.org.uk/markets/market-news/2017/march/09/prospects-policy-fuels-uncertainty-for-biodiesel-current-situation.aspx. Retrieved 9th August 2017.

AHDB (2017b) https://cereals.ahdb.org.uk/markets/market-news/2017/march/23/prospects-policy-fuels-uncertainty-for-biodiesel-implications-for-eu-and-us-veg-oil-demand.aspx. Retrieved 9th August 2017.

Alves BJR, Smith KA, Flores RA *et al.* (2012) Selection of the most suitable sampling time for static chambers for the estimation of daily mean N_2O flux from soils. Soil Biology & Biochemistry, **46**, 129-135.

Ambus P, Christensen S (1993) Denitrification variability and control in a riparian fen irrigated with agricultural drainage water. Soil Biology & Biochemistry, **25**, 915-923.

Ambus P, Skiba U, Drewer J, Jones SK, Carter MS, Albert KR, Sutton MA (2010) Development of an accumulation-based system for cost-effective chamber measurements of inert trace gas fluxes. European Journal of Soil Science, **61**, 785-792.

Asgedom H, Tenuta M, Flaten DN, Gao XP, Kebreab E (2014) Nitrous oxide emissions from a clay soil receiving granular urea formulations and dairy manure. Agronomy Journal, **106**, 732-744.

Barton L, Kiese R, Gatter D, Butterbach-Bahl K, Buck R, Hinz C, Murphy DV (2008) Nitrous oxide emissions from a cropped soil in a semi-arid climate. Global Change Biology, **14**, 177-192.

Barton L, Murphy DV, Kiese R, Butterbach-Bahl K (2010) Soil nitrous oxide and methane fluxes are low from a bioenergy crop (canola) grown in a semi-arid climate. Global Change Biology Bioenergy, **2**, 1-15.

Bateman EJ, Baggs EM (2005) Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biology and Fertility of Soils, **41**, 379-388.

Beaudette C, Bradley RL, Whalen JK, Mcvetty PBE, Vessey K, Smith DL (2010) Tree-based intercropping does not compromise canola (*Brassica napus* L.) seed oil yield and reduces soil nitrous oxide emissions. Agriculture Ecosystems & Environment, **139**, 33-39.

Blackmer AM, Robbins SG, Bremner JM (1982) Diurnal variability in rate of emission of nitrous oxide from soils. Soil Science Society of America Journal, **46**, 937-942.

Bouchet AS, Laperche A, Bissuel-Belaygue C, Snowdon R, Nesi N, Stahl A (2016) Nitrogen use efficiency in rapeseed. A review. Agronomy for Sustainable Development, **36**, 20.

Breuer L, Papen H, Butterbach-Bahl K (2000) N_2O emission from tropical forest soils of Australia. Journal of Geophysical Research-Atmospheres, **105**, 26353-26367.

Carlton JS, Perry-Hill R, Huber M, Prokopy LS (2015) The climate change consensus extends beyond climate scientists. Environmental Research Letters, **10**, 12.

Castaldi S (2000) Responses of nitrous oxide, dinitrogen and carbon dioxide production and oxygen consumption to temperature in forest and agricultural light-textured soils determined by model experiment. Biology and Fertility of Soils, **32**, 67-72.

Chadwick DR, Cardenas L, Misselbrook TH *et al.* (2014) Optimising chamber methods for measuring nitrous oxide emissions from plot-based agricultural experiments. European Journal of Soil Science, **65**, 295-307.

Christensen S (1983) Nitrous oxide emission from a soil under permanent grass – seasonal and diurnal fluctuations as influenced by manuring and fertilization. Soil Biology & Biochemistry, **15**, 531-536.

Ciais P, Wattenbach M, Vuichard N *et al.* (2010) The European carbon balance. Part 2: croplands. Global Change Biology, **16**, 1409-1428.

Committee on Climate Change (2017) Quantifying greenhouse gas emissions. Committee on Climate Change, London UK.

Conrad R (2009) The global methane cycle: recent advances in understanding the microbial processes involved. Environmental Microbiology Reports, **1**, 285-292.

Dalal RC, Wang WJ, Robertson GP, Parton WJ (2003) Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. Australian Journal of Soil Research, **41**, 165-195.

Das BT, Hamonts K, Moltchanova E, Clough TJ, Condron LM, Wakelin SA, O'callaghan M (2012) Influence of photosynthetically active radiation on diurnal N_2O emissions under ruminant urine patches. New Zealand Journal of Agricultural Research, **55**, 319-331.

Davidson EA, Matson PA, Vitousek PM, Riley R, Dunkin K, Garciamendez G, Maass JM (1993) Processes regulating soil emissions of NO and N_2O in a seasonally dry tropical forest. Ecology, **74**, 130-139.

De Klein C, Novoa RS, Ogle S *et al.* (2006) N_2O emissions from managed soils, and CO_2 emissions from lime and urea application. IPCC Guidelines for National Greenhouse Gas Inventories, Prepared by the National Greenhouse Gas Inventories Programme, **4**.

De Vries SC, Van De Ven GWJ, Van Ittersum MK (2014) First or second generation biofuel crops in Brandenburg, Germany? A model-based comparison of their production-ecological sustainability. European Journal of Agronomy, **52**, 166-179.

DECC (2015) Annual statement of emissions for 2013, Her Majesty's Stationery Office, UK.

Defra (2010) Fertiliser manual (RB209) 8th edition, Her Majesty's Stationery Office, UK.

Defra (2014a) Agricultural emissions reporting, Her Majesty's Stationery Office, UK.

Defra (2014b) Farming statistics, final crop areas, yields, livestock populations and agricultural workforce at June 2014, Her Majesty's Stationery Office, UK.

Del Grosso S, Smith P, Galdos M, Hastings A, Parton W (2014) Sustainable energy crop production. Current Opinion in Environmental Sustainability, **9-10**, 20-25.

Dobbie KE, Smith KA (2003a) Impact of different forms of N fertilizer on N_2O emissions from intensive grassland. Nutrient Cycling in Agroecosystems, **67**, 37-46.

Dobbie KE, Smith KA (2003b) Nitrous oxide emission factors for agricultural soils in Great Britain: the impact of soil water-filled pore space and other controlling variables. Global Change Biology, **9**, 204-218.

Dondini M, Richards Mia, Pogson M *et al.* (2016) Simulation of greenhouse gases following land-use change to bioenergy crops using the ECOSSE model: a comparison between site measurements and model predictions. Global Change Biology Bioenergy*,* **8**, 925-940.

Drewer J, Finch JW, Lloyd CR, Baggs EM, Skiba U (2012) How do soil emissions of N_2O, CH_4 and CO_2 from perennial bioenergy crops differ from arable annual crops? Global Change Biology Bioenergy*,* **4**, 408-419.

ec.europa.eu http://ec.europa.eu/eurostat/statistics-explained/index.php/Main_annual_crop_statistics. Accessed 23rd June 2017

Environment Agency (2015) The nitrate pollution prevention regulations 2015. Controller of Her Majesty's Stationery Office, UK.

EU (2009) Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. Official Journal of the European Union.

FAO 2017 http://www.fao.org/faostat/en/#data/QC retrieved 6th November 2017

Farquharson R, Baldock J (2008) Concepts in modelling N_2O emissions from land use. Plant and Soil*,* **309**, 147-167.

Firestone MK, Davidson EA (1989) Microbiological basis of NO and N_2O production and consumption in soil.

Fitton N, Datta A, Hastings A *et al.* (2014a) The challenge of modelling nitrogen management at the field scale: simulation and sensitivity analysis of N_2O fluxes across nine experimental sites using DailyDayCent. Environmental Research Letters*,* **9**, 15.

Fitton N, Datta A, Smith K *et al.* (2014b) Assessing the sensitivity of modelled estimates of N_2O emissions and yield to input uncertainty at a UK cropland experimental site using the DailyDayCent model. Nutrient Cycling in Agroecosystems*,* **99**, 119-133.

Gauder M, Butterbach-Bahl K, Graeff-Honninger S, Claupein W, Wiegel R (2012) Soil-derived trace gas fluxes from different energy crops - results from a field experiment in Southwest Germany. Global Change Biology Bioenergy, **4**, 289-301.

Gerasimchuk I (2013) Biofuel Policies and Feedstock in the EU. Chatham House, London UK.

Gerber JS, Carlson Km, Makowski D *et al.* (2016) Spatially explicit estimates of N_2O emissions from croplands suggest climate mitigation opportunities from improved fertiliser management. Global Change Biology, **22**, 3383-3394.

Harrison J, Matson P (2003) Patterns and controls of nitrous oxide emissions from waters draining a subtropical agricultural valley. Global Biogeochemical Cycles, **17**, 13.

Healy RW, Striegl RG|, Russell TF, Hutchinson GL, Livingston GP (1996) Numerical evaluation of static-chamber measurements of soil-atmosphere gas exchange: Identification of physical processes. Soil Science Society of America Journal, **60**, 740-747.

Heinemeyer A, Gornall J, Baxter R, Huntley B, Ineson P (2013) Evaluating the carbon balance estimate from an automated ground-level flux chamber system in artificial grass mesocosms. Ecology and Evolution, **3**, 4998-5010.

Hellebrand HJ, Kern J, Scholz V (2003) Long-term studies on greenhouse gas fluxes during cultivation of energy crops on sandy soils. Atmospheric Environment, **37**, 1635-1644.

IPCC (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the IPCC (eds Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor T, Miller HL), pp 996. Cambridge, UK: Cambridge University Press.

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (eds Pachauri RK, Meyer LA). IPCC, Geneva, Switzerland.

Jeuffroy MH, Baranger E, Carrouee B *et al.* (2013) Nitrous oxide emissions from crop rotations including wheat, oilseed rape and dry peas. Biogeosciences, **10**, 1787-1797.

Johnson JMF, Archer D, Barbour N (2010) Greenhouse gas emission from contrasting management scenarios in the Northern corn belt. Soil Science Society of America Journal, **74**, 396-406.

Juszczak R, Augustin J (2013) Exchange of the greenhouse gases methane and nitrous oxide between the atmosphere and a temperate peatland in Central Europe. Wetlands, **33**, 895-907.

Kaiser EA, Eiland F, Germon JC *et al.* (1996) What predicts nitrous oxide emissions and denitrification N-loss from European soils? Journal of Plant Nutrition and Soil Science, **159**, 541-547.

Kavdir Y, Hellebrand HJ, Kern J (2008) Seasonal variations of nitrous oxide emission in relation to nitrogen fertilisation and energy crop types in sandy soil. Soil & Tillage Research, **98**, 175-186.

Keane JB & Ineson P (2017) Technical note: Differences in the diurnal pattern of soil respiration under adjacent *Miscanthus x giganteus* and barley crops reveal potential flaws in accepted sampling strategies. Biogeosciences, **14**, 1181- 1187.

Kravchenko AN, Robertson GP (2015) Statistical Challenges in Analyses of Chamber-Based Soil CO_2 and N_2O Emissions Data. Soil Science Society of America Journal, **79**, 200-211.

Kutsch WI, Aubinet M, Buchmann N *et al.* (2010) The net biome production of full crop rotations in Europe. Agriculture Ecosystems & Environment, **139**, 336-345.

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: A review. European Journal of Soil Biology, **37**, 25-50.

Lemke RL, Izaurralde RC, Nyborg M (1998) Seasonal distribution of nitrous oxide emissions from soils in the Parkland region. Soil Science Society of America Journal, **62**, 1320-1326.

Levy PE, Meir P, Allen SJ, Jarvis PG (1999) The effect of aqueous transport of CO_2 in xylem sap on gas exchange in woody plants. Tree Physiology, **19**, 53-58.

Liu XJ, Mosier AR, Halvorson AD, Zhang FS (2005) Tillage and nitrogen application effects on nitrous and nitric oxide emissions from irrigated corn fields. Plant and Soil, **276**, 235-249.

Livesley SJ, Kiese R, Graham J, Weston CJ, Butterbach-Bahl K, Arndt SK (2008) Trace gas flux and the influence of short-term soil water and temperature dynamics in Australian sheep grazed pastures of differing productivity. Plant and Soil, **309**, 89-103.

Lognoul M, Theodorakopoulos N, Hiel MP *et al.* (2017) Impact of tillage on greenhouse gas emissions by an agricultural crop and dynamics of N_2O fluxes: Insights from automated closed chamber measurements. Soil & Tillage Research, **167**, 80-89.

Marsden KA, Jones DL Chadwick DR (2017) DMPP is ineffective at mitigating N_2O emissions from sheep urine patches in a UK grassland under summer conditions. Agriculture Ecosystems & Environment, **246**, 1-11.

Morris SG, Kimber SWL, Grace P, Van Zwieten L (2013) Improving the statistical preparation for measuring soil N_2O flux by closed chamber. Science of the Total Environment*,* **465**, 166-172.

Muller J, Behrens T, Diepenbrock W (2005) Measurement and modelling of canopy gas exchange of oilseed rape. Agricultural and Forest Meteorology*,* **132**, 181-200.

Mummey DL, Smith JL, Bolton H (1997) Small-scale spatial and temporal variability of N_2O flux from a shrub-steppe ecosystem. Soil Biology & Biochemistry*,* **29**, 1699-1706.

Myhre G, D. Shindell, F.-M. Bréon, W. Collins, J. Fuglestvedt, J. Huang, D. Koch, J.-F. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura and H. Zhang (2013) Anthropogenic and Natural Radiative Forcing. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Nishimura S, Sudo S, Akiyama H, Yonemura S, Yagi K, Tsuruta H (2005) Development of a system for simultaneous and continuous measurement of carbon dioxide, methane and nitrous oxide fluxes from croplands based on the automated closed chamber method. Soil Science and Plant Nutrition*,* **51**, 557-564.

Ostrom NE, Sutka R, Ostrom PH *et al.* (2010) Isotopologue data reveal bacterial denitrification as the primary source of N_2O during a high flux event following cultivation of a native temperate grassland. Soil Biology & Biochemistry*,* **42**, 499-506.

Parton WJ, Mosier AR, Ojima DS, Valentine DW, Schimel DS, Weier K, Kulmala AE (1996) Generalised model for N_2 and N_2O production from nitrification and denitrification. Global Biogeochemical Cycles, **10**, 401-412.

Paul MJ, Lawlor DW, Driscoll SP (1990) The effect of temperature on photosynthesis and carbon fluxes in sunflower and rape. Journal of Experimental Botany, **41**, 547-555.

Perdomo C, Irisarri P, Ernst O (2009) Nitrous oxide emissions from an Uruguayan argiudoll under different tillage and rotation treatments. Nutrient Cycling in Agroecosystems, **84**, 119-128.

Petersen SO, Schjonning P, Thomsen IK, Christensen BT (2008) Nitrous oxide evolution from structurally intact soil as influenced by tillage and soil water content. Soil Biology & Biochemistry, **40**, 967-977.

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

Pivec J, Brant V, Becka D, Cihlar P (2011) Consumptive use of water in *Brassica napus* from flowering to ripening stage under rainless region conditions. Irrigation and Drainage, **60**, 493-498.

Reay DS, Davidson EA, Smith KA, Smith P, Melillo JM, Dentener F, Crutzen PJ (2012) Global agriculture and nitrous oxide emissions. Nature Climate Change, **2**, 410-416.

Reay DS, Grace J (2007) Carbon Dioxide: Importance, Sources and Sinks. In: *Greenhouse Gas Sinks.* (eds Reay DS, Hewitt CN, Smith KA, Grace J) pp Page., Cabi Publishing-C a B Int, Cabi Publishing, Wallingford OX10 8DE, Oxon, UK.

Rees RM, Baddeley JA, Bhogal A *et al.* (2013) Nitrous oxide mitigation in UK agriculture. Soil Science and Plant Nutrition, **59**, 3-15.

Ryden JC, Lund LJ, Focht DD (1978) Direct in-field measurement of nitrous oxide fluxes from soils. Soil Science Society of America Journal, **42**, 731-737.

Schulze ED, Ciais P, Luyssaert S *et al.* (2010) The European carbon balance. Part 4: integration of carbon and other trace-gas fluxes. Global Change Biology, **16**, 1451-1469.

Shurpali NJ, Rannik U, Jokinen S *et al.* (2016) Neglecting diurnal variations leads to uncertainties in terrestrial nitrous oxide emissions. Scientific Reports, **6**, 9.

Simek M, Brucek P, Hynst J (2010) Diurnal fluxes of CO_2 and N_2O from cattle-impacted soil and implications for emission estimates. Plant Soil and Environment, **56**, 451-457.

Skiba U, Smith KA (2000) The control of nitrous oxide emissions from agricultural and natural soils. Chemosphere - Global Change Science, **2**, 379-386.

Skiba UM, Sheppard LJ, Macdonald J, Fowler D (1998) Some key environmental variables controlling nitrous oxide emissions from agricultural and semi-natural soils in Scotland. Atmospheric Environment, **32**, 3311-3320.

Smeets EMW, Bouwmanw LF, Stehfest E, Van Vuuren DP, Posthuma A (2009) Contribution of N_2O to the greenhouse gas balance of first-generation biofuels. Global Change Biology, **15**, 1-23.

Smith KA (2017) Changing views of nitrous oxide emissions from agricultural soil: key controlling processes and assessment at different spatial scales. European Journal of Soil Science, **68**, 137-155.

Smith KA, Thomson PE, Clayton H, Mctaggart IP, Conen F (1998) Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. Atmospheric Environment, **32**, 3301-3309.

Von Arnold K, Nilsson M, Hanell B, Weslien P, Klemedtsson L (2005) Fluxes of CO_2, CH_4 and N_2O from drained organic soils in deciduous forests. Soil Biology & Biochemistry, **37**, 1059-1071.

Walter K, Don A, Fuss R, Kern J, Drewer J, Flessa H (2015) Direct nitrous oxide emissions from oilseed rape cropping - a meta-analysis. Global Change Biology Bioenergy, **7**, 1260-1271.

Wrage N, Velthof GL, Van Beusichem ML, Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology & Biochemistry, **33**, 1723-1732.

Xu LK, Furtaw MD, Madsen RA, Garcia RL, Anderson DJ, Mcdermitt DK (2006) On maintaining pressure equilibrium between a soil CO_2 flux chamber and the ambient air. Journal of Geophysical Research-Atmospheres, **111**.

Zhang XB, Wu LH, Sun N, Ding XS, Li JW, Wang BR, Li DC (2014) Soil CO_2 and N_2O emissions in maize growing season under different fertilizer regimes in an upland red soil region of South China. Journal of Integrative Agriculture, **13**, 604-614.

Zhou MH, Zhu B, Bruggemann N, Bergmann J, Wang YQ, Butterbach-Bahl K (2014) N_2O and CH_4 emissions, and NO_3^- leaching on a crop-yield basis from a subtropical rain-fed wheat-maize rotation in response to different types of nitrogen fertiliser. Ecosystems, **17**, 286-301.

Fig. 1 Aerial and side-profile schematics of the SkyLine2D system showing **(a)**, the trellis arch supports at either end, supporting the Kevlar ropes between. The motorised trolley is depicted at the midpoint of the two supports **(b)**. Cross section of the *in situ* system at the OSR field site and **(c)** the N_2O and CH_4 Los Gatos CRD analysers were housed in the green garden box by the right hand trellis support.

Fig. 2 The SkyLine2D chamber *in situ* during a measurement over the OSR crop (left hand panel). Note the PAR sensor mounted within the chamber (white circle). The schematic of the chamber (right hand panel) highlights the components and dimensions: **A**- manifold with attached gas lines. Arrows denote direction of flow; the sampling line draws from near the top (*circa* 10 cm) of the chamber and the return pipe opens near the base of the chamber. **B-** vent for pressure equalisation, after Xu *et al.* (2006). **C**- chamber constructed from clear Perspex. **D**- gasket to ensure gas-tight seal between chamber and **E**- landing base. The base (ring) had an inner diameter (38 cm) smaller than that of the chamber (41 cm), affording a greater margin of error when the chamber landed.

Fig. 3 Fluxes of N_2O **(a)** CO_2 **(b)** and CH_4 **(c)** from the oilseed rape crop, following application of three types of mineral nitrogen (NH_4NO_3 (FER), closed circles, NH_4Cl (NH_4), open circles, $NaNO_3$ (NO_3), closed triangles) measured using the SkyLine2D. Values shown are mean (n= 5, ± 1SE). Vertical arrows indicate timing of nitrogen additions.

Fig. 4 Diurnal variation of N_2O flux in relation to PAR **(a)** and soil temperature at 5 cm depth **(b)**. Data shown are for the collars treated with $NaNO_3$ (NO_3). Fluxes of N_2O can be seen to increase prior to soil temperature and in close relations to PAR.

Fig. 5 Diurnal variation of the mean (n=5) daily-normalised N_2O **(a)** and NEP **(b)** averaged over the entire study period. Data are shown for each of the three nitrogen treatments applied, and a third order Gaussian function has been fitted: FER- closed circles, solid line: N_2O R^2= 0.74, p< 0.0001; NEE R^2= 0.94, p< 0.0001; NH_4- open circles, long dashes: N_2O R^2= 0.70, p< 0.0001; NEE R^2= 0.97, p< 0.0001; NO_3- closed triangles, short dashes: N_2O R^2= 0.75, p< 0.0001; NEE R^2= 0.97, p< 0.0001.

Fig. 6 Relationship of the mean hourly normalised flux of N_2O to the mean hourly normalised flux CO_2 (expressed as net ecosystem production (NEP)) across the study period. Data shown are for three nitrogen treatments: FER- closed circles, solid line: R^2= 0.77 p< 0.0001; NH_4- open circles, long dashes: R^2= 0.64 p< 0.0001; NO_3- closed triangles, short dashes: R^2= 0.75, p< 0.0001.

Fig. 7 Response of N_2O flux from OSR to soil temperature at 5 cm depth **(a)** under two nitrogen treatments: FER- (closed circles, solid line) R^2= 0.35, p< 0.0001; NH_4- (open circles, long dashed line) R^2= 0.34, p< 0.0001 and **(b)** relationship of N_2O flux to PAR from OSR under NO_3 addition (closed triangles, short dashed line), R^2= 0.35, p< 0.0001. Relationship of the hourly mean (n= 5) normalised NEP **(c)** and N_2O **(d)** to PAR, averaged over the study period for three nitrogen treatments with a second order polynomial function fitted: NEP- FER- closed circles, solid line, R^2= 0.98, p< 0.0001; NH_4- open circles, long dashes, R^2= 0.98, p< 0.0001; NO_3- closed triangles, short dashes, R^2= 0.98, p< 0.0001. N_2O- FER- closed circles, solid line, R^2= 0.79, p< 0.0001; NH_4- open circles, long dashes, R^2= 0.62, p< 0.0001; NO_3- closed triangles, short dashes, R^2= 0.71, p< 0.0001.

Fig. 8 The effect of sampling time on the estimate of mean (± 1 SE) cumulative flux of N_2O from OSR under three different nitrogen treatments. Significant (p< 0.001) differences in sampling time are shown (two-way ANOVA testing for effect of treatment and sampling time), times with similar letters

do not differ (Duncan multiple range *post hoc* test). Time bins are: **0-** 00-03:59 **4-** 04-07:59 **8-** 08-11:59 **12-** 12-15:59 **16-** 16-19:59 **20-** 20-23:59. Cumulative flux of N_2O was significantly lower ($p< 0.04$) from NO_3 than from FER treatment (*).

Fig. 9 Mean ± 1SE total fluxes of CO_2, N_2O and CH_4 from oilseed rape under three different nitrogen regimes, n=5 shown in terms of global warming potential (GWP) as calculated over a 100 year period (IPCC 2013) and is expressed in terms of CO_2- equivalents. Negative values indicate net uptake from the atmosphere and positive fluxes net emission