Differential effects of environmental stressors on physiological processes and methane emissions in pea (Pisum sativum) plants at various growth stages

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**Abstract**

Many studies have investigated the effects of one or two environmental factors on methane (CH₄) emissions from plants at a single growth stage, but the impact that multiple co-occurring stress factors may have on emissions at different growth stages has rarely been studied. The objective of this study was to examine the effects of temperature, ultraviolet-B (UVB) radiation, and watering regime on CH₄ emissions and some relevant physiological characteristics of pea (Pisum sativum L. cv. 237 J Sundance) plants at three growth stages. We grew plants under two temperature regimes (22/18 °C and 28/24 °C; 16 h light/8 h dark), two UVB levels [0 and 5 kJ m⁻² d⁻¹] and two watering regimes (well-watered, watering plants to field capacity, and water-stressed, watering plants at wilting point). Measurements were then taken after 10, 20, and 30 days of growth under experimental conditions, following seven days of initial growth under 22/18 °C. Higher temperatures, UVBS, and water stress adversely affected photosynthesis and chlorophyll fluorescence, but increased CH₄ emissions, which decreased with increased plant age. Also, interaction of higher temperatures and UVBS reversed the pattern of CH₄ emissions at growth stages, compared to that of other treatments. We conclude that CH₄ emission decreases with plant age, and it is affected by stress factors through changes in physiological activities of plants.

1. Introduction

Methane (CH₄) is the second important anthropogenic greenhouse gas after carbon dioxide (CO₂), with a global warming potential of nearly 34 times greater than that of CO₂ (Myhre et al., 2013). Prior to 2006, the biological formation of CH₄ was attributed to the metabolic activities of microorganisms under anaerobic conditions (Reay et al., 2010). However, in 2006, Keppler et al. published their experimental results, suggesting that plants can also produce and emit CH₄ under aerobic conditions with non-microbial origins, and opened the door for further studies of the subject. Although initially this finding was met with skepticism (e.g., Dueck et al., 2007), evidence has increased substantially in support of this phenomenon (see Bruhn et al., 2012; Wang et al., 2013; Liu et al., 2015). For the non-microbial plant-derived CH₄, earlier studies have suggested a number of potential precursors, including methoxyl groups of plant pectins (Keppler et al., 2006; McLeod et al., 2008), cellulose and lignin (Vigano et al., 2008), leaf surface wax (Bruhn et al., 2014), and methionine (Lenhart et al., 2015).

Many studies have shown the non-microbial CH₄ formation in plants grown under different environmental conditions (see Bruhn et al., 2012; Qaderi and Reid, 2014; Martel and Qaderi, 2017, 2019). These studies confirmed the emissions of CH₄ from detached and/or attached leaves of plants (see Liu et al., 2015) that were grown under stress factors, such as high temperature (Vigano et al., 2008; Bruhn et al., 2012; Abdulmajeed et al., 2017), enhanced ultraviolet-B radiation (McLeod et al., 2008; Vigano et al., 2008; Bruhn et al., 2014; Fraser et al., 2015), and water stress (Qaderi and Reid, 2009, 2011). The effects of these factors can partially be mitigated by elevated CO₂ (Qaderi and Reid, 2011). Environmental stress factors affect plant metabolic activities, including the production of reactive oxygen species (ROS), leading to increased CH₄ emissions, although the underlying mechanism of its formation remains unknown (Liu et al., 2015; Martel and Qaderi, 2017). In earlier studies, temperature has been shown to affect CH₄ emissions from plants (see Liu et al., 2015). As temperature increases above...
the plant optimum level, it adversely affects plant morphological and physiological characteristics (Jones, 2013). Higher temperatures reduce total chlorophyll and change the ratio of chlorophyll to carotenoids, leading to decreased CO₂ assimilation (Azoulay-Shemer et al., 2015) and increased transpiration and stomatal conductance (Jones, 2013) and, in turn, to decreased plant biomass (Cui et al., 2006; Abdulmajeed et al., 2017). Higher temperatures can also shorten developmental periods of plants (Qaderi et al., 2003). Other environmental stressors, such as UVB radiation and water stress, have also been shown to increase CH₄ emissions from plants by negatively affecting plant growth (Liu et al., 2015). Enhanced UVB radiation adversely affects plant physiological processes (Day and Neale, 2002), alters chemical composition of plant cells (Reifenrath and Müller, 2007), changes leaf anatomy and thickness, modifies developmental rate (Robson et al., 2015), and results in decreased growth (Day and Neale, 2002). Plants respond differently to UVB radiation during their growth stages (Qaderi et al., 2008). Also, plant growth, metabolism and yield are negatively affected by drought (Reddy et al., 2004a), because this stress factor influences chlorophyll fluorescence and gas exchange (Xu et al., 2008; Guan et al., 2015). Since plant responses to environmental stress factors vary with developmental stage (Gray and Brady, 2016), it is logical to expect that plants during their life cycle emit different CH₄ levels.

In the past, most studies had been designed to investigate the effect of single or double environmental stress factors on CH₄ emissions from plants (see Bruhn et al., 2012; Liu et al., 2015) and, to our knowledge, none of these studies considered measuring CH₄ emissions during plant growth. It is, therefore, crucial to understand the effects of multiple factors on CH₄ emissions from plants at different growth stages. We hypothesized that higher temperature, UVB radiation and water stress adversely affect physiological processes in plants and would lead to increased CH₄ emissions, which can vary with plant growth stage. The objective of this study was to examine the combined effects of temperature, UVB radiation and watering regime on CH₄ emission rates and relevant physiological parameters at three growth stages of pea plants. This study will contribute to our understanding of CH₄ emissions during growth stages of plants exposed to multiple abiotic stressors.

2. Materials and methods

2.1. Plant material and growth conditions

In this study, we essentially used the same growth conditions that were described previously (Abdulmajeed and Qaderi, 2017). We also worked with pea (Pisum sativum L. cv. 237 J Sundance) as a model plant because in the previous studies this species had the highest CH₄ emission, compared to that of other crops (Qaderi and Reid, 2009, 2011). We planted pea seeds in pots that contained a mixture of Vermiculite, Perlite and peat moss (1:1:2, by volume). After emergence, the seedlings were kept for one week in controlled-environment growth chamber (ATC26, Conviron, Winnipeg, MB) with the following conditions: temperature regime (22/18 °C), photoperiod (16 h light/8 h dark), relative humidity (RH, about 65%), and photosynthetically active radiation (PAR, 300 μmol m⁻² s⁻¹). The seedlings were fertilized with a modified Hoagland’s solution (Zioni et al., 1971). In the chamber, the source of light was a mix of cool white fluorescent tubes (Philips Master TL-D58 W/840, Amsterdam) and incandescent lamps (Litemor, Boston, MA). We measured RH with an Oakton thermo-hygrometer (WD-35612-00, Vernon Hills, IL) and PAR (at the shoot apex) with an LI-250A quantum radiometer/photometer (LI-COR Biosciences, Lincoln, NE). Plants were randomly assigned to eight treatments with combinations of the following factors: two temperature regimes (22/18 °C and 28/24 °C, 16 h light/8 h dark), two UVB levels (0 and 5 kJ m⁻² d⁻¹), and two watering regimes (well-watered, watering plant to field capacity; and water-stressed, watering plants at wilting point). Each treatment combination had a minimum of 27 plants; of which nine plants were grown for 10 days, nine plants for 20 days, and the remaining plants for 30 days. The supplemental UVB radiation was provided by four fluorescent lamps (UVB 313 EL, Q-Panel, Cleveland, OH), after pre-burning for 96 h to stabilize their output. Then, the lamps were wrapped with two layers of cellulose dif acetate film (thickness: 0.127 mm; Grafix Plastics, Cleveland, OH) to filter radiation below 280 nm. The exact UVB radiation (5 kJ m⁻² d⁻¹) was achieved by adjusting the distance between lamps and plants. The Caldwell’s (1971) procedure was used to estimate the levels of biologically effective UVB irradiation (UVBₑ). UVBₑ was measured with a calibrated PMA2100 photometer/radiometer (Solar Light Co., Philadelphia, PA). Daily UVB radiation was between 8:00 and 14:00 h. The plants were rotated two times per week to reduce positional effects within treatments. All experiments were conducted three times.

2.2. Measurement of methane emissions

Methane emission rates were measured following a modification of the method used to assess ethylene evolution from plant tissues (Qaderi et al., 2010). From each treatment, three leaf samples of each of the three plant growth stages (17, 27, and 37 days old) were detached, placed within 3-ml syringes and flushed with CH₄-free air, and incubated for 2 h inside a growth chamber at 22 °C and 300-μmol photons m⁻² s⁻¹. Then, the CH₄ gas was collected, following our previous procedure (see Abdulmajeed et al., 2017). Before running the samples, the gas chromatography column was conditioned for 98 min. From each syringe, 1 ml of gas was collected and injected onto a Varian 3900 gas chromatograph-flame ionization detector system (GC-FID; Varian Canada, Mississauga, ON) stocked with a Carboxen 1006 PLOT capillary column (30 m × 0.53 mm ID; Supelco, Bellefonte, PA). The temperatures of injector and detector were set to 200 and 230 °C, respectively. Helium was used, as a carrier gas, at 10 ml min⁻¹. The GC-FID was programmed as following: 1 min isothermal heating at 35 °C, which increased to 225 °C at 24 °C min⁻¹. Methane eluted at about 2.6 min of the total run time of 9 min. Methane from plant samples was identified by the retention time of external standard CH₄ gas (Air Liquide, Dartmouth, NS), and quantified by using the standard curve obtained from the injection of CH₄ standard. Leaf samples were dried (60 °C for 96 h) to calculate the rates of CH₄ emission (ng g⁻¹ DM (dry mass) h⁻¹; Qaderi and Reid, 2011). In this study, plants were grown in a well-oxygenated environment; therefore, it appears most likely that we measured nonmicrobial plant-derived CH₄ emissions. Although CH₄ production by methanogens in the phyllosphere cannot be ruled out, recent studies have shown that archaea are more abundant in the rhizosphere than in the phyllosphere (see Knief et al., 2012).

2.3. Gas exchange

Gas exchange was measured with a portable 6400XT photosynthesis system (LI-COR Inc., Lincoln, NE), calibrated with 400 μmol mol⁻¹ of CO₂ at a flow rate of 400 ml s⁻¹. From each treatment, three fully-developed leaves (each from a different plant) were used for the measurement of net CO₂ assimilation (Aₛ; μmol CO₂ m⁻² s⁻¹), transpiration (E; mmol H₂O m⁻² s⁻¹), and stomatal conductance (gₛ; mol H₂O m⁻² s⁻¹). The water use efficiency (WUE; μmol CO₂ mmol⁻¹ H₂O) was calculated as Aₛ/E (Lambers et al., 2008).

2.4. Chlorophyll fluorescence

Chlorophyll fluorescence was measured with a portable Fluorpen FP 100 fluorometer (Photon Systems Instruments, Drasov). Three fully-developed leaves (each from a different plant) were selected from each treatment. The light-adapted leaves were used to measure the effective quantum yield of photosystem II (φPSII; Fᵥ/Fₘₑₐₙ), and the dark-adapted leaves (kept for 30 min in clamps) were used to measure the maximum quantum yield of PSII (Fᵥ/Fₘₑₐₙ), non-photochemical quenching (qNP; (Fₘₑₐₙ − Fᵥ)/Fₘₑₐₙ) and photochemical quenching (qP; Fᵥ/Fₘₑₐₙ) (Baker, 2008).
2.5. Nitrogen balance index, total chlorophyll, and flavonoids

Nitrogen balance index (NBI), total chlorophyll (μg cm$^{-2}$), and flavonoids (μg cm$^{-2}$) were measured with a Dualex Scientific® System (Force-A, Orsay Cedex). Three fully-developed leaves (each from a different plant) were selected for the measurements (see Martel and Qaderi, 2016).

2.6. Data analysis

Data were analyzed by means of a four-way analysis of variance (ANOVA). A repeated measures ANOVA was used to determine differences among treatments over growth stages, applying Scheffé's test at \(P < 0.05\) (SAS Institute, 2011). The relationship between CH$_4$ emission and physiological characteristics was determined by Pearson's correlation coefficient (Minitab Inc., 2014).

3. Results

3.1. Methane emissions

All stress factors – higher temperatures, UVB5 and water stress – increased CH$_4$ emissions from plants (Table 1). Although not significant, CH$_4$ emissions progressively decreased with increased plant age (Tables 1–2). Temperature, UVB radiation, watering regime, the two-way interactions of T (temperature) × G (growth stage), U (UVB radiation) × G, and W (watering regime) × G, and the three-way interaction of T × U × G significantly affected CH$_4$ emissions (Table 3). Methane emission rates in the well-watered plants grown under lower temperatures at UVB5 ranged from 24.27 ± 2.56 ng g$^{-1}$ DM h$^{-1}$ (37-day-old plants) to 50.94 ± 7.98 ng g$^{-1}$ DM h$^{-1}$ (17-day-old plants), and increased by 4.8 and 1.3 times, respectively, when plants were exposed to the combination of water stress, UVB5 and higher temperatures. Regardless of watering regime, interaction of higher temperatures and UVB5 reversed the pattern of CH$_4$ emissions at growth stages, compared to that of other treatments (Fig. 1).

3.2. Gas exchange

Net CO$_2$ assimilation (AN) was significantly higher for the 37-day-old plants than for the 17- and 27-day-old plants, indicating an increase of 7.6 and 7.7 times, respectively (Tables 1–3). The two-way interaction of U × G had significant effects on AN (Table 3). AN was highest in the 37-day-old plants grown at UVB5 (80.44 ± 14.63 μmol CO$_2$ m$^{-2}$ s$^{-1}$), but lowest in the 17-day-old plants grown at the same UVB level (68.28 ± 6.22 μmol CO$_2$ m$^{-2}$ s$^{-1}$; Fig. 2A).

Higher temperatures, UVB5 and water stress significantly increased transpiration (E; Table 3). E was 7.8 and 9.4 times higher from the 37-day-old plants than from the 17- and 27-day-old plants, respectively (Tables 1–2). Also, the two-way interactions of T × U, T × W, T × G, U × G and W × G, and the three-way interactions of T × U × G and T × W × G significantly affected E (Table 3). These interactions indicate that the 37-day-old water-stressed plants grown under higher temperatures at UVB5 had highest E (36.46 ± 2.69 mmol H$_2$O m$^{-2}$ s$^{-1}$), whereas the 27-day-old water-stressed plants grown under lower temperatures at UVB5 had lowest E (0.85 ± 0.14 mmol H$_2$O m$^{-2}$ s$^{-1}$; Fig. 2B).

Higher temperatures and UVB5 increased stomatal conductance (gs), which was 9.1 and 12.8 times higher in the 37-day-old plants than in the 17- and 27-day-old plants, respectively (Tables 1–3). Also, the two-way interactions of T × U, T × G and U × G, and the three-way interaction of T × U × G had significant effects on gs (Table 3). On the basis of these interactions, the 37-day-old plants grown under higher temperatures at UVB5 had highest gs (1.45 ± 0.23 mol H$_2$O m$^{-2}$ s$^{-1}$), whereas the 27-day-old plants grown under lower temperatures at UVB5 had lowest gs (0.04 ± 0.01 mol H$_2$O m$^{-2}$ s$^{-1}$; Fig. 2C).

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Temperature</th>
<th>UVB radiation</th>
<th>Watering regime</th>
<th>Growth stage</th>
<th>CH$_4$ (ng g$^{-1}$ DM h$^{-1}$)</th>
<th>AN (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>E (mmol H$_2$O m$^{-2}$ s$^{-1}$)</th>
<th>gs (mol H$_2$O m$^{-2}$ s$^{-1}$)</th>
<th>WUE (mol CO$_2$ mmol H$_2$O$^{-1}$)</th>
<th>ϕ$_{Fv/Fm}$</th>
<th>qNP</th>
<th>qP</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-day-old</td>
<td>Higher</td>
<td>UVB5</td>
<td>Well-watered</td>
<td>54.07 ± 3.398</td>
<td>79.74 ± 4.628</td>
<td>4.09 ± 0.818</td>
<td>0.14 ± 0.030</td>
<td>4.00 ± 0.30</td>
<td>0.75 ± 0.01</td>
<td>31.38 ± 0.82</td>
<td>3.34 ± 0.04</td>
<td>0.72 ± 0.01</td>
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<tr>
<td>27-day-old</td>
<td>Lower</td>
<td>UVB0</td>
<td>Water-stressed</td>
<td>39.13 ± 0.76</td>
<td>49.75 ± 2.16</td>
<td>0.75 ± 0.01</td>
<td>0.20 ± 0.01</td>
<td>3.16 ± 0.20</td>
<td>0.79 ± 0.01</td>
<td>40.93 ± 0.98</td>
<td>3.54 ± 0.06</td>
<td>0.72 ± 0.01</td>
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<tr>
<td>37-day-old</td>
<td>Higher</td>
<td>UVB5</td>
<td>Well-watered</td>
<td>47.92 ± 4.13</td>
<td>73.08 ± 4.73</td>
<td>4.09 ± 0.818</td>
<td>0.14 ± 0.030</td>
<td>4.00 ± 0.30</td>
<td>0.75 ± 0.01</td>
<td>31.38 ± 0.82</td>
<td>3.34 ± 0.04</td>
<td>0.72 ± 0.01</td>
</tr>
</tbody>
</table>

Plants were grown under experimental conditions, after one week of initial growth under 22/18 °C. Data are means ± SE of three trials (three replications per treatment per trial). Means followed by different letters within each parameter and factor are significantly different (\(p < 0.05\)) according to Scheffé’s test.
Plants were grown under experimental conditions, after one week of initial growth under 22/18 °C. Significantly decreased the maximum quantum yield of photosystem II (Fv/Fm; Tables 1 and 4), which was 1.1 times higher in the 17-day-old plants than in the 27-day-old plants (Tables 1–2). Fv/Fm was significantly affected by the two-way interaction of T × U and T × G, and the three-way interaction of U × W × G (Table 4). These interactions revealed that Fv/Fm was highest in plants grown under lower temperatures at UVB0 (0.76 ± 0.02), in the 17-day-old plants under lower temperatures (0.79 ± 0.02), and in the 17-day-old well-watered plants at UVB5 (0.77 ± 0.02); however, it was lowest in plants grown under higher temperatures at UVB5 (0.72 ± 0.03), in the 27-day-old plants under lower temperatures (0.68 ± 0.03), and in the 27-day-old water-stressed plants at UVB5 (0.64 ± 0.06), respectively (Fig. 3B).

Based on the two-way interaction of C × G in repeated measures ANOVA (Table 2), Fv/Fm was highest in the 17-day-old well-watered plants that were grown under lower temperatures at UVB0 (0.80 ± 0.00), and lowest in the 37-day-old water-stressed plants that were grown under higher temperatures at UVB5 (0.60 ± 0.08; Fig. 3B).

Higher temperatures significantly decreased non-photochemical quenching (qNP; Tables 1 and 4), which was 1.3 and 1.1 times lower in the 17-day-old plants than in the 27-day-old plants (Tables 1–2). Fv/Fm was significantly affected by the two-way interaction of T × U and T × G, and the three-way interaction of U × W × G (Table 4). These interactions revealed that Fv/Fm was highest in plants grown under lower temperatures at UVB0 (0.76 ± 0.02), in the 17-day-old plants under lower temperatures (0.79 ± 0.02), and in the 17-day-old well-watered plants at UVB5 (0.77 ± 0.02); however, it was lowest in plants grown under higher temperatures at UVB5 (0.72 ± 0.03), in the 27-day-old plants under lower temperatures (0.68 ± 0.03), and in the 27-day-old water-stressed plants at UVB5 (0.64 ± 0.06), respectively (Fig. 3B).

None of the interactions were significant for qNP.
Table 4; Fig. 3C).

Higher temperatures significantly increased, but water stress decreased, photochemical quenching (qP; Tables 1 and 4). qP progressively decreased with increased plant age; it was 1.5 times lower in the latest growth stage than in the earliest stage (Tables 1–2). Also, the two-way interaction of T × U, and the three-way interaction of T × U × G significantly affected qP (Table 4). The three-way interaction showed that the 17-day-old plants grown under higher temperatures at UVB5 had highest qP (0.32 ± 0.05), whereas the 37-day-old plants grown under lower temperatures at UVB0 had lowest qP (0.13 ± 0.03; Fig. 3D).

3.4. Nitrogen balance index, total chlorophyll, and flavonoids

UVB5 significantly decreased nitrogen balance index (NBI; Tables 1 and 4). NBI progressively decreased with increased plant age; it was 1.5 times lower in the 37-day-old plants than in the 17-day-old plants (Tables 1–2). The two-way interactions of T × G, U × W and U × G significantly affected NBI (Table 4). On the basis of these interactions, NBI was highest in the 17-day-old plants under lower temperatures (57.21 ± 3.68), in the water-stressed plants at UVB0 (51.59 ± 4.38), and in the 17-day-old plants at UVB0 (64.74 ± 4.81); however, it was lowest in the 37-day-old plants under lower temperatures (32.52 ± 4.63), in the water-stressed plants at UVB5 (37.98 ± 3.86), and in the 37-day-old plants at UVB5 (35.13 ± 5.31), respectively.
Based on the two-way interaction of C × G in repeated measures ANOVA (Table 2), NBI was highest in the 17-day-old water-stressed plants that were grown under higher temperatures at UVB0 (68.32 ± 5.87); however, it was lowest in the 37-day-old well-watered plants that were grown under lower temperatures at UVB0 (29.74 ± 4.80; Fig. 4A).

As plant growth progressed, the total chlorophyll concentration significantly decreased; the total chlorophyll content was 1.1 and 1.3 times lower, respectively, in the 27- and 37-day-old plants than in the 17-day-old plants (Tables 1, 2 and 4). None of the interactions were

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Chlorophyll fluorescence</th>
<th>Nitrogen balance index</th>
<th>Total chlorophyll</th>
<th>Flavonoids</th>
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<tr>
<td></td>
<td></td>
<td>ϕPSII</td>
<td>Fv/Fm</td>
<td>qNP</td>
<td>qP</td>
</tr>
<tr>
<td>Temperature (T)</td>
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<td>24.35**</td>
<td>2.10</td>
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<td>9.15**</td>
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<td>Watering regime (W)</td>
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<td>Growth stage (G)</td>
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<td>16.55****</td>
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<td>W × G</td>
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<td>T × U × W × G</td>
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<td>8.55***</td>
<td>3.59</td>
<td>0.43</td>
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Plants were grown under experimental conditions, after one week of initial growth under 22/18 °C. ϕPSII, effective quantum yield of photosystem II; Fv/Fm, maximum quantum yield of photosystem II; qNP, non-photochemical quenching; qP, photochemical quenching. Significance values: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
significant for total chlorophyll (Table 4; Fig. 4B).

UVB5 increased flavonoids (Tables 1 and 4); it was 1.2 and 1.3 times higher, respectively, in the last two growth stages than in the first stage (Tables 1–2). Only the three-way interaction of T × U × W significantly affected flavonoids (Table 4), indicating that the well-watered plants grown under lower temperatures at UVB5 had increased flavonoids (0.86 ± 0.03 μg cm⁻²), whereas the water-stressed plants grown under higher temperatures at UVB0 had decreased flavonoids (0.64 ± 0.04 μg cm⁻²; Fig. 4C).

3.5. Relationship between methane emissions and other plant parameters

Pearson’s correlation coefficients were significant for many relationships of physiological characteristics. For instance, CH₄ emission was positively correlated with E (r = 0.323, P = 0.006) and gₛ (r = 0.381, P = 0.001), but was negatively correlated with WUE (r = -0.456, P = 0.000) and qNP (r = -0.309, P = 0.008).

4. Discussion

It has long been known that methane is the primary end-product of anaerobic mineralization process, which can take place naturally through methanogenesis (Reay et al., 2010). However, recent studies have shown that plants under aerobic conditions can also produce and emit CH₄ (Keppler et al., 2006; Bruhn et al., 2014; Liu et al., 2015), which increases with environmental stress factors (Abdulmajeed et al., 2017; Martel and Qaderi, 2017, 2019).

In this study, we investigated the effects of temperature, UVB radiation, watering regime and plant growth stage on CH₄ emissions and some relevant physiological characteristics of pea plants. All stress factors increased CH₄ emissions from plants (Table 1; Fig. 1). It has been suggested that under high UV stress conditions, CH₄ can be released from the spontaneous break down of plant material (Nisbet et al., 2009). Higher temperatures can harm plants by damaging DNA or through the production of ROS (Stapleton and Walbot, 1994), which, in turn, lead to the release of CH₄ from the pectic substances (Keppler et al., 2006; McLeod et al., 2008; Messenger et al., 2009). We also found that CH₄ emissions decrease with increased plant age. It is, therefore, true that the 17-day-old plants emitted 1.2 times more CH₄ than the 37-day-old plants (Table 1), which might have acclimated to stress over time (Martel and Qaderi, 2017).

Findings from this study are relatively similar to those of other studies on plant-derived CH₄, which have been reviewed recently (see Bruhn et al., 2012; Wang et al., 2013; Liu et al., 2015; Covey and Megonigal, 2019). For example, Keppler et al. (2006) have shown that CH₄ emission rates from detached tissues of some C₃ and C₄ plants ranged from 1.6 ng g⁻¹ DW h⁻¹ to 8.7 ng g⁻¹ DW h⁻¹, and those from the intact plants of the same species ranged from 119 ng g⁻¹ DW h⁻¹ to 374 ng g⁻¹ DW h⁻¹, in darkness and sunlight, respectively. McLeod et al. (2008) have demonstrated that the excised leaves of tobacco (Nicotiana tabacum L. cv. Xanthi) plants, which were exposed to UV irradiation, emitted 12.3 ng CH₄ g⁻¹ DW h⁻¹. In another study, CH₄ emission rates from the detached leaves of canola (Brassica napus) plants, exposed to 0 and 4 mW cm⁻² of blue light, were 62 and 101 ng CH₄ g⁻¹ DW h⁻¹, respectively (Martel and Qaderi, 2019). In a previous study, the detached leaves of pea (Pisum sativum L. var. 234 Lincoln)
plants that were grown under higher temperatures and experienced water stress emitted 106.2 CH$_4$ ng g$^{-1}$ DW h$^{-1}$ (Qaderi and Reid, 2011). In our current study, the rates of CH$_4$ emission were in the range of 24.27 ng g$^{-1}$ DW h$^{-1}$ and 116.60 ng g$^{-1}$ DW h$^{-1}$, suggesting some similarities to or differences from earlier findings. Differences among studies on plant-derived CH$_4$ might have been related to differences in plant species, plant genotype and/or experimental condition and setup.

In this study, plant physiological performance, including gas exchange, chlorophyll fluorescence and production of UV-screening compounds, could have been influenced by environmental stressors, which, in turn, would have affected CH$_4$ emission rates. It was found that the effects of stress factors on gas exchange varied with parameters (Tables 1 and 3; Fig. 2). Although the three stress factors did not have a drastic effect on AN, they all increased E and to a large extent g, (Table 1). A higher E usually indicates lower WUE, which was the case under higher temperature regime in our current study, and in other earlier studies (Bacon, 2004; Qaderi and Reid, 2008). Overall, photosynthetic activities of plants vary with plant species and developmental stage (Xu et al., 2015). In our study, the 37-day-old plants had at least 7.6, 7.8 and 9.1 times more AN, E and g, respectively, compared to the younger plants (Table 1). This may be attributed to the fact that older plants were acclimated to the stress conditions during this time period. Decreased AN in younger plants, compared to older ones, might be due to a higher stomatal closure in young leaves than in old leaves (Davis and McCree, 1978), resulting in decreased g, and, in turn, E (Caird et al., 2007). These findings suggest that plants can be more sensitive to climatic factors in the earlier developmental stages than in the later stages (Donohue et al., 2010; Gray and Brady, 2016), and such response might have influenced plants to emit relatively more CH$_4$ in the earlier life stage than in the later stages.

Also, chlorophyll fluorescence was variably influenced by the environmental factors (Tables 1 and 4; Fig. 3). In this study, higher temperatures decreased $\Phi$PSII and $q$NP, but increased $q$P, which are similar to an earlier finding, showing the negative effects of high temperature on chlorophyll fluorescence (Gui et al., 2006). Moreover, water stress decreased $\Phi$PSII, F$_{v}$/F$_{m}$ and $q$P, as previously reported (Qaderi et al., 2013; Guan et al., 2015). Across growth stages, plants in their earliest stage had highest $\Phi$PSII, F$_{v}$/F$_{m}$ and $q$P, but lowest $q$NP. This suggests that stress-induced stomatal closure (He et al., 2011; Tosi et al., 2014) leads to decreased CO$_2$ concentration inside the leaf. All these processes in the cell thus negatively affect the photosynthetic activity of PSII, leading to decreased chlorophyll fluorescence activity and increased CH$_4$ emissions. Plants with short-term exposure, 10 days, still had a good state of $\Phi$PSII, F$_{v}$/F$_{m}$ and $q$P, which decreased with exposure time. Our results support the findings by Mauroimacale et al. (2006), who showed that, in potato (Solanum tuberosum L.), chlorophyll fluorescence during plant growth varies with temperature. Obviously, heat stress causes injuries to the photosynthetic apparatus and adversely affect crop yield, as shown in wheat (Harding et al., 1990), but increases CH$_4$ emissions (Bruhn et al., 2012; Abdulmajied et al., 2017).

In the present study, UVBs decreased NBI, but increased flavonoids. NBI and total chlorophyll progressively decreased, whereas flavonoids increased with increased plant age (Tables 1 and 4; Fig. 4). Decreased NBI might have correlated to reduced leaf nitrogen content, as NBI is used to predict the status of nitrogen nutrition in plants (Agati et al., 2013). An earlier study, with garden pea, has shown that the chlorophyll a/b ratios decrease as pea plants are aged (Day et al., 1996). Our findings on total chlorophyll is similar to that of an earlier study on potato plants in which chlorophyll content decreased with increased plant age (Mauroimacale et al., 2006). Also, in our study, increased flavonoids with increased plant age and under supplemental UVB support our argument about plant acclimatization to stress factors in later growth stages. It is well documented that flavonoids act as UV-absorbing agents, protecting plants from damaging effects of UVB radiation (Stapleton and Walbot, 1994; A.-H.-Mackerness, 2000). In our study, the longer-term exposure to UVB influenced plants to accumulate more flavonoids than the shorter-term exposure, as reported in the past (Kakani et al., 2003; Reddy et al., 2004b; Reifenrath and Müller, 2007). The result of our study on flavonoids is again in agreement with earlier reports (see Di Ferdinando et al., 2012), showing that flavonoid biosynthesis is upregulated in response to UVB radiation; thus reducing stress on the 37-day-old plants, leading to relatively decreased CH$_4$ emission rates from these plants than emission rates from younger plants (Figs. 1 and 4).

5. Conclusion

This study revealed that CH$_4$ emission from pea plants is regulated by stress factors through changes in plant physiological processes, including gas exchange, chlorophyll fluorescence and the accumulation of UV-screening compounds. Also, interaction between/among environmental stress factors affects plants, leading to increased CH$_4$ emissions, which decrease with increased plant age. Further studies should consider measurement of CH$_4$ emissions during developmental stages of multiple plant species for better understanding of the phenomenon and estimation of plant contribution to global CH$_4$ budget.

Author contributions

MMQ conceived and designed the experiments; AMA performed the experiments; AMA and MMQ analyzed the data, wrote the manuscript and approved it for publication.

Conflicts of interest

The authors declare no conflict of interest.

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