UV-induced N$_2$O emission from plants

Dan Bruhna, b, * , Kristian R. Albert a, Teis N. Mikkelsen a, Per Ambus a

a Department of Chemical and Biochemical Engineering, Centre for Ecosystems and Environmental Sustainability (ECO), Technical University of Denmark (DTU), DK-2800 Kgs. Lyngby, Denmark
b Centre for Earth, Planetary, Space and Astronomical Research, The Open University, Walton Hall, Milton Keynes MK76AA, UK

HIGHLIGHTS

• Plants released N$_2$O in natural sunlight, mostly due to UV.
• The emission rate is temperature dependent.
• The N$_2$O formation appears to be at the surface of leaves.
• Ecosystem emission of N$_2$O may be up to c. 30% higher than hitherto assumed.

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ABSTRACT

Nitrous oxide (N$_2$O) is an important long-lived greenhouse gas and precursor of stratospheric ozone-depleting mono-nitrogen oxides. The atmospheric concentration of N$_2$O is persistently increasing; however, large uncertainties are associated with the distinct source strengths. Here we investigate for the first time N$_2$O emission from terrestrial vegetation in response to natural solar ultra violet radiation. We conducted field site measurements to investigate N$_2$O atmosphere exchange from grass vegetation exposed to solar irradiance with and without UV-screening. Further laboratory tests were conducted with a range of species to study the controls and possible loci of UV-induced N$_2$O emission from plants. Plants released N$_2$O in response to natural sunlight at rates of c. 20–50 nmol m$^{-2}$ h$^{-1}$, mostly due to the UV component. The emission response to UV-A is of the same magnitude as that to UV-B. Therefore, UV-A is more important than UV-B given the natural UV-spectrum at Earth’s surface. Plants also emitted N$_2$O in darkness, although at reduced rates. The emission rate is temperature dependent with a rather high activation energy indicative for an abiotic process. The prevailing zone for the N$_2$O formation appears to be at the very surface of leaves. However, only c. 26% of the UV-induced N$_2$O appears to originate from plant-N. Further, the process is dependent on atmospheric oxygen concentration. Our work demonstrates that ecosystem emission of the important greenhouse gas, N$_2$O, may be up to c. 30% higher than hitherto assumed.

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1. Introduction

Estimates of the natural terrestrial source strengths of the long-lived greenhouse gas nitrous oxide (N$_2$O) range between 3.3 and 9.0 TgN yr$^{-1}$, which accounts for more than one third of total global N$_2$O emissions to the atmosphere (Solomon et al., 2007). The natural terrestrial sources are explained entirely as N$_2$O release via upward diffusion through the soil profile resulting from microbial activity (Solomon et al., 2007). Plants may, however, also play an important, although not yet quantified, role in N$_2$O emission either indirectly as conduits of soil derived N$_2$O or directly via generation of N$_2$O in leaves. This is important as a recent study by Syakila and Kroeze (2011) indicates that the increase in atmospheric concentration of N$_2$O cannot be explained by emission inventories based on the current IPCC Guidelines, as these result in global emission estimates that are too low to explain atmospheric trends. Plants can transpire N$_2$O enriched water from the soil and mediate the release of N$_2$O into the atmosphere, with the N$_2$O being of microbial origin (Chang et al., 1998; Pihlatie et al., 2005). Indeed, in flooded rice paddy soils up to 87% of the emitted N$_2$O can be through plants rather than the soil surface (Yan et al., 2000). Plants may also release N$_2$O produced from NO$_3$ reduction and assimilation in leaves during photosynthesis (Dean and Harper, 1986; Smart...
and Bloom, 2001; Hakata et al., 2003), which is important as shoots can account for 55% of the whole-plant NO$_3$ assimilation over the entire day (Cen and Layzell, 2003). Both of these pathways of plant N$_2$O release would be dependent on sunlight due to the nature of the processes; however, the two types of plant N$_2$O release are yet unaccounted for in global N$_2$O budgets (Solomon et al., 2007). Thus, assessment of ecosystem emissions of N$_2$O ought to include direct sunlight as a potential stimulation of plant-mediated N$_2$O release via transpiration and N$_2$O produced from NO$_3$ reduction and assimilation in leaves during photosynthesis, as well as the indirect sunlight effect on microbial activity via temperature increases. As pointed out by Chen et al. (2002), chamber based measurements of ecosystem N$_2$O emission is underestimated when plants are not included.

Lately, a number of traces gasses are reported to be released by plants in response to UV-radiation, such as carbon monoxide (CO) (Derendorp et al., 2011a, b; Bruhn et al., 2013), methane (CH$_4$) (e.g. Keppler et al., 2006; Vigano et al., 2008; Bruhn et al. 2009, 2012, 2014), but also N-species such as mono-nitrogen oxides (NO$_x$) (Hari et al. 2003). These observations raise the question of whether UV-radiation may also induce emission of the important greenhouse gas, N$_2$O, from surfaces in terrestrial vegetation, which is addressed in the current study.

Hu et al. (2010) reported a 33–42% reduction in measured soil-soybean ecosystem N$_2$O emission rate in response to an artificial 20% increase in UV-B radiation during the growing season. This decrease in soil-soybean ecosystem N$_2$O emission in response to long-term enhanced UV-B radiation was ascribed to i) inhibition of plant growth, ii) potential decrease in foliar NO$_3$ reductase activity, and iii) reduced soil microbial activity (Hu et al., 2010). However, in their study Hu et al. (2010) compared N$_2$O emission rates in the dark of study objects with different pretreatments of UV-radiation and thereby addressed the indirect effect of UV-radiation.

In contrast, the current study addresses the direct relationship between UV-radiation and N$_2$O emission by deployment of UV-radiation transparent chambers and vials in combination with controlled levels of UV-radiation and ambient solar UV. We performed field site in situ measurements to investigate N$_2$O atmospheric exchange from grass vegetation exposed to solar irradiance with and without UV-screening. Laboratory tests were conducted to study the controls of UV-induced N$_2$O emission from plants.

The research questions of our study were:

i) Does natural solar UV-radiation directly affect N$_2$O emission at the ecosystem level?
ii) Is there interspecific variation in plant UV-induced N$_2$O emission?
iii) Does plant N$_2$O emission depend on irradiance intensity and wavelength, and is there a potential interaction with temperature?
iv) What are the possible loci and mechanism behind UV-induced N$_2$O emission?

2. Materials and methods

2.1. Field site description

Ecosystem-atmosphere N$_2$O exchange measurements in situ on natural vegetation and under ambient UV-B conditions, were conducted in September and October 2011 at DTU Risø campus (55°44’ N, 12°05’ E). We conducted these measurements in a grass covered location, adjacent to the laboratory (dominated by Deschampsia flexuosa and with minor occurrence of Achillea millefolium and Plantago lanceolata). The soil was a sandy loam; the location was not subject to fertilization or other chemical treatment.

2.2. Field site measurements setup

A UV-transparent acrylic plastic chamber (45 × 45 × 25 cm$^3$) was placed in a water filled groove on top of a 10 cm high metal collar pushed 5 cm into the ground. The chamber headspace temperature was continuously monitored and air was mixed by a fan. The average temperature increase in the chamber was 2.98 °C with standard error of 1.29 °C. The potential emission of N$_2$O from the acrylic plastic chamber per se was characterized in the laboratory (Fig. 1a), and this background contribution was subtracted from all in situ observations.

2.3. Field site irradiation treatments

For the exclusion of solar UV-radiation, a larger UV-filtering chamber transmitting only 30% UV-B but 95% PAR was mounted around the acrylic plastic chamber. Irradiance was measured adjacent to the chamber with a PAR sensor (400–700 nm; LI-250A Light Meter, LI-COR, Lincoln, Nebraska USA) and a UV-B detector (280–315 nm; UV-1102, Gigahertz-Optik GmbH, Tükenfeld, Germany). These irradiance measurements were adjusted according to transmittance of the chamber material. This was verified also after each measurement of gas exchange.

2.4. Leaf material

Fresh leaf material was collected within 30–60 min before any experiment from well watered potted plants grown in the greenhouse (Asplenium nidus, Brassica oleracea capitata f. alba, Brassica juncea cv. Columbo, Ficus elastic, Zea mays), from trees (Acer platanoides, Corylus avellana) and cut vegetation from the ecosystem grass vegetation.

2.5. Laboratory setup

A laboratory scale experiment was set up using a temperature controlled (Peltier technology) and well mixed leaf scale leaf chamber (0.32 l) with a UV-transparent quartz-glass lid (3010-GWK1, Heinz Walz GmbH, Effeltrich, Germany). The emission of N$_2$O from the leaf chamber per se was characterized at temperatures from 5 to 45 °C using an empty chamber (example for 25 °C is given in Fig. 1b). The temperature specific background emissions have been subtracted from all reported N$_2$O emission rates.

2.6. Laboratory irradiance treatments

For dark measurements the entire lid was covered with layers of black cloth. For light measurements a cover with a well-defined aperture was mounted on the lid to ensure that only the sample of interest inside the chamber was exposed to radiation. For exposure to natural sunlight, the chamber was placed on a table outside the laboratory. A Plexiglas UV-filter transmitting 17% UV-B and 91% PAR was used to examine the UV-effect. For artificial UV exposure, lamps were positioned at varying distances to the lid to obtain different intensities (Bruhn et al., 2009). The lamps were fitted with UV-B (PL-S 9W/01 2 P 1 CT, Philips, Eindhoven, The Netherlands) or UV-A (PL-S 9W/10/2P UNP, Philips, Eindhoven, The Netherlands) tubes. The spectral distribution of the tubes was measured with a UV spectrometer (detector range: 190–520 nm; USB4000 Miniature Fiber Optic UV Spectrometer, Ocean optics, Inc. Dunedin, Florida, USA). The UV spectrometer was calibrated against a 200 W quartz halogen lamp by the use of an Optical radiation
calibrator (model 1800-02, LI-COR Environmental, Lincoln, Nebraska USA). The quartz halogen lamp has been calibrated against reference standard lamps traceable to the U.S. National Institute of Standards and Technology (NIST). The fluorescent tubes (UV-A & UV-B) were designed by Philips for medical purposes to treat humans suffering from e.g. psoriasis. These lamps do not produce UV-C and as a consequence it was not necessary to use UV-C filters e.g. cellulose diacetate. This was also confirmed by measurements (Bruhn et al., 2009). For PAR exposure, an LED lamp (Model HL-BL_150-D 150W Color 5000–7000 K, Hesalight, Roskilde, Denmark) emitting no UV-light was used.

2.7. Additional laboratory treatments

The effect of leaf excision was examined by comparing UV-induced N₂O emission from attached leaves with those excised.

Also, the effect of drying was examined by comparing UV-induced N₂O emission from leaves before and after 24 h oven drying at 70 °C.

Ammonium-nitrate (NH₄NO₃) was applied to the surface of B. oleracea leaves in single doses by spraying them with a 2 ml solution, resulting in 0.5 g NH₄NO₃ m⁻² or 7.2 kg NH₄NO₃ m⁻². Leaves were let to dry 5 min prior to incubations.

Carbon monoxide (CO) was scrubbed from the atmosphere of the air entering the leaf chamber with Schütze reagent in a sub-cabinet, resulting in 0.5 g NH₄NO₃ m⁻². This was also confirmed by measurements (Bruhn et al., 2009). For PAR exposure, an LED lamp (Model HL-BL_150-D 150W Color 5000–7000 K, Hesalight, Roskilde, Denmark) emitting no UV-light was used.

2.8. Analysis of N₂O

Real-time measurements of N₂O concentrations, corrected for H₂O interference, were conducted by off-axis enhanced cavity spectroscopy by a N₂O/CO analyser (Los Gatos Research Inc, Mountain View, CA, USA) connected in closed-loop to either the ecosystem chamber or the leaf chamber. Air flow in the closed loop was provided by the internal pump in the N₂O/CO analyzer at a nominal flow of 3 l min⁻¹. The exchange of N₂O between the surface and atmosphere was calculated based on the changes in chamber N₂O concentration. Preliminary tests demonstrated a constant slope of traces within 1 min of initiating a new treatment, which lasted for at least 2 h. Therefore steady N₂O concentration changes (R² > 0.95) within time-windows of 5–15 min (see Fig. 1 for examples of this) were used to derive rates from linear integrations of several replicates. We used the N₂O/CO analyser in the low flow configuration with a low flow rate of 3.3 l min⁻¹, 55 cm³ s⁻¹. The N₂O/CO analyser internal volume was 411 cm³. The tubing connecting the LGR to the chamber (leaf/ecosystem) was a 6 mm outer diameter, 3 mm inner diameter polyethylene coated aluminum Synflex™ tubing (Eaton Synflex®, Eaton Denmark, Seborg, DK). Total tubing length was 6.2 m, split into 3.2 m at the inlet side and 3.0 m at the outlet side.

2.9. Exchange of N₂O by leaf wax

Samples of wax (0.002–0.01 g) were removed gently from B. oleracea capitata f. alba leaf surfaces using a scalpel and placed in UV-transparent (93% for UV-A; 61% for UV-B) 4 ml glass vials fitted with septum caps and then placed in temperature controlled growth cabinets and exposed to 17 W m⁻² of UV-B (309–314 nm) for 331 h. The vials were purged with ambient air, pure nitrogen gas (N₂), or helium (He), to test the dependency on atmospheric composition. The N₂O development in vials was detected directly by gas chromatographic analysis of the headspace. All data are corrected for background emissions in blank vials exposed to similar treatments as ([Produced N₂O in vials with wax] – [produced N₂O in empty vials])/time (Bruhn et al., 2009).

2.10. ¹⁵N:¹⁴N isotope ratio of emitted N₂O

The ¹⁵N:¹⁴N isotope ratio was measured in N₂O emitted from B. juncea cv. Columbus plants that had been grown in the greenhouse with the use of ¹⁵N-enriched ¹⁵NH₄NO₃ fertilizer resulting in plant ¹⁵N content of 4.275 atom% excess (APE), i.e. the enrichment of ¹⁵N in excess to the natural abundance level (0.3663%). During incubations with ¹⁵N-enriched plant material a 120-ml top-crimped serum bottle sealed with a butyl rubber cover was connected in serial to the closed gas-loop connecting the N₂O/CO analyzer and leaf chamber. The headspace aliquot collected in the serum bottle was subsequently analyzed for ¹⁵N-content in the N₂O by continuous flow isotope-ratio-mass-spectrometry (IRMS) upon cryotrapping of the N₂O present in the 120 ml samples (PreCon with
DeltaPLUS IRMS, Thermo Scientific, Bremen, Germany). A laboratory air sample was collected at the beginning of the incubation to quantify starting conditions. The $^{15}$N isotopic excess enrichment of the developed $\text{N}_2\text{O}$ ($\text{APE}_{\text{N}_2\text{O}}$) was assessed by estimation of the $Y$-interception point of the linear regression between the $^{15}$N-$\text{N}_2\text{O}$ atom% in headspace ($D$) on the reciprocal headspace $\text{N}_2\text{O}$ concentration ($C$) according to the equation:

$$\text{APE}_{\text{N}_2\text{O}} = C_1 \times \left( \frac{(D_2 - D_1)}{(C_1 - C_2)} \right) + D_1,$$

where 1 and 2 denote values at starting and ending points, respectively.

Plant derived $\text{N}_2\text{O}$ ($\text{N}_2\text{O}\text{-Od}, \text{Od}$) was calculated as the proportion between $\text{APE}_{\text{N}_2\text{O}}$ and the APE of the plant material (4.275%) assuming that any $\text{N}_2\text{O}$ produced from other sources contained $^{15}$N at the natural abundance level:

$$\text{N}_2\text{O}\text{-Od} = \left( \frac{\text{APE}_{\text{N}_2\text{O}}}{4.275} \right) \times 100\%$$

The incubation with $^{15}$N enriched plant material was carried out in triple or quadruplicate with typical exposure times between 0.4 and 1.5 h. One set of samples ($n = 4$) was exposed to UV-B, and one set ($n = 3$) to darkness. As working standard for the $^{15}$N quantification in $\text{N}_2\text{O}$ we used pure $\text{N}_2\text{O}$ gas that had been isotopically calibrated against atmospheric air. The precision for analysis of $^{15}$N in $\text{N}_2\text{O}$ at near-ambient concentrations was 0.0003 atom%.

2.11. Statistical analysis

Analyses of variance were calculated in SAS (SAS Institute Inc. 2004, version 9.1) using Proc Mixed to test for the effect difference to dark values.

### 3. Results

#### 3.1. Natural solar UV-radiation effects on $\text{N}_2\text{O}$ emission at the ecosystem level

We investigated the effect of natural solar UV-radiation on $\text{in situ}$ $\text{N}_2\text{O}$ emission from a grassland (dominated by D. flexuosa and with minor occurrence of A. millefolium and P. lanceolata) by altering the light regime at random order between darkness, full sunlight, and sunlight with natural UV-radiation screened (see Fig. 1a for an example). In darkness, the grassland emitted 104 ± 9 nmol m$^{-2}$ h$^{-1}$ (Fig. 2). In full sunlight, the grassland $\text{N}_2\text{O}$ emission increased by c. 30% to 135 ± 9 nmol m$^{-2}$ h$^{-1}$ (Fig. 2). However, when the UV-radiation was screened off and the vegetation thus only received visible light (PAR), the $\text{N}_2\text{O}$ emission decreased to rates similar to those measured in the dark (Fig. 2). Thus, the effect of solar UV-radiation on $\text{in situ}$ grass field $\text{N}_2\text{O}$ emission was 26 ± 5 nmol m$^{-2}$ h$^{-1}$ (Fig. 2).

As the UV-filtering field chamber indeed did transmit 32% UV-B, the $\text{N}_2\text{O}$ emission from the natural grass field in response to only natural solar PAR was estimated by calculating the value at 0% UV assuming a linear extrapolation (Fig. 3a) of the relationship between the data points (100% PAR & 32% UV, 109 ± 9 nmol $\text{N}_2\text{O}$ m$^{-2}$ h$^{-1}$) and (100% PAR & 100% UV, 135 ± 9 nmol $\text{N}_2\text{O}$ m$^{-2}$ h$^{-1}$). This resulted in a calculated value of 96 nmol $\text{N}_2\text{O}$ m$^{-2}$ h$^{-1}$. Thus the actual effect of solar UV-radiation on $\text{in situ}$ grass field $\text{N}_2\text{O}$ emission was 34 ± 6 nmol m$^{-2}$ h$^{-1}$.

#### 3.2. Natural solar UV-radiation effects on $\text{N}_2\text{O}$ emission from leaves

We also investigated the effect of natural solar UV-radiation on $\text{N}_2\text{O}$ emission from cut vegetation from grass field (see Fig. 1b for an example) in a similar fashion to that for $\text{in situ}$ measurements. In darkness, the cut grass field vegetation emitted 18.5 ± 2.8 nmol m$^{-2}$ h$^{-1}$ (Fig. 2). In full sunlight, the leaf $\text{N}_2\text{O}$ emission increased by c. 262% to 67.0 ± 4.7 nmol m$^{-2}$ h$^{-1}$ (Fig. 2). Again, when the UV-radiation was screened off and the leaves thus only received PAR, the $\text{N}_2\text{O}$ emission decreased to rates similar to those measured in the dark (Fig. 2). Thus, the effect of solar UV-radiation on grass field leaf $\text{N}_2\text{O}$ emission was 48.5 nmol m$^{-2}$ h$^{-1}$ (Fig. 2).

We measured rates of leaf $\text{N}_2\text{O}$ emission in response to natural sunlight in another five species. The leaf $\text{N}_2\text{O}$ emission rates varied from 18.5 ± 4.7 nmol m$^{-2}$ h$^{-1}$ by F. elastica to 48.5 ± 21.5 nmol m$^{-2}$ h$^{-1}$ in cut vegetation from the grass field (Table 1). However, an ANOVA showed no significant effect of species on the leaf $\text{N}_2\text{O}$ emission in response to natural sunlight ($P = 0.1066$).

#### 3.3. Dependency of light-induced $\text{N}_2\text{O}$ emission on irradiance intensity and wavelength, and the interaction with temperature

The $\text{N}_2\text{O}$ emission rates of B. oleracea leaves increased with increasing intensities of UV-B (Fig. 3a), UV-A (3b), and PAR (3c). The irradiance intensity dependence of leaf $\text{N}_2\text{O}$ emission rates was linear for UV-B (Fig. 3a) and UV-A (Fig. 3b), whereas for PAR it was curvilinear (Fig. 3c). T-tests were used to test the differences of emission rate (In-transformed) between irradiance dose (W m$^{-2}$) of PAR, UVB and UVA. The slopes did not differ significantly between UV-B and UV-A ($p = 0.76$), whereas the slopes in both UV-B and UV-A tended to differ from PAR ($p = 0.053$ and $p = 0.062$, respectively).

In darkness, all the nine different species including cut vegetation emitted $\text{N}_2\text{O}$ with a mean value of 8.6 ± 5.6 nmol m$^{-2}$ h$^{-1}$ (mean ± SE, Table 1). Relative to their corresponding dark value the
examined species emitted N₂O at rates on average 1209% (n = 9) higher in response to artificial UV-B and 662% (n = 7) in response to natural sunlight incl. UV (Table 1). Relative to their corresponding values of N₂O emission rate in artificial UV-B the examined species emitted 22% (n = 2) in response to artificial UV-A, 10% (n = 2) in response to PAR, and 56% in response to natural sunlight incl. UV (n = 6) (Table 1).

Both the UV-induced and light independent (darkness) N₂O emissions increased with increasing temperatures (Fig. 3d). The calculated activation energies (Eₐ) for N₂O emissions was in darkness 68.6 kJ mol⁻¹ (mean of the two species) and in UV-B irradiation 62.9 kJ mol⁻¹ (mean of the two species), thus very similar. T-tests were used to test whether slopes of temperature response curves differed (ln-transformed). Dark slopes did not differ from each other (p = 0.26) and UV-B slopes did not differ from each other (p = 0.25), however the slopes in dark differed from the slopes in UV-B (p = 0.004).

3.4. Possible loci and mechanism behind UV-induced N₂O emission

Incubation of ¹⁵N-enriched leaves in the dark revealed a N₂O concentration increment of 2.2 ppb h⁻¹ and reaching 0.367 atom% ¹⁵N in the N₂O pool indicated that the emitted N₂O had a ¹⁵N atom% excess (APE) of 0.107 (Table 3). For ¹⁵N plant material exposed to UV-B N₂O was emitted at 9.3 ppb h⁻¹ reaching a final ¹⁵N atom% of 0.372 indicating an APE of 0.736 in emitted N₂O. Compared with
the 4.64 atom% $^{15}$N concentration in the leaf material, these observations suggest that 17.2% of the emitted N$_2$O in UV-B was derived from the N in the leaf material and in darkness 2.5% was derived from the leaf N (Table 3). Assuming that the leaf N$_2$O emission rate in darkness (11 nmol m$^{-2}$ h$^{-1}$; Table 1) was not inhibited during UV-B exposure and thus constitute the same derived from the leaf N ($^{15}$N concentration in the leaf material, these observations suggest that 17.2% of the emitted N$_2$O in UV-B was derived from the N in the leaf material and in darkness 2.5% was derived from the leaf N. The emission rates (unit is nmol N$_2$O m$^{-2}$ h$^{-1}$) were measured with a temperature controlled chamber at 25 °C. Not determined is shown by ‘nd’.

Table 1 Leaf N$_2$O emissions from different species in darkness and under different light conditions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dark</th>
<th>UV-B</th>
<th>UV-A</th>
<th>PAR</th>
<th>Sun incl UV</th>
<th>Sun excl UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± se</td>
<td>Mean ± se</td>
<td>Mean ± se</td>
<td>Mean ± se</td>
<td>Mean ± se</td>
<td>Mean ± se</td>
<td>Mean ± se</td>
</tr>
<tr>
<td>Acer platanoides</td>
<td>3.5 ± 2.2</td>
<td>10</td>
<td>81.1 ± 3.5</td>
<td>5</td>
<td>nd</td>
<td>40.1 ± 17.1</td>
</tr>
<tr>
<td>Aspidistra eliator</td>
<td>14.1 ± 2.7</td>
<td>18</td>
<td>101.7 ± 5.5</td>
<td>6</td>
<td>6.6 ± 5.6</td>
<td>6</td>
</tr>
<tr>
<td>Asplenium nidus</td>
<td>9.8 ± 3.4</td>
<td>10</td>
<td>76.3 ± 15.3</td>
<td>10</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Ficus elastica</td>
<td>2.0 ± 1.1</td>
<td>10</td>
<td>43.7 ± 12.1</td>
<td>3</td>
<td>nd</td>
<td>18.5 ± 4.7</td>
</tr>
<tr>
<td>Corylus avellana</td>
<td>7.2 ± 1.9</td>
<td>11</td>
<td>38.1 ± 5.7</td>
<td>6</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Brassica juncea cv. columbo</td>
<td>11.0 ± 6.2</td>
<td>4</td>
<td>34.1 ± 5.9</td>
<td>4</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Brassica oleracea capitata f. alba</td>
<td>4.1 ± 1.1</td>
<td>44</td>
<td>87.3 ± 14.2</td>
<td>21</td>
<td>32.0 ± 6.9</td>
<td>6</td>
</tr>
<tr>
<td>Zea mays</td>
<td>6.5 ± 2.6</td>
<td>9</td>
<td>110.5 ± 4.4</td>
<td>4</td>
<td>nd</td>
<td>40.7 ± 11.5</td>
</tr>
<tr>
<td>Cut vegetation</td>
<td>19.3 ± 1.3</td>
<td>8</td>
<td>40.8 ± 7.6</td>
<td>4</td>
<td>nd</td>
<td>48.5 ± 21.5</td>
</tr>
</tbody>
</table>

The emission rates (unit is nmol N$_2$O m$^{-2}$ h$^{-1}$) were measured with a temperature controlled chamber at 25 °C. Not determined is shown by ‘nd’.

Table 2 Leaf N$_2$O emissions from different species in response to different pretreatments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dark</th>
<th>UV-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± se</td>
<td>Mean ± se</td>
<td></td>
</tr>
<tr>
<td>A. eliator</td>
<td>23.6 ± 5.7</td>
<td>6</td>
</tr>
<tr>
<td>Attached</td>
<td>20.4 ± 6.9</td>
<td>6</td>
</tr>
<tr>
<td>A. nidus</td>
<td>9.8 ± 3.4</td>
<td>10</td>
</tr>
<tr>
<td>Detached</td>
<td>−0.1 ± 1.0</td>
<td>4</td>
</tr>
<tr>
<td>B. oleracea</td>
<td>8.8 ± 1.9</td>
<td>10</td>
</tr>
<tr>
<td>Detached</td>
<td>2.8 ± 1.4</td>
<td>5</td>
</tr>
<tr>
<td>B. oleracea</td>
<td>1.6 ± 5.0</td>
<td>6</td>
</tr>
<tr>
<td>Fresh</td>
<td>−0.9 ± 4.0</td>
<td>6</td>
</tr>
<tr>
<td>B. oleracea +NH$_4$NO$_3$</td>
<td>nd</td>
<td>146.4 ± 6.8</td>
</tr>
<tr>
<td>[CO] &lt; 10 ppb</td>
<td>nd</td>
<td>226.1 ± 17.9</td>
</tr>
<tr>
<td>B. oleracea +NH$_4$NO$_3$ (7.2 kg m$^{-2}$)</td>
<td>590</td>
<td>1</td>
</tr>
<tr>
<td>B. oleracea No NH$_4$NO$_3$</td>
<td>nd</td>
<td>27.6 ± 6.0</td>
</tr>
<tr>
<td>+0.5 g m$^{-2}$ NH$_4$NO$_3$</td>
<td>nd</td>
<td>32.4 ± 4.5</td>
</tr>
<tr>
<td>F. elastica</td>
<td>nd</td>
<td>19.9 ± 3.4</td>
</tr>
<tr>
<td>No NH$_4$NO$_3$</td>
<td>nd</td>
<td>18.9 ± 1.9</td>
</tr>
<tr>
<td>+0.5 g m$^{-2}$ NH$_4$NO$_3$</td>
<td>nd</td>
<td>32.4 ± 4.5</td>
</tr>
</tbody>
</table>

The emission rates (unit is nmol N$_2$O m$^{-2}$ h$^{-1}$) were measured with a temperature controlled chamber at 25 °C. Not determined is shown by ‘nd’.

4. Discussion

4.1. Natural solar UV-radiation effects on N$_2$O emission at the ecosystem level

The N$_2$O emission rate measured in the grassland in darkness was of the same magnitude as other European grasslands, which are not intensively fertilized and grazed (Skiba et al., 2009). Therefore, we do not suspect any significant problems with our setup resulting in artificially high rates of N$_2$O emission from the soil due to artificial pressure changes and/or gradients. Importantly, any such problems would not affect the conclusions regarding the effect of UV irradiation on the ecosystem.
During ecosystem measurements, the average temperature increase in the chamber was 2.98 °C with standard error of 1.29 °C. Nonetheless, the changes in rates of ecosystem N₂O emission (Fig. 1a) are unlikely to be temperature responses but rather UV-responses. The reasons for this are i) the rate changes are abrupt, ii) the rate changes are stable (linear increase) almost immediately upon change in irradiance regime, iii) the rate increase when switching from darkness to sun with UV is matched by an almost similar decrease upon change to sun without UV, when temperature keeps rising, iv) the temperature sensitivity of leaf N₂O emission rates are very low (Fig. 3c), and v) most importantly, the ecosystem rate changes in response to change in irradiance regime (Fig. 1a) are very similar to those at leaf level (Fig. 1b) when cuvette temperature was maintained constant during measurements.

It is concluded that radiation in the UV-range contributed significantly to the stimulation of N₂O emission under sunlight conditions. Such UV-induced N₂O emission from plant surfaces is hitherto unrecognized (Solomon et al., 2007). For chamber based N₂O exchange experiments in vegetated systems it is therefore important to use UV-transparent materials rather than opaque chambers.

4.2. Natural solar UV-radiation effects on N₂O emission from leaves

The effect of natural solar UV-radiation on N₂O emission from the cut vegetation from the grass field (Fig. 1b and Fig. 2) could account for the observed effect of natural solar UV-radiation the grass field in situ measurements. Further, we measured rates of leaf N₂O emission in response to natural sunlight in another five species and found that the interspecific variation was low as earlier found for leaf CO emission (Bruhn et al., 2013). Thus, we expect leaf N₂O emission in response to natural sunlight including UV to be a common phenomenon of terrestrial vegetation globally. Further, although we found no significant interspecific variation in emission rates, there is a trend of such, which ought to be examined in more detail in future studies.

4.3. Dependency of light-induced N₂O emission on irradiance intensity and wavelength

The rate of N₂O emission increased with increasing irradiance intensity (Fig. 3a–c), a phenomenon also known for plant emission of CH₄ (McLeod et al., 2008; Vigano et al., 2008; Bruhn et al., 2009) and CO (Bruhn et al., 2013) in response to UV-radiation. For PAR and UV-A treatments it was easy to obtain varying irradiance intensities within levels of light at the Earth’s surface by varying the distance between lamps and cuvette. However, this was difficult to obtain accurately with the UV-B lamps. Instead, we chose to obtain many, albeit high, levels of accurate UV-B intensities and interpolate to measurements in darkness (Fig. 3a) in order to investigate the dependency on irradiance intensity. Given the linear response of N₂O emission to varying UV-B intensities (Fig. 3a), we chose for subsequent screenings (Tables 1 and 2) to apply high intensities in order to increase signal-to-noise ratio when examining potential effects of other treatments. The lamp spectra were tested previously and no UV-C was present. Similarity in N₂O emission rate changes in response to changes in irradiance regimes between use of UV-B lamps and natural sunlight (Fig. 1b) further indicates that the observations were not artefacts caused by UV-C.

We found that when screening off the UV component from natural sunlight there appeared to be no contribution to leaf N₂O emission from the natural PAR component (Figs. 1b and 2 & Table 1) in the cut vegetation (dominated by D. flexuosa and with minor occurrence of A. millefolium and D. lanceolata). This indicates that under natural sunlight conditions most of the photo-induced N₂O was caused by UV rather than PAR. Natural levels of light at the Earth’s surface e.g. in the tropics only reach ca. 2 W m⁻² UV-B, but can reach 45 W m⁻² UV-A and 2000 μmol m⁻² PAR. Thus it seems likely that in many environments photo-induced leaf N₂O emission will be caused more by the lower energy wavelengths, UV-A, than by UV-B. The same phenomenon was found for leaf CO emission (Bruhn et al., 2013). Further, in some species, e.g. B. oleracea, even PAR might cause substantial N₂O emission (Fig. 3, Table 2). The crude action spectrum, which can be deduced from Fig. 3a–c indicates that at common intensities, the leaf N₂O emission is highest for UV-A, followed by UV-B, and less so for PAR. This observation is in contrast to what is known for e.g. photo-induced CH₄ by purified pectin (Bruhn et al., 2009). Further, given the possibility of a curvilinear response function observed for UV-A light (Fig. 3b) indicates that at canopy level, the effect of UV-A reaches further into the canopy than an effective leaf area index of unity. Again, this contradicts what has been assumed for scaling of UV-induced CH₄ emission from vegetation (Bloom et al., 2010).

More detailed studies in the future into the relative contribution of each wavelength are needed in order to model global current and future N₂O emissions. This is because UV-A:UV-B ratios change on a temporal scale (Dring et al., 2001) and further changes are predicted to occur due to ozone depletion.

4.4. Possible loci and mechanism behind UV-induced N₂O emission

Any potential N₂O emissions following the route of transpiration or physiological processes were not significantly mediated by UV-B exposure as there were no differences in N₂O emission rates between fresh and oven dry material exposed to UV-B (Table 2). Also, across all examined plant species there was no significant difference in light induced N₂O emission rate between leaves still attached to plants and excised leaves (Table 2). Together with the observation of relatively high activation energies for N₂O emissions (Fig. 3d) an underlying abiotic process is implied (Obermeyer and Tyerman, 2005). Indeed, we are not aware of any enzymatic process directly stimulated by UV-irradiation. The δ¹⁵N:N₂O isotopic signature in N₂O emitted from leaves of ¹⁵N-enriched plants may suggest that the substrate for plant emitted N₂O in response to UV-irradiation is both of a plant origin and from other sources.
There are photolytic studies, which lend support to UV-induced N$_2$O production from both plant origin and from other sources. In solution NO$_3^-$ can result in NO$_2$ upon UV-irradiation (305 nm) (Goldstein and Rabani, 2007; Schuttlefield et al., 2008). This can lead to heterogeneous formation of N$_2$O from the hydrolysis of gas-phase NO$_2$ via HONO on acidic and oxide surfaces (Kleffmann et al., 1998). Leaf NO$_3^-$ is typically $^{15}$N enriched (Gauthier et al., 2013), which somewhat contrast our $^{15}$N–N$_2$O measurements. However, the leaf NO$_3^-$–$^{15}$N enrichment may be counteracted by the wavelength-dependent discrimination against $^{15}$N in NO$_3^-$ photolysis by UV irradiation (Frey et al., 2009). Another potential plant originated N-source might be alkaloids, which typically are $^{15}$N depleted (Gauthier et al., 2013) and absorb UV-irradiation. It remains to be investigated whether diazotrophic entophytes perhaps contribute via production of alkaloids.

Other sources of UV-induced leaf N$_2$O emission may be photo-desorption of N$_2$O per se from the surface and photolytic production of N$_2$O from different surface bound N-species. Photodesorption of N$_2$O from aerosol surfaces is a known phenomenon at wavelengths below 300 nm, i.e. in the UV-B spectrum (Kim et al., 2010). Further, UV-induced N$_2$O production from aerosol bound NO$_3^-$ (Rubasinghege and Grassian, 2009) and NH$_4$NO$_3$ (Rubasinghege et al., 2011) is known to occur within the UV-spectrum at Earth’s surface. We confirmed the potential of this process when NH$_4$NO$_3$ was applied at high surface concentrations to leaves (Table 2).

As UV-radiation, in particular UV-B, in some species is almost completely screened off at the leaf epidermis (Cen and Bornman, 1993), it can be speculated that N$_2$O might be produced by surface photolytic reactions at the boundary between epicuticular leaf wax and atmosphere. Indeed, we observed N$_2$O emissions from leaf surface wax exposed to UV-radiation. The reduced N$_2$O emissions under oxygen-free conditions may suggest the involvement of oxygen radicals in the UV-induced N$_2$O formation. The positive dependence on the presence of oxygen and/or oxygen radicals appears to be common in UV driven photolytic formation of N$_2$O (Prasad, 2002; Prasad and Zipf, 2008; Rubasinghege and Grassian, 2009). Also, the fact that scrubbing the CO in the air to below 10 ppb resulted in much increased rates of UV-induced N$_2$O emission rates (Table 2) demonstrates a strong interaction with the chemical properties of the air-medium per se.

We speculate that relative humidity (RH) of the air in the chamber may in part have been a reason for variance in values within one species. We did not control RH during measurements to a discrete value, but rather to a range between 40% and 80%. This may potentially have influenced the measured rates as Rubasinghege and Grassian (2009) found that the relative ratio and product yield of NO$_2$, NO, and N$_2$O changes with RH upon UV-radiation of NO$_3$ on aerosols. Future experiments are needed to clarify this.

Altogether, our findings and those of others imply the occurrence of multiple loci and processes responsible for the UV-induced N$_2$O emitted from leaf surfaces. Importantly, the measured rates of UV-induced plant surface N$_2$O emission from a) grassland ecosystem and b) cut vegetation presented in Figs. 1 and 2 are indeed representative of natural rather than special measurement conditions. This is because these rates were obtained by simply subtracting dark + PAR emission from the emission naturally occurring, i.e. without any surface-deposited NH$_4$NO$_3$ caused by the experimental/measurement conditions. The rates presented in Figs. 1 and 2 are therefore to be expected to occur for long periods in situ as the grassland ecosystem investigated naturally receive UV-irradiation without an exhaustion of vegetation surface deposited N-pool. However, as we did find indications from isotopic studies that only 26% of the UV-induced N$_2$O emission originated from intrinsic N, we do speculate that background surface-deposited NH$_4$NO$_3$ may be a substantial part of the source to UV-induced N$_2$O emission. Therefore, there is a need for future work in order for an understanding of the kinetics of a potential exhaustion of a surface deposited N-pool.

4.5. UV-induced N$_2$O emission in a global perspective

A recent study by Syakila and Kroze (2011) indicates that there seems to be a missing N$_2$O source in the IPCC Guidelines. A part of this missing source may be the not yet quantified role of plants in N$_2$O emission either indirectly as conduits of soil derived N$_2$O or directly via generation of N$_2$O in leaves from NO$_3^-$ reduction in leaves during photosynthesis as well as the newly discovered source of UV-induced N$_2$O emission demonstrated in the current study.

Chen et al. (2002) pointed out that chamber based measurements of ecosystem N$_2$O emission is underestimated when plants are not included. We found that natural UV irradiation caused the ecosystem N$_2$O emission to be c. 30% higher than otherwise assumed using darkened chambers as per usual. This discovery of a significant emission of N$_2$O in response to UV irradiation from terrestrial ecosystems has an important value for current understanding of the global N$_2$O budget.

It can be speculated that a photolytic conversion of mineral nitrogen on the vegetation surfaces may occupy a significant role in periods/areas of fertilization, suggesting a link between UV induced N$_2$O emissions and natural- and anthropogenic N-sources which may be affected by land management, crop selection, husbandry practices, and associated dry-wet depositions. Careful investigations are needed to reveal this further.

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