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Title: Greenhouse gas emissions from the energy crop oilseed rape (Brassica napus); the role of photosynthetically active radiation in diurnal N₂O flux variation.

Running title: Diel N₂O flux variation driven by PAR in OSR

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Primary research article
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$_2$O) 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$_2$O 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$_2$O emission. Three treatments were applied twice, one week apart: ammonium nitrate fertiliser (NH$_4$NO$_3$, 69 kg-N ha$^{-1}$) mimicking the farm management, ammonium chloride (NH$_4$Cl, 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$_2$O 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$_2$O emissions (*ca.* 120 g CO$_2$-eq m$^{-2}$) from NH$_4$NO$_3$ were significantly greater (*p* < 0.04) than from NaNO$_3$ (*ca.* 80 g CO$_2$-eq m$^{-2}$), but did not differ from NH$_4$Cl (*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$_2$O 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$_2$O emission and thus should be considered in future process-based models of GHG emissions.
Introduction

Carbon dioxide (CO\textsubscript{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 \textit{et al.}, 2015). Other biogenic greenhouse gases (GHGs), nitrous oxide (N\textsubscript{2}O) and methane (CH\textsubscript{4}), having global warming potentials over 100 years (GWP) of 298 and 34 times that of CO\textsubscript{2} (Myrhe \textit{et al.}, 2013) have also increased from pre-industrial levels by more than 50 and 250\% respectively (Conrad, 2009; Myrhe \textit{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\textsubscript{2}O emissions (Committee on Climate Change, 2017) is needed.

Generally, during growth, crops in Europe sequester carbon (C) from the atmosphere (Schulze \textit{et al.}, 2010), and European agricultural land is also a net sink for CH\textsubscript{4} (Ciais \textit{et al.}, 2010). In contrast, one of the biggest global sources of N\textsubscript{2}O is agriculture (Reay \textit{et al.}, 2012) and, in 2013, agricultural N\textsubscript{2}O 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\textsubscript{2} equivalents per year, N\textsubscript{2}O 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\textsubscript{2}O.

Oilseed rape (OSR, \textit{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$_2$O 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, focused 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 CO₂ (Reay & Grace, 2007) and CH₄ (Le Mer & Roger, 2001) fluxes are well understood, those controlling N₂O fluxes are less clear. Of several microbial N₂O production pathways, nitrification and denitrification are considered the most important in soils (Smith, 2017). The former is the aerobic oxidation of ammonium (NH₄⁺) to nitrate (NO₃⁻), whilst the latter is an anaerobic sequence of heterotrophic reactions through which NO₃⁻ is reduced to dinitrogen gas (N₂) via N₂O, 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 N₂O 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 (dentification), oxygen (O₂) concentration, water-filled pore space (WFPS) (Davidson et al., 1993) and soil respiration (Castaldi, 2000) (denitrification) ensure that N₂O fluxes are notoriously difficult to predict, especially at fine temporal resolution (Fitton et al., 2014b).

Despite this lack of understanding of variation in N₂O 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 N₂O emissions but could benefit markedly from a fuller understanding of the processes governing N₂O 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$_2$O 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$_2$O 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$_2$O flux may be emitted in just 48 hours (Mummey et al., 1997). In agricultural systems N$_2$O 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$_2$O 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\textsubscript{2}, CH\textsubscript{4} and N\textsubscript{2}O 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\textsubscript{2}O production from OSR following application of N fertiliser. Three mineral N treatments (ammonium nitrate (NH\textsubscript{4}NO\textsubscript{3}), ammonium chloride (NH\textsubscript{4}Cl) and sodium nitrate (NaNO\textsubscript{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 I 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 $1.33 \pm 0.20 \text{ g cm}^{-3}$ (0-10 cm depth) and $1.49 \pm 0.14 \text{ g cm}^{-3}$ (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$_2$O fluxes were measured on 18th March from the experimental transect and shown to be $144 \pm 50 \mu\text{g m}^{-2} \text{ h}^{-1}$. The experimental applications mimicked the NH$_4$NO$_3$ fertiliser (‘FER’) treatment on five replicate plots (within 40 cm diameter collars), with additional ammonium-only (‘NH$_4$’) as NH$_4$Cl and nitrate-only (‘NO$_3$’), as NaNO$_3$ treatments. The treatments were applied in pellet (NH$_4$NO$_3$) 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 CO₂ 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 CO₂ fluxes, adjusted for temperature, chamber volume and enclosed soil area, following Healy et al. (1996). In order to also measure the fluxes of N₂O and CH₄, 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 N₂O and CH₄ flux measurements (LGR isotopic N₂O 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 CH₄ 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 CO₂ flux and a four minute period used for N₂O and CH₄ 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, CO₂ 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 R² value of the CO₂ flux was below 0.9, fluxes were discarded; for N₂O and
CH₄ 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, N₂O flux rates were log transformed
and the reciprocal of the CO₂ 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\textsubscript{2}O 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\textsubscript{2} and N\textsubscript{2}O 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\textsubscript{2}O-N emitted over the study was calculated as a percentage of the total mineral N applied in the two experimental applications (24\textsuperscript{th} March and 1\textsuperscript{st} 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$_2$O 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$_2$O showed a net emission from the soil to the atmosphere (by convention referred to here as a positive flux). Initial fluxes (24$^{\text{th}}$ -30$^{\text{th}}$ 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$_4$NO$_3$ (‘FER’), NH$_4$ only (‘NH$_4$’) and NO$_3$ only (‘NO$_3$’) fertiliser additions on 27$^{\text{th}}$ March, fluxes began to increase and, during the afternoon of March 29$^{\text{th}}$ N$_2$O 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 1$^{\text{st}}$. By the second N addition, fluxes were approaching 1000 µg m$^{-2}$ h$^{-1}$ (Fig. 3a) with distinct peaks in N$_2$O emission during the afternoons of March 31$^{\text{st}}$ to April 6$^{\text{th}}$. These peaks increased steadily from ca. 500 µg m$^{-2}$ h$^{-1}$ on the 31$^{\text{st}}$ March to a maximum of 3131 µg m$^{-2}$ h$^{-1}$ on the 6$^{\text{th}}$ April and the highest mean flux (4266 µg m$^{-2}$ h$^{-1}$) was recorded from the NH$_4$ collars on April 6$^{\text{th}}$, with a further peak in N$_2$O emissions from all treatments seen on April 12$^{\text{th}}$.

There was a significant effect of the N treatments on N$_2$O emissions, F$_{[2,356]}= 9.76$, p<0.0001, and there was a significant interaction between treatment and time over the study,
307 \( F_{[122,356]} = 1.35, p < 0.02; \) 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 28\(^{th}\) March and 5\(^{th}\) April), fluxes were greatest from the FER treatment; over several cycles N\(_2\)O 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.

315 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 30-31\(^{st}\) (700 mg m\(^{-2}\) h\(^{-1}\)) and April 5-6\(^{th}\) (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).

323 N treatments did not have a significant effect on NEE throughout the study, \(F_{[2,574]} = 1.38, p > 0.29\).

325 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 diurnal pattern of NEE, throughout the study a clear and repeating diurnal trend in N₂O emissions was also observed, with peaks in the afternoon and lows throughout the night (Fig. 4). Analysis of this diurnal variation in N₂O 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 N₂O). Normalising the flux data showed that the maximum N₂O emission consistently occurred during the afternoon, peaking around 13:00 for the FER treatment, 14:00 for NH₄ and around 12:00 for the NO₃ treatment (Fig. 5a) which coincided with maximum net ecosystem production (NEP) (greatest net uptake of CO₂) for all three nitrogen treatments (Fig. 5b). This characteristic is further reinforced by the strong positive relationship between the normalised fluxes of N₂O and CO₂ 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 N₂O fluxes for the FER and NH₄ treatments was with soil temperature (Fig. 7a) whilst PAR also correlated with N₂O fluxes in the NO₃ 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 N₂O 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 N₂O emissions as well, again across all three N treatments (R² > 0.62; Fig. 7d).

Cumulative fluxes and GHG balance
The strong diurnal pattern in N\textsubscript{2}O flux raises concerns about the choice of sampling time used to estimate cumulative fluxes for N\textsubscript{2}O. 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\textsubscript{2}O 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\textsubscript{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\textsubscript{3} treatment than for the other two treatments. These fluxes represented a total loss over 14 days of FER 1.06 (± 0.23), NH\textsubscript{4} 0.86 (± 0.23) and NO\textsubscript{3} 0.64 (± 0.21) kg N\textsubscript{2}O-N ha\textsuperscript{-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\textsubscript{2} from 24\textsuperscript{th} March to 6\textsuperscript{th} April, accumulating FER 107.5 (± 23.5), NH\textsubscript{4} 170.4 (± 16.94) and NO\textsubscript{3} 115.1 (± 16.0) g CO\textsubscript{2} m\textsuperscript{2}, with no significant effect of N treatment ($F_{[2,12]}= 2.24$, $p< 0.15$, Fig. 9). The contribution of CH\textsubscript{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\textsubscript{2}O emissions the GHG sink was greatly reduced in the NO\textsubscript{3} and NH\textsubscript{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\textsubscript{2}O flux to fertiliser, no effect was apparent in NEE, and CH\textsubscript{4} fluxes were so small their contribution to the GHG balance was negligible. The increase in NEE between 28\textsuperscript{th} and 30\textsuperscript{th} 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\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O fluxes are included in any lifecycle analysis (LCA), especially as a net
GHG source was seen in the FER treatment (NH$_4$NO$_3$) 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$_2$O fluxes at an appropriate temporal frequency report diurnal patterns (e.g. Barton et al., 2008; Lognoul et al., 2017), but several have shown N$_2$O 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$_2$O 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$_2$O at 15˚C (5.95 10$^{-4}$ mol mol$^{-1}$), suggest that whilst a transpiration-mediated flux of ca. 10,000 µg N$_2$O 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$_2$O 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$_2$O flux variation. Christensen (1983) suggested that PAR influenced N$_2$O flux and Das et al. (2012) specifically investigated its role on N$_2$O 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$_2$O 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$_2$O fluxes were
negligible even under high rates of mineral N addition. However, we have not found any
explanatory models of measured N$_2$O fluxes which use PAR, while soil organic carbon
(SOC) or dissolved organic carbon (DOC) has only occasionally been used to explain N$_2$O
fluxes from soils (e.g. Ambus & Christensen, 1993; Kaiser et al., 1996; Lemke et al., 1998;
Harrison & Matson, 2003; Petersen et al., 2008).

N$_2$O 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$_2$O 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$_2$O 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$_2$O fluxes and soil moisture, which is often cited as one of the key
drivers of N$_2$O 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$_2$O fluxes presented here was captured due to the high
temporal resolution of SkyLine2D. The automated system measured CO$_2$, CH$_4$ and N$_2$O 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.,

The high N$_2$O 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$_2$O 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\textsubscript{2}O 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\textsubscript{2}O fluxes. Additionally, the pronounced diurnal pattern
in N\textsubscript{2}O 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\textsubscript{2}O 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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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 N₂O and CH₄ 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 N₂O (a) CO₂ (b) and CH₄ (c) from the oilseed rape crop, following application of three types of mineral nitrogen (NH₄NO₃ (FER), closed circles, NH₄Cl (NH₄), open circles, NaNO₃ (NO₃), 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 N₂O flux in relation to PAR (a) and soil temperature at 5 cm depth (b). Data shown are for the collars treated with NaNO₃ (NO₃). Fluxes of N₂O 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 N₂O (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: N₂O R² =
0.74, p < 0.0001; NEE $R^2 = 0.94$, p < 0.0001; NH$_4^-$ open circles, long dashes: N$_2$O $R^2 = 0.70$, p < 0.0001; NEE $R^2 = 0.97$, p < 0.0001; NO$_3^-$ closed triangles, short dashes: N$_2$O $R^2 = 0.75$, p < 0.0001.

Fig. 6 Relationship of the mean hourly normalised flux of N$_2$O 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$_2$O 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$_2$O 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$_2$O (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$_2$O- 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 N₂O 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 N₂O was significantly lower (p< 0.04) from NO₃ than from FER treatment (*).

Fig. 9 Mean ± 1SE total fluxes of CO₂, N₂O and CH₄ 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 CO₂ equivalents. Negative values indicate net uptake from the atmosphere and positive fluxes net emission.
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$_2$O and CH$_4$ Los Gatos CRD analysers were housed in the green garden box by the right hand trellis support.
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476x317mm (300 x 300 DPI)
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Fig. 6 Relationship of the mean hourly normalised flux of N\textsubscript{2}O to the mean hourly normalised flux CO\textsubscript{2} (expressed as net ecosystem production (NEP)) across the study period. Data shown are for three nitrogen treatments: FER- closed circles, solid line: R\textsuperscript{2} = 0.77 p< 0.0001; NH\textsubscript{4}- open circles, long dashes: R\textsuperscript{2} = 0.64 p< 0.0001; NO\textsubscript{3}- closed triangles, short dashes: R\textsuperscript{2} = 0.75, p< 0.0001.
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Fig. 8 The effect of sampling time on the estimate of mean (± 1 SE) cumulative flux of N\textsubscript{2}O 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\textsubscript{2}O was significantly lower (p< 0.04) from NO\textsubscript{3} than from FER treatment (*).
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