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Research article

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

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A B S T R A C T

Many studies have investigated the effects of one or two environmental factors on methane (CH_4) 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 CH_4 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, UVB5, and water stress adversely affected photosynthesis and chlorophyll fluorescence, but increased CH_4 emissions, which decreased with increased plant age. Also, interaction of higher temperatures and UVB5 reversed the pattern of CH_4 emissions at growth stages, compared to that of other treatments. We conclude that CH_4 emission decreases with plant age, and it is affected by stress factors through changes in physiological activities of plants.

1. Introduction

Methane (CH_4) is the second important anthropogenic greenhouse gas after carbon dioxide (CO_2), with a global warming potential of nearly 34 times greater than that of CO_2 (Myhre et al., 2013). Prior to 2006, the biological formation of CH_4 was attributed only 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 CH_4 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 CH_4, 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., 2009), leaf surface wax (Bruhn et al., 2014), and methionine (Lenhart et al., 2015).

Many studies have shown the non-microbial CH_4 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 CH_4 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 CO_2 (Qaderi and Reid, 2011). Environmental stress factors affect plant metabolic activities, including the production of reactive oxygen species (ROS), leading to increased CH_4 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 CH_4 emissions from plants (see Liu et al., 2015). As temperature increases above

Abbreviations: A_N, net CO_2 assimilation; C, growth condition; DM, dry mass; E, transpiration; FM, fresh mass; F_v/F_m, maximum quantum yield of PSII; G, growth stage; g_s, stomatal conductance; HT, higher temperatures; LT, lower temperatures; NBI, nitrogen balance index; ϕPSII, effective quantum yield of PSII; qNP, non-photochemical quenching; qP, photochemical quenching; T, temperature; U and UVB, ultraviolet-B radiation; W, watering regime; WUE, water use efficiency

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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 CO_2 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 CH_4 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 CH_4 levels.

In the past, most studies had been designed to investigate the effect of single or double environmental stress factors on CH_4 emissions from plants (see Bruhn et al., 2012; Liu et al., 2015) and, to our knowledge, none of these studies considered measuring CH_4 emissions during plant growth. It is, therefore, crucial to understand the effects of multiple factors on CH_4 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 CH_4 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 CH_4 emission rates and relevant physiological parameters at three growth stages of pea plants. This study will contribute to our understanding of CH_4 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 CH_4 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-D-58 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 diacetate 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 (UVB_{BE}). UVB_{BE} 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 CH_4-free air, and incubated for 2 h inside a growth chamber at 22 °C and 300-μmol photons m^{-2} s^{-1}. Then, the CH_4 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 CH_4 gas (Air Liquide, Dartmouth, NS), and quantified by using the standard curve obtained from the injection of CH_4 standard. Leaf samples were dried (60 °C for 96 h) to calculate the rates of CH_4 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 CH_4 emissions. Although CH_4 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 CO_2 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 CO_2 assimilation (A_N; μmol CO_2 m^{-2} s^{-1}), transpiration (E; mmol H_2O m^{-2} s^{-1}), and stomatal conductance (g_s; mol H_2O m^{-2} s^{-1}). The water use efficiency (WUE; μmol CO_2 $mmol^{-1}$ H_2O) was calculated as A_N/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; F_q'/F_m'), and the dark-adapted leaves (kept for 30 min in clamps) were used to measure the maximum quantum yield of PSII (F_v/F_m), non-photochemical quenching (qNP; $(F_m\text{-}F_m')^{-1}$) and photochemical quenching (qP; F_q'/F_v') (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 UVB0 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 (A_N) 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 A_N (Table 3). A_N 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_2O 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_2O m^{-2} s^{-1}; Fig. 2B).

Higher temperatures and UVB5 increased stomatal conductance (g_s), 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 g_s (Table 3). On the basis of these interactions, the 37-day-old plants grown under higher temperatures at UVB5 had highest g_s (1.45 ± 0.23 mol H_2O m^{-2} s^{-1}), whereas the 27-day-old plants grown under lower temperatures at UVB5 had lowest g_s (0.04 ± 0.01 mol H_2O m^{-2} s^{-1}; Fig. 2C). Overall,

Table 1
Effects of temperature, UVB radiation, watering regime and growth stage on methane emissions and some physiological characteristics of pea (*Pisum sativum*) plants.

Parameter	Temperature		UVB radiation		Watering regime		Growth stage		
	Lower	Higher	UVB0	UVB5	Well-watered	Water-stressed	17-day-old	27-day-old	37-day-old
CH_4 (ng g^{-1} DM h^{-1})	54.07 ± 3.35b	79.74 ± 4.62a	59.88 ± 4.01b	73.92 ± 4.81a	60.73 ± 4.18b	73.08 ± 4.73a	72.42 ± 4.11a	67.57 ± 4.38a	60.72 ± 7.54a
A_N (μmol CO_2 m^{-2} s^{-1})	15.28 ± 2.60a	14.58 ± 2.55a	14.54 ± 2.32a	15.32 ± 2.81a	14.75 ± 2.37a	15.11 ± 2.77a	4.65 ± 0.27b	4.60 ± 0.41b	35.53 ± 1.52a
E (mmol H_2O m^{-2} s^{-1})	4.09 ± 0.81b	8.68 ± 1.90a	4.77 ± 0.91b	8.00 ± 1.89a	5.53 ± 1.13b	7.24 ± 1.80a	1.99 ± 0.20b	1.65 ± 0.15b	15.52 ± 2.22a
g_s (mol H_2O m^{-2} s^{-1})	0.14 ± 0.03b	0.36 ± 0.09a	0.17 ± 0.03b	0.34 ± 0.09a	0.24 ± 0.07a	0.27 ± 0.07a	0.07 ± 0.01b	0.05 ± 0.01b	0.64 ± 0.11a
WUE (μmol CO_2 $mmol^{-1}$ H_2O)	4.00 ± 0.30a	2.20 ± 0.17b	3.42 ± 0.30a	2.78 ± 0.26a	3.08 ± 0.25a	3.12 ± 0.32a	2.57 ± 0.21b	3.23 ± 0.33 ab	3.49 ± 0.46a
ϕPSII	0.70 ± 0.01a	0.63 ± 0.02b	0.66 ± 0.02a	0.67 ± 0.01a	0.68 ± 0.01a	0.65 ± 0.02b	0.70 ± 0.01a	0.65 ± 0.02b	0.65 ± 0.02b
F_v/F_m	0.75 ± 0.01a	0.73 ± 0.01a	0.73 ± 0.01a	0.74 ± 0.01a	0.75 ± 0.01a	0.72 ± 0.01b	0.77 ± 0.01a	0.70 ± 0.01b	0.74 ± 0.02 ab
qNP	1.61 ± 0.04a	1.47 ± 0.05b	1.53 ± 0.05a	1.54 ± 0.04a	1.58 ± 0.05a	1.50 ± 0.05a	1.33 ± 0.05b	1.56 ± 0.05a	1.73 ± 0.05a
qP	0.20 ± 0.01b	0.25 ± 0.01a	0.23 ± 0.02a	0.22 ± 0.01a	0.24 ± 0.02a	0.21 ± 0.01b	0.27 ± 0.02a	0.22 ± 0.02 ab	0.18 ± 0.02b
NBI	43.31 ± 2.25a	46.47 ± 1.98a	49.47 ± 2.40a	40.31 ± 1.46b	44.99 ± 2.06a	44.79 ± 2.21a	55.46 ± 2.40a	43.28 ± 1.86b	35.93 ± 1.77c
Total chlorophyll (μg cm^{-2})	31.38 ± 0.82a	31.15 ± 0.87a	31.41 ± 0.81a	31.12 ± 0.88a	31.07 ± 0.90a	31.47 ± 0.79a	34.78 ± 0.71a	31.54 ± 0.82b	27.48 ± 0.97c
Flavonoids (μg cm^{-2})	0.77 ± 0.02a	0.72 ± 0.03a	0.69 ± 0.02b	0.80 ± 0.02a	0.74 ± 0.02a	0.75 ± 0.03a	0.64 ± 0.03b	0.78 ± 0.03a	0.82 ± 0.02a

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.

Table 2
Repeated measures analysis of variance (F value) for effects of temperature, UVB radiation, watering regime, growth stage, and their interactions on methane emission, gas exchange, chlorophyll fluorescence, nitrogen balance index, total chlorophyll and flavonoids of pea (*Pisum sativum*) plants.

Source		Gas exchange				
	d.f.	Methane	Net CO_2 assimilation	Transpiration	Stomatal conductance	Water use efficiency
Growth condition (C)	7	9.61****	0.59	12.05****	10.78****	7.38****
Growth stage (G)	2	3.03	422.35****	157.97****	108.56****	3.99*
C × G	14	5.03****	1.52	10.99****	10.21****	1.81

		Chlorophyll fluorescence						
Source	d.f.	ϕPSII	F_v/F_m	qNP	qP	Nitrogen balance index	Total chlorophyll	Flavonoids
Growth condition (C)	7	6.73****	1.95	1.64	2.59*	7.09****	0.68	5.21***
Growth stage (G)	2	4.07*	9.05***	17.06****	7.91**	54.58****	18.46****	18.91****
C × G	14	4.20***	2.16*	0.73	0.85	3.16**	0.86	0.94

Plants were grown under experimental conditions, after one week of initial growth under 22/18 °C. ϕPSII, effective quantum yield of photosystem II; F_v/F_m, 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.

g_s was lowest in the 27-day-old water-stressed plants that were grown under lower temperatures at UVB5 (0.02 ± 0.00 mol H_2O m^{-2} s^{-1}), considering the two-way interaction of C (growth condition) × G (growth stage) in repeated measures ANOVA (Table 2).

Higher temperatures reduced water use efficiency (WUE) of plants; it was 1.4 times higher in the latest growth stage than in the earliest growth stage (Tables 1–3). The two-way interaction of T × G significantly affected WUE (Table 3), which was highest in the 37-day-old plants under lower temperatures (4.82 ± 1.35 μmol CO_2 $mmol^{-1}$ H_2O), but lowest in plants with the age under higher temperatures (2.15 ± 0.24 μmol CO_2 $mmol^{-1}$ H_2O; Fig. 2D).

3.3. Chlorophyll fluorescence

Higher temperatures and water stress significantly decreased the effective quantum yield of PSII (ϕPSII; Tables 1 and 4), which was 1.1 times higher in the 17-day-old plants than in both the 27- and 37-day-old plants (Tables 1–2). The two-way interactions of U × W and W × G, the three-way interactions of T × U × W and U × W × G, and the four-way interaction significantly affected ϕPSII (Table 4). On the basis of four-way interaction, ϕPSII was highest in the 37-day-old water-stressed plants grown under lower temperatures at UVB5 (0.76 ± 0.00), but lowest in the 37-day-old well-watered plants grown under higher temperatures at UVB0 (0.41 ± 0.08; Fig. 3A).

Only water stress significantly decreased the maximum quantum yield of PSII (F_v/F_m; 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). F_v/F_m 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 F_v/F_m was highest in plants grown under lower temperatures at UVB5 (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), F_v/F_m 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- and 37-day-old plants, respectively (Tables 1–2). None of the interactions were significant for qNP

Table 3
Analysis of variance (F value) for effects of temperature, UVB radiation, watering regime, growth stage, and their interactions on methane emission and gas exchange of pea (*Pisum sativum*) plants.

Source	d.f.	Methane	Gas exchange			
			Net CO_2 assimilation	Transpiration	Stomata conductance	Water use efficiency
Temperature (T)	1	41.78****	0.44	37.61****	32.30****	29.43****
UVB radiation (U)	1	12.50***	0.55	18.54****	19.34****	3.74
Watering regime (W)	1	9.67**	0.12	5.25*	0.38	0.02
Growth stage (G)	2	2.92	383.17****	149.03****	102.55****	2.72
T × U	1	0.02	0.00	11.05**	17.90***	0.94
T × W	1	0.03	0.01	6.73*	0.47	1.00
T × G	2	7.81**	0.47	27.27****	27.15****	3.74*
U × W	1	0.27	1.27	0.36	0.14	0.00
U × G	2	12.18****	3.43*	19.70****	20.71****	1.74
W × G	2	2.09****	2.02	8.83***	1.06	0.42
T × U × W	1	0.54	1.40	0.03	0.73	0.10
T × U × G	2	10.76***	0.22	8.83***	16.02****	0.25
T × W × G	2	0.35	0.26	7.69**	0.71	1.32
U × W × G	2	0.54	1.26	0.09	0.23	0.28
T × U × W × G	2	0.17	1.97	0.18	1.66	0.88

Plants were grown under experimental conditions, after one week of initial growth under 22/18 °C. Significance values: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

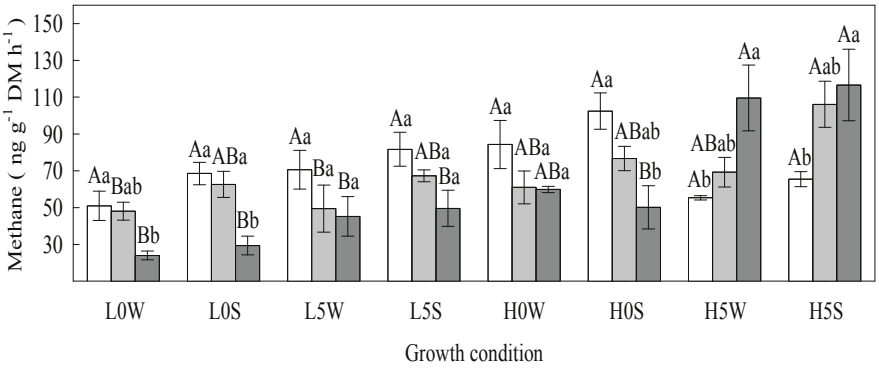

Fig. 1. Methane emissions from three growth stages of pea (*Pisum sativum*) plants. Plants were grown under two temperature regimes (22/18 °C and 28/24 °C; 16 h light/8 h dark), two levels of ultraviolet-B radiation (0 and 5 kJ m^{-2} d^{-1}) and two watering regimes (well-watered and water-stressed) for 10, 20 and 30 days, after one week of initial growth under 22/18 °C. Open bars represent the 17-day-old plants, light-gray bars the 27-day-old plants, and dark-gray bars the 37-day-old plants. L, lower temperatures; H, higher temperatures; 0, UVB0; 5, UVB5; W, well-watered; S, water-stressed. Data are means ± SE of nine samples from three trials. Bars (mean ± SE) with different upper-case letters within growth stages and with different lower-case letters within treatments are significantly different ($P < 0.05$; Scheffé's test).

(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

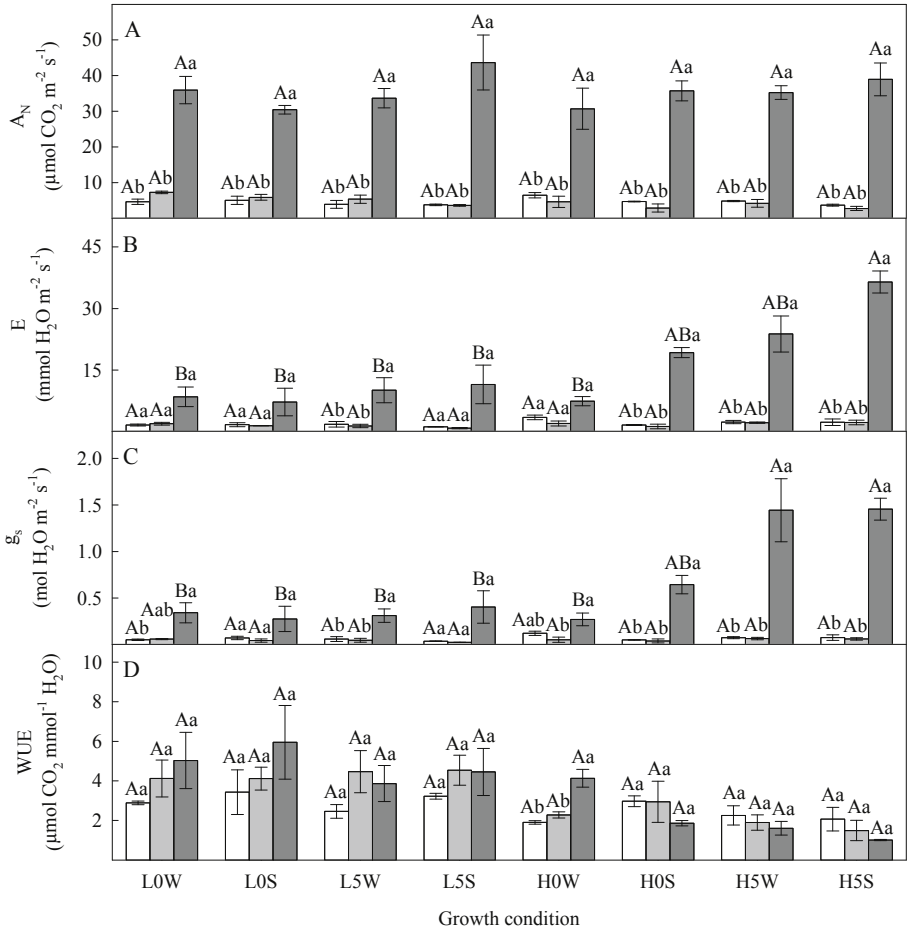

Fig. 2. Photosynthetic parameters from different growth stages of pea (*Pisum sativum*) plants. (A) A_N, net CO_2 assimilation; (B) E, transpiration; (C) g_s, stomatal conductance; and (D) WUE, water use efficiency. Otherwise, as for Fig. 1.

Table 4
Analysis of variance (F value) for effects of temperature, UVB radiation, watering regime, growth stage, and their interactions on chlorophyll fluorescence, nitrogen balance index, total chlorophyll and flavonoids of pea (*Pisum sativum*) plants.

Source	d.f.	Chlorophyll fluorescence				Nitrogen balance index	Total chlorophyll	Flavonoids
		ϕPSII	F_v/F_m	qNP	qP			
Temperature (T)	1	24.35****	2.10	6.24*	9.15**	3.07	0.05	3.99
UVB radiation (U)	1	0.24	0.37	0.03	0.09	25.89****	0.08	17.50***
Watering regime (W)	1	4.85*	6.08*	2.25	4.24*	0.01	0.15	0.40
Growth stage (G)	2	4.53*	10.56***	16.55****	8.91***	40.05****	17.61****	17.29****
T × U	1	0.29	4.87*	1.03	4.95*	0.75	1.00	1.63
T × W	1	2.10	0.44	0.07	1.34	0.46	1.98	0.43
T × G	2	2.00	5.73**	0.37	1.49	3.41*	0.15	1.92
U × W	1	6.42*	0.62	0.22	0.06	6.11*	1.12	2.89
U × G	2	1.75	2.90	3.03	0.02	7.64**	0.11	1.61
W × G	2	12.10****	0.29	0.34	0.16	2.67	2.53	0.04
T × U × W	1	14.23***	1.48	1.31	0.55	0.09	0.14	6.47*
T × U × G	2	1.62	0.31	0.11	3.99*	0.70	0.58	0.20
T × W × G	2	1.60	0.38	0.16	0.53	0.41	0.73	0.03
U × W × G	2	5.12**	4.45*	0.50	0.32	0.07	0.31	0.20
T × U × W × G	2	8.55***	3.59	0.43	0.18	1.31	1.32	2.03

Plants were grown under experimental conditions, after one week of initial growth under 22/18 °C. ϕPSII, effective quantum yield of photosystem II; F_v/F_m, 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.

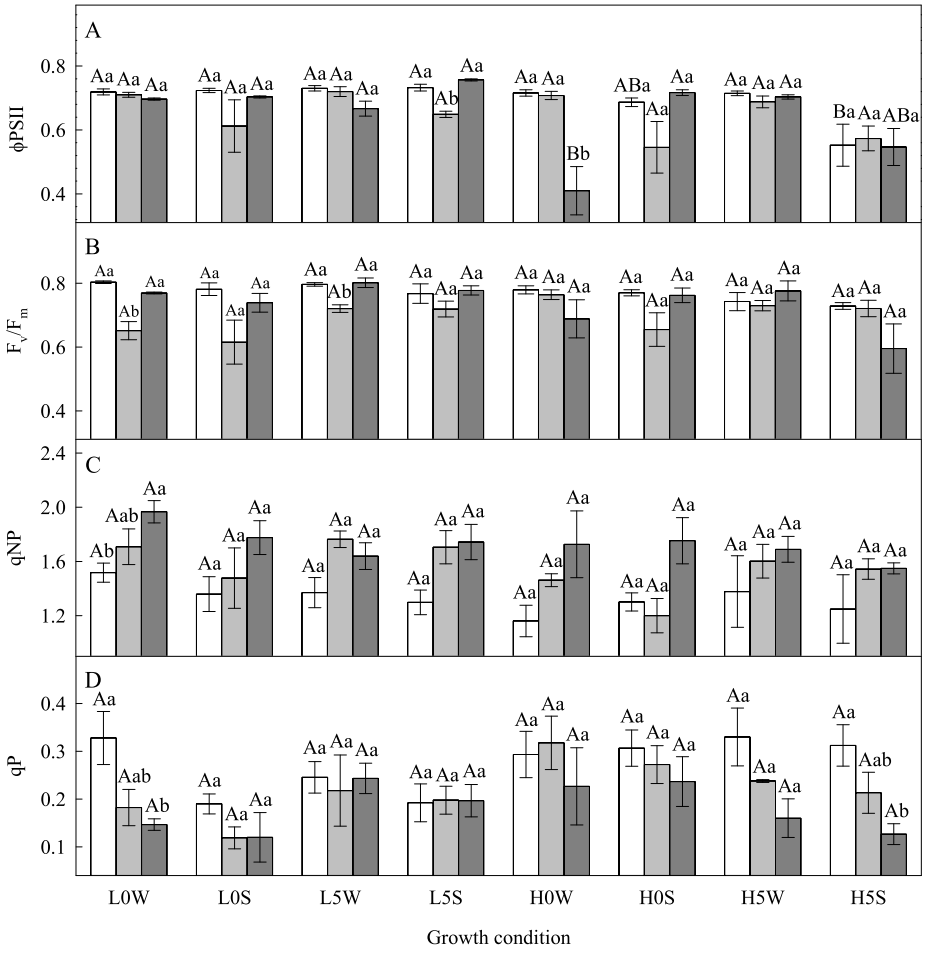

Fig. 3. Chlorophyll fluorescence from different growth stages of pea (*Pisum sativum*) plants. (A) ϕPSII, effective quantum yield of PSII; (B) F_v/F_m, maximum quantum yield of PSII; (C) qNP, non-photochemical quenching; and (D) qP, photochemical quenching. Otherwise, as for Fig. 1.

(Fig. 4A). 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

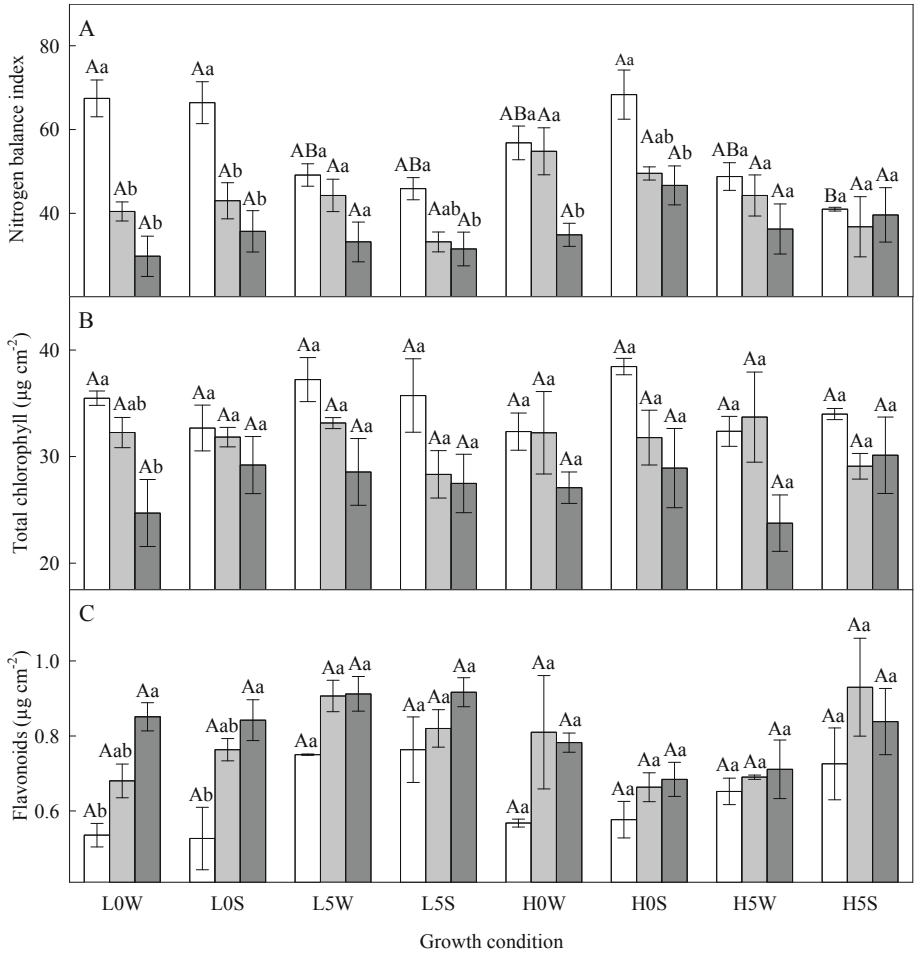

Fig. 4. Nitrogen balance index, total chlorophyll and flavonoids from different growth stages of pea (*Pisum sativum*) plants. (A) Nitrogen balance index; (B) total chlorophyll; and (C) flavonoids. Otherwise, as for Fig. 1.

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 ($0.86 \pm 0.03\,\mu g\,cm^{-2}$), whereas the water-stressed plants grown under higher temperatures at UVB0 had decreased flavonoids ($0.64 \pm 0.04\,\mu g\,cm^{-2}$; 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, CH_4 emission was positively correlated with E ($r = 0.323$, $P = 0.006$) and g_s ($r = 0.381$, $P = 0.001$), 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 CH_4 (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 CH_4 emissions and some relevant physiological characteristics of pea plants. All stress factors increased CH_4 emissions from plants (Table 1; Fig. 1). It has been suggested that under high UV stress conditions, CH_4 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 CH_4 from the pectic substances (Keppler et al., 2006; McLeod et al., 2008; Messenger et al., 2009). We also found that CH_4 emissions decrease with increased plant age. It is, therefore, true that the 17-day-old plants emitted 1.2 times more CH_4 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 CH_4, 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 CH_4 emission rates from detached tissues of some C_3 and C_4 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 CH_4 g^{-1} DW h^{-1}. In another study, CH_4 emission rates from the detached leaves of canola (*Brassica napus*) plants, exposed to 0 and 4 mW cm^{-1} of blue light, were 62 and 101 ng CH_4 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 A_N, they all increased E and to a large extent g_s (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 A_N, E and g_s, 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 A_N in younger plants, compared to older ones, might be due to higher stomatal closure in young leaves than in old leaves (Davis and McCree, 1978), resulting in decreased g_s 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 ϕPSII and qNP, but increased qP, which are similar to an earlier finding, showing the negative effects of high tempearture on chlorophyll fluorescence (Cui et al., 2006). Moreover, water stress decreased ϕPSII, F_v/F_m and qP, as previously reported (Qaderi et al., 2013; Guan et al., 2015). Across growth stages, plants in their earliest stage had highest ϕPSII, F_v/F_m, and qP, but lowest qNP. This suggests that stress-induced stomatal closure (He et al., 2011; Tossi et al., 2014) leads to decreased CO_2 concentration inside the leaf. All these processes in the cell thus negatively affect the photochemical 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 ϕPSII, F_v/F_m and qP, which decreased with exposure time. Our results support the findings by Mauromicale 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; Abdulmajeed et al., 2017).

In the present study, UVB5 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 (Mauromicale 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-absorbents, 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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References

Abdulmajeed, A.M., Derby, S.R., Strickland, S.K., Qaderi, M.M., 2017. Interactive effects of temperature and UVB radiation on methane emissions from different organs of pea plants grown in hydroponic system. J. Photochem. Photobiol. B Biol. 166, 193–201.
Abdulmajeed, A.M., Qaderi, M.M., 2017. Intrashoot variation in aerobic methane emissions from pea plants exposed to multiple abiotic stresses. Acta Physiol. Plant. 39, 124.
Agati, G., Foschi, L., Grossi, N., Guglielminetti, L., Cerovic, Z.G., Volterrani, M., 2013. Fluorescence-based versus reflectance proximal sensing of nitrogen content in *Paspalum vaginatum* and *Zoysia matrella* turfgrasses. Eur. J. Agron. 45, 39–51.
A.H.Mackerness, S., 2000. Plant responses to ultraviolet-B (UV-B: 280–320 nm) stress: what are the key regulators? J. Plant Growth Regul. 32, 27–39.
Azoulay-Shemer, T., Palomares, A., Bagheri, A., Israelsson-Nordstrom, M., Engineer, C.B., Bargmann, B.O., Stephan, A.B., Schroeder, J.I., 2015. Guard cell photosynthesis is critical for stomatal turgor production, yet does not directly mediate CO_2- and ABA-induced stomatal closing. Plant J. 83, 567–581.
Bacon, M.A. (Ed.), 2004. Water Use Efficiency in Plant Biology. Blackwell Publishing, Oxford.
Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. Annu. Rev. Plant Biol. 59, 89–113.
Bruhn, D., Mikkelsen, T.N., Rolsted, M.M.M., Egsgaard, H., Ambus, P., 2014. Leaf surface wax is a source of plant methane formation under UV radiation and in the presence of oxygen. Plant Biol. 6, 512–516.
Bruhn, D., Møller, I.M., Mikkelsen, T.N., Ambus, P., 2012. Terrestrial plant methane production and emission. Physiol. Plant. 144, 201–209.
Caird, M.A., Richards, J.H., Donovan, L.A., 2007. Nighttime stomatal conductance and transpiration in C_3 and C_4 plants. Plant Physiol. 143, 4–10.
Caldwell, M.M., 1971. Solar UV irradiation and the growth and development of higher plants. In: In: Giese, A.C. (Ed.), Photophysiology, vol. 6. Academic Press, New York,

pp. 131–177.
Covey, K.R., Megonigal, J.P., 2019. Methane production and emissions in trees and forests. New Phytol. 222, 35–51.
Cui, L., Li, J., Fan, Y., Xu, S., Zhang, Z., 2006. High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two *Festuca arundinacea* cultivars with different heat susceptibility. Bot. Stud. 47, 61–69.
Davis, S.D., McCree, K.J., 1978. Photosynthetic rate and diffusion conductance as a function of age in leaves of bean plants. Crop Sci. 18, 280–282.
Day, T.A., Howells, B.W., Ruhland, C.T., 1996. Changes in growth and pigment concentrations with leaf age in pea under modulated UV-B radiation field treatments. Plant Cell Environ. 19, 101–108.
Day, T.A., Neale, P.J., 2002. Effects of UV-B radiation on terrestrial and aquatic primary producers. Annu. Rev. Ecol. Systemat. 33, 371–396.
Donohue, K., de Casas, R.R., Burghardt, L., Kovach, K., Willis, C.G., 2010. Germination, postgermination adaptation, and species ecological ranges. Annu. Rev. Ecol. Evol. Syst. 41, 293–319.
Di Ferdinando, M., Brunetti, C., Fini, A., Tattini, M., 2012. Flavonoids as antioxidants in plants under abiotic stresses. In: Ahmad, P., Prasad, M.N.V. (Eds.), Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability. Springer Science +Business Media, LLC, New York, pp. 159–179.
Dueck, T.A., de Visser, R., Poorter, H., Persijn, S., Gorissen, A., deVisser, W., Schapendonk, A., Verhagen, J., Snel, J., Harren, F.J.M., Ngai, A.K.Y., Verstappen, F., Bouwmeester, H., Voesenek, L.A.C.J., van der Werf, A., 2007. No evidence for substantial aerobic methane emission by terrestrial plants: a ^{13}C-labelling approach. New Phytol. 175, 29–35.
Fraser, W.T., Blei, E., Fry, S.C., Newman, M.F., Reay, D.S., Smith, K.A., McLeod, A.R., 2015. Emissions of methane, carbon monoxide, carbon dioxide and short-chain hydrocarbons from vegetation foliage under ultraviolet irradiation. Plant Cell Environ. 38, 980–989.
Gray, S.B., Brady, S.M., 2016. Plant developmental responses to climate change. Dev. Biol. 419, 64–77.
Guan, X.K., Song, L., Wang, T.C., Turner, N.C., Li, F.M., 2015. Effect of drought on the gas exchange, chlorophyll fluorescence and yield of six different-era spring wheat cultivars. J. Agron. Crop Sci. 201, 253–266.
Harding, S.A., Guikema, J.A., Paulsen, G.M., 1990. Photosynthetic decline from high temperature stress during maturation of wheat: 1. Interaction with senescence processes. Plant Physiol. 92, 648–653.
He, J., Yue, X., Wang, R., Zhang, Y., 2011. Ethylene mediates UV-B-induced stomatal closure via peroxidase-dependent hydrogen peroxide synthesis in *Vicia faba* L. J. Exp. Bot. 62, 2657–2666.
Jones, H.G., 2013. Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology, third ed. Cambridge University Press, Cambridge.
Kakani, V.G., Reddy, K.R., Zhao, D., Sailaja, K., 2003. Field crop responses to ultraviolet-B radiation: a review. Agric. For. Meteor. 120, 191–218.
Keppler, F., Hamilton, J.T.G., Braß, M., Röckmann, T., 2006. Methane emissions from terrestrial plants under aerobic conditions. Nature 439, 187–191.
Knief, C., Delmotte, N., Chaffron, S., Stark, M., Innerebner, G., Wassmann, R., von Mering, C., Vorholt, J.A., 2012. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME J. 6, 1378–1390.
Lambers, H., Chapin III., F.S., Pons, T.L., 2008. Plant Physiological Ecology, second ed. Springer Science + Business Media, LLC, New York.
Lenhart, K., Althoff, F., Greule, M., Keppler, F., 2015. Technical note: methionine, a precursor of methane in living plants. Biogeosciences 12, 1907–1914.
Liu, J., Chen, H., Zhu, Q., Shen, Y., Wang, X., Wang, M., Peng, C., 2015. A novel pathway of direct methane production and emission by eukaryotes including plants, animals and fungi: an overview. Atmos. Environ. 115, 26–35.
Martel, A.B., Qaderi, M.M., 2016. Does salicylic acid mitigate the adverse effects of temperature and ultraviolet-B radiation on pea (*Pisum sativum*) plants? Environ. Exp. Bot. 122, 39–48.
Martel, A.B., Qaderi, M.M., 2017. Light quality and quantity regulate aerobic methane emissions from plants. Physiol. Plant. 159, 313–328.
Martel, A.B., Qaderi, M.M., 2019. Unravelling the effects of blue light on aerobic methane emissions from canola. J. Plant Physiol. 233, 12–19.
Mauromicale, G., Ierna, A., Marchese, M., 2006. Chlorophyll fluorescence and chlorophyll content in field-grown potato as affected by nitrogen supply, genotype, and plant age. Photosynthetica 44, 76–82.
McLeod, A.R., Fry, S.C., Loake, G.J., Messenger, D.S., Reay, D.S., Smith, K.A., Yun, B.-W., 2008. Ultraviolet radiation drives methane emissions from terrestrial plant pectins. New Phytol. 180, 124–132.
Messenger, D.J., McLeod, A.R., Fry, S.C., 2009. Reactive oxygen species in aerobic methane formation from vegetation. Plant Signal. Behav. 4, 629–630.
Minitab Inc, 2014. Minitab® Release 17. Statistical Software for Windows®. Minitab Inc., State College, PA.
Myhre, G., Shindell, D., Bréon, F.-M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.-F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Takemura, T., Zhang, H., 2013. Anthropogenic and natural radiative forcing. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge and New York, pp. 659–740.
Nisbet, R.E.R., Fisher, R., Nimmo, R.H., Bendall, D.S., Crill, P.M., Gallego-Sala, A.V., Hornibrook, E.R.C., López-Juez, E., Lowry, D., Nisbet, P.B.R., Shuckburgh, E.F., Srikantharajah, S., Howe, C.J., Nisbet, E.G., 2009. Emissions of methane from plants. Proc. R. Soc. Lond. Ser. B Biol. Sci. 276, 1347–1354.
Qaderi, M.M., Basraon, N.K., Chinnappa, C.C., Reid, D.M., 2010. Combined effects of temperature, ultraviolet-B radiation, and watering regime on growth and physiological processes in canola (*Brassica napus*) seedlings. Int. J. Plant Sci. 171, 466–481.
Qaderi, M.M., Cavers, P.B., Bernards, M.A., 2003. Pre- and post-dispersal factors regulate germination patterns and structural characteristics of Scotch thistle (*Onopordum acanthium*) cypselas. New Phytol. 159, 263–278.
Qaderi, M.M., Lynch, A.L., Godin, V.J., Reid, D.M., 2013. Single and interactive effects of temperature, carbon dioxide, and watering regime on the invasive weed black knapweed (*Centaurea nigra*). Ecoscience 20, 328–338.
Qaderi, M.M., Reid, D.M., 2008. Combined effects of temperature and carbon dioxide on plant growth and subsequent seed germinability of *Silene noctiflora*. Int. J. Plant Sci. 169, 1200–1209.
Qaderi, M.M., Reid, D.M., 2009. Methane emissions from six crop species exposed to three components of global climate change: temperature, ultraviolet-B radiation, and water stress. Physiol. Plant. 137, 139–147.
Qaderi, M.M., Reid, D.M., 2011. Stressed crops emit more methane despite the mitigating effects of elevated carbon dioxide. Funct. Plant Biol. 38, 97–105.
Qaderi, M.M., Reid, D.M., 2014. Aerobic methane emissions from stinkweed (*Thlaspi arvense*) capsules. Plant Signal. Behav. 9, e970095.
Qaderi, M.M., Yeung, E.C., Reid, D.M., 2008. Growth and physiological responses of an invasive alien species, *Silene noctiflora*, during two developmental stages to four levels of ultraviolet-B radiation. Ecoscience 15, 150–159.
Reay, D., Smith, P., van Amstel, A. (Eds.), 2010. Methane and Climate Change. Earthscan Ltd., London.
Reddy, A.R., Chaitanya, K.V., Vivekanandan, M., 2004a. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol. 161, 1189–1202.
Reddy, K.R., Kakani, V.G., Zhao, D., Koti, S., Gao, W., 2004b. Interactive effects of ultraviolet-B radiation and temperature on cotton physiology, growth, development and hyperspectral reflectance. Photochem. Photobiol. 79, 416–427.
Reifenrath, K., Müller, C., 2007. Species-specific and leaf-age dependent effects of ultraviolet radiation on two Brassicaceae. Phytochemistry 68, 875–885.
Robson, T., Klem, K., Urban, O., Jansen, M.A., 2015. Re-interpreting plant morphological responses to UV-B radiation. Plant Cell Environ. 38, 856–866.
SAS Institute, 2011. SAS/STAT User's Guide. SAS Institute, Cary, NC Version 9.3.
Stapleton, A.E., Walbot, V., 1994. Flavonoids can protect maize DNA from the induction of ultraviolet radiation damage. Plant Physiol. 105, 881–889.
Tossi, V., Lamattina, L., Jenkins, G.I., Cassia, R.O., 2014. Ultraviolet-B-induced stomatal closure in Arabidopsis is regulated by the UV RESISTANCE LOCUS8 photoreceptor in a nitric oxide-dependent mechanism. Plant Physiol. 164, 2220–2230.
Vigano, I., Röckmann, T., Holzinger, R., Van Dijk, A., Keppler, F., Greule, M., Brand, W.A., Geilmann, H., Van Weelden, H., 2009. The stable isotope signature of methane emitted from plant material under UV irradiation. Atmos. Environ. 43, 5637–5646.
Vigano, I., van Weelden, H., Holzinger, R., Keppler, F., McLeod, A., Röckmann, T., 2008. Effect of UV radiation and temperature on the emission of methane from plant biomass and structural components. Biogeosciences 5, 937–947.
Wang, Z.-P., Chang, S.X., Chen, H., Han, X.-G., 2013. Widespread non-microbial methane production by organic compounds and the impact of environmental stresses. Earth Sci. Rev. 127, 193–202.
Xu, Z., Jiang, Y., Zhou, G., 2015. Response and adaptation of photosynthesis, respiration, and antioxidant systems to elevated CO_2 with environmental stress in plants. Front. Plant Sci. 6, 701.
Xu, Z.Z., Zhou, G.S., Wang, Y.L., Han, G.X., Li, Y.J., 2008. Changes in chlorophyll fluorescence in maize plants with imposed rapid dehydration at different leaf ages. J. Plant Growth Regul. 27, 83–92.
Zioni, A.B., Vaadia, Y., Lips, S.H., 1971. Nitrate uptake by root as regulated by nitrate reduction products of the shoot. Physiol. Plant. 24, 288–290.