scientific reports

OPEN

Evaluation of secondary sexual dimorphism of the dioecious *Amaranthus palmeri* under abiotic stress

Nicholas E. Korres[1✉], Jason K. Norsworthy[2], Toby FitzSimons[3], Trenton L. Roberts[2], Derrick M. Oosterhuis[2] & Govindjee Govindjee[4]

The evolution of secondary sex-specific traits of dioecious species under abiotic stress conditions has received limited research, especially in the case of *Amaranthus palmeri*, a fast adapting and highly competing plant. Here, we have examined the interactive effects of abiotic stress on mineral accumulation, chlorophyll *a* and *b* content, and the operating capacity of Photosystem II (PSII) in both male and female *A. palmeri* plants grown under three different intensities of white light, and under N, K or P deficiency. Mineral profiling of the leaves and stems (with inflorescence) highlighted intra- and intersexual differences in their accumulation pattern and mineral associations. Chlorophyll *a* and chlorophyll *b* were different between the male and the female plants, being slightly lower in the latter, at high light intensity towards maturity, or under K or P deficiency. Further, slight, although statistically significant differences were recorded in the chlorophyll *a*/*b* ratio, which was lower at the higher light intensity in the female, over that in the male, plants towards maturity. Chlorophyll fluorescence parameters, i.e., steady state and maximum fluorescence increased under high light intensity, whereas the PSII operating efficiency decreased in the female plants, indicating reduced PSII capacity. Sex-specific differences in *A. palmeri* showed a differential response to stressful conditions because of differences in their ontogeny and physiology, and possibly due to the cost of reproduction. We suggest that the breeding system of dioecious species has weaknesses that can be used for the ecological management of dioecious weeds without relying on the use of herbicides.

Amaranthus palmeri, one of the most problematic weeds in many row and vegetable crops worldwide, exhibits remarkable biological and physiological characteristics such as rapid growth rate, high prolific capacity, and great adaptability under biotic (e.g., crop competition) or abiotic (e.g., environmental, or chemical) stressful conditions[1,2]. These characteristics enhance its invasive potential, competitive ability[1,3,4] and its capability to develop multiple herbicide resistance to a range of herbicide families[5] resulting in high infestation levels of natural habitats and agroecosystems[3,4]. Consequently, its presence leads to increases in food production costs[3].

As a summer ephemeral species, *A. palmeri* grows well under both xerophytic and heliophytic conditions[6]. It prefers warm or moderate temperatures and thrives in soils rich in nitrogen content[6]. In addition, climate change imposes a strong selection pressure on weeds[7] that eventually adapt rapidly to changes through alterations in their biology, physiology, and phenology[8]. This is particularly true for C4 weed species, as is the case for *A. palmeri*, which has already expanded its habitable range in the northern states of USA, as well as in the southern Canadian provinces[8]. Fitzpatrick et al.[9] have emphasized the need for trait-based studies since phenotypic variations can affect plant-environment trade-offs, thus deepening our understanding of species interaction with its environment and consequently the species habituation. However, the habituation of a species, an important phenomenon per se, does not intrinsically expand the behavioral repertoire of an organism[10]. This is of a particular importance since the obligatory outcrossing of *A. palmeri*, due to its dioecious nature, results in high genetic diversity, broad adaptability, and enhanced evolutionary capacity[11,12]. Furthermore, *A. palmeri* is known to exhibit great plasticity to external stimuli and has distinguishable sex differentiation under a wide range of environments[13,14]. Research

[1]School of Agriculture, Department of Agriculture, University of Ioannina, Kostakii, 47100 Arta, Greece. [2]Crop Soil and Environmental Sciences, University of Arkansas, Fayetteville, AR 72704, USA. [3]PepsiCo Inc., St. Paul, MN 55108, USA. [4]Plant Biology, Biochemistry and Biophysics, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA. ✉email: nkorres@uoi.gr; nkorres@yahoo.co.uk

on the plasticity of plants has revealed morphological and allocation responses to environmental stimuli along with ontogenetic trajectories and offspring traits[15]. Further, dioecious species have evolved sex-specific secondary traits, i.e., secondary sexual dimorphism and functional differences[16], some of them attributed to differences in their reproductive functions[17], but also to physiological and ontogenetic functions during their vegetative growth[18–20]. For example, greater height and biomass production in the female than in the male *A. palmeri* plants grown under field conditions have been reported[4,21]. In addition, as reported by Delph et al.[22] and Morales et al.[23], sexual dimorphism in dioecious species is expressed through many ecophysiological traits including vegetative mass, leaf mass, leaf thickness, flower size, and CO_2 exchange rate. This differential response is a fitness strategy by the female plants in their "effort" to reproduce[17,24] and is related to physiological functions of the plant such as photosynthetic performance, water use efficiency or even phenology[25,26]. Another characteristic of dioecious species, "division of labor"[27], is expressed through the manifestation of resource acquisition and allocation. In complex and ever-changing environment, resources are often scarce with unequal spatial and temporal distribution[28]. As plants are simultaneously exposed to diverse abiotic stresses, such as mineral deficiency, and/or inadequate light environment, they have developed specific mechanisms to tackle different types of abiotic stress they are exposed to during their growth[29].

Deficiency of any of the essential minerals, such as nitrogen (N), phosphorous (P) and potassium (K), restricts plant growth and development through reduction in photosynthetic efficiency. However, plants are concurrently exposed to various abiotic stresses such as mineral deficiency and varying regimes of light intensity[30]. In *A. palmeri*, light environment affects acclimation through adjustments in plant ontogeny, especially of morphological traits during both its vegetative and reproductive stages[31]. Investigating and characterizing the complex interactions between weeds and their environment in natural habitats and agroecosystems is vital for understanding the dynamics of species occurrence in both natural and agricultural-based habitats[31]. To the best of our knowledge, limited research has demonstrated the differential response of dioecious male and female *A. palmeri* plants to abiotic stress related to N, P and K deficient environment, as well as to varying light intensities[14]. The research described here clearly reveals key differences in sex-specific secondary traits related to biological and functional characteristics between the male and the female *A. palmeri* plants grown under different light intensities and NPK deficient environment. We suggest that the male and the female plants, grown under stress conditions, initiate secondary sex-specific traits related to mineral content accumulation, chlorophyll content and photosynthetic capacity. Specific research questions addressed here include: (i) Does *A. palmeri* mineral content accumulation differ between male and female plant organs (leaves vs. stems including inflorescences) when they are exposed to abiotic stress? (ii) Do male and female *A. palmeri* plants exhibit differences in chlorophyll content when grown under abiotic stress? (iii) Is the operating efficiency of PSII, as evaluated by measurements of chlorophyll fluorescence, of *A. palmeri* male plants less functionally plastic than that of the female plants under abiotic stress? Answers to these questions have revealed important information on the ontogenetic and physiological secondary traits that allow for greater competitive ability and invasive potential of dioecious species such as *A. palmeri*. However, the dioecious nature of the species conceals weaknesses that can be used for the ecological management of dioecious weeds without relying solely on the use of herbicides.

Results

We present below experimental results related to the similarities and differences between the male and female plants of *A. palmeri* grown under abiotic stress; effects where sex is not involved are not included here.

Inter- and intrasexual accumulation of minerals in *A. palmeri*. To begin with, analysis of the mineral content of untreated male and female *A. palmeri* plants showed no significant differences (P = 0.451), both in the leaves and the stems. However, mineral content between the leaves and the stems was significantly (P < 0.001) affected by NPK deficiency, which was different in male and female *A. palmeri* plants (Table 1 and SI, Table S1). Light intensity did not cause any effect on the mineral content of stems and leaves, either in the male or the female plant (SI, Table S1). Under NPK deficient condition, the mineral content of male and female plants was significantly different for all the elements measured, except for Fe, Cu and B (Table 1 and SI, Table S1). Mineral content in the stems, mostly under K and P deficiency, was found to be greater in the female compared to the male plants. For example, the stem N content was 46% and 91% greater in the female than that in the male plants, under K and P deficiency respectively. Similarly, Ca content in the stems of the female plants was 30% greater than that in the male plants for both K and P deficiency. Likewise, Mg was higher by 35% in the female plants compared to the male plants for each NPK treatment. Similarly higher differences were recorded for all other stem mineral contents in the female compared to the male plants except Fe, Cu and B (Table 1).

Further, we observed intrasexual differences (P < 0.001) between the stem and the leaf mineral content of *A. palmeri* female plants. In particular, the mineral content in the stems of the female plants was on the average 8.4-, 3.3- and 5.7-fold greater than that in the leaves under N, P and K deficiency respectively (Table 1). On the contrary, the mineral content in the stem of the male plants was 4.5-fold, 2.4-fold, and threefold than that in the leaves under N, P and K deficient environment (Table 1 and SI, Table S1) respectively.

In the female plants, we observed positive correlations between Cu–Zn, Zn–Mn, and Cu–Fe (in the leaves), and Zn–Ca, B–Mg (in the stems) (Fig. 1A,C and SI, Tables S2, S3) whereas in the male plants the positive correlations were between N–P, N–K, P–Mg, Ca–S, and B–Mn contents in both stem and leaves (Fig. 1B, D). Negative correlations between B–Fe (in stems and leaves), K–Fe (in stems only) and P–Cu (in leaves only) contents were observed in the male plants (Fig. 1B, D and SI, Tables S2, S3). In the female plants, however, negative correlations were recorded between B–Cu, Cu–Mn, Zn–S, Zn–Fe, N–S (in leaves only) and P–Zn, Zn–S and Ca–Fe (in stems only) (Fig. 1A, C and SI, Tables S2, S3). On the other hand, a negative correlation between the leaf P–K content, and positive correlations between the Mn–Fe content (in stems and leaves), N–Cu content (in leaves

A. palmeri sex	NPK deficiency	Plant organ	N	P	K	Ca	Mg	S	Na	Mn	Zn
			ppm								
Female	N	L	0.004[e]	0.001[fg]	0.009[g]	0.006[f]	0.005[e]	0.002[e]	0.002[d]	0.030[d]	0.002[f]
		S	0.031[d]	0.011[c]	0.065[de]	0.051[de]	0.041[d]	0.017[d]	0.019[c]	0.233[c]	0.014[de]
	P	L	0.037[d]	0.002[efg]	0.057[def]	0.061[d]	0.043[d]	0.020[d]	0.013[cd]	0.282[c]	0.021[d]
		S	0.123[b]	0.008[cd]	0.170[b]	0.212[b]	0.150[b]	0.071[b]	0.041[b]	0.964[a]	0.069[a]
	K	L	0.034[d]	0.005[de]	0.039[efg]	0.048[de]	0.037[d]	0.017[d]	0.014[cd]	0.186[cd]	0.011[ef]
		S	0.201[a]	0.029[a]	0.230[a]	0.280[a]	0.219[a]	0.102[a]	0.077[a]	1.120[a]	0.063[a]
Male	N	L	0.004[e]	0.001[fg]	0.008[g]	0.006[f]	0.006[e]	0.002[e]	0.002[d]	0.029[d]	0.002[f]
		S	0.018[de]	0.006[d]	0.035[efg]	0.029[ef]	0.026[de]	0.009[de]	0.010[cd]	0.135[cd]	0.009[ef]
	P	L	0.037[d]	0.002[efg]	0.051[ef]	0.056[de]	0.039[d]	0.019[d]	0.013[cd]	0.246[c]	0.018[de]
		S	0.010[c]	0.006[d]	0.130[c]	0.150[c]	0.104[c]	0.049[c]	0.033[b]	0.647[b]	0.049[b]
	K	L	0.032[d]	0.004[def]	0.026[fg]	0.045[de]	0.042[d]	0.015[d]	0.010[cd]	0.158[cd]	0.008[ef]
		S	0.108[bc]	0.016[b]	0.090[d]	0.162[c]	0.147[b]	0.054[c]	0.033[b]	0.552[b]	0.031[c]

Table 1. Effects L = leaf, S = stem (incl. inflorescences). Values with the same letter (lower case and superscript) within each column are not different at $a = 0.05$ of *A. palmeri* sex × NPK deficiency × plant organ on leaf and stem mineral content at harvest averaged across light intensities.
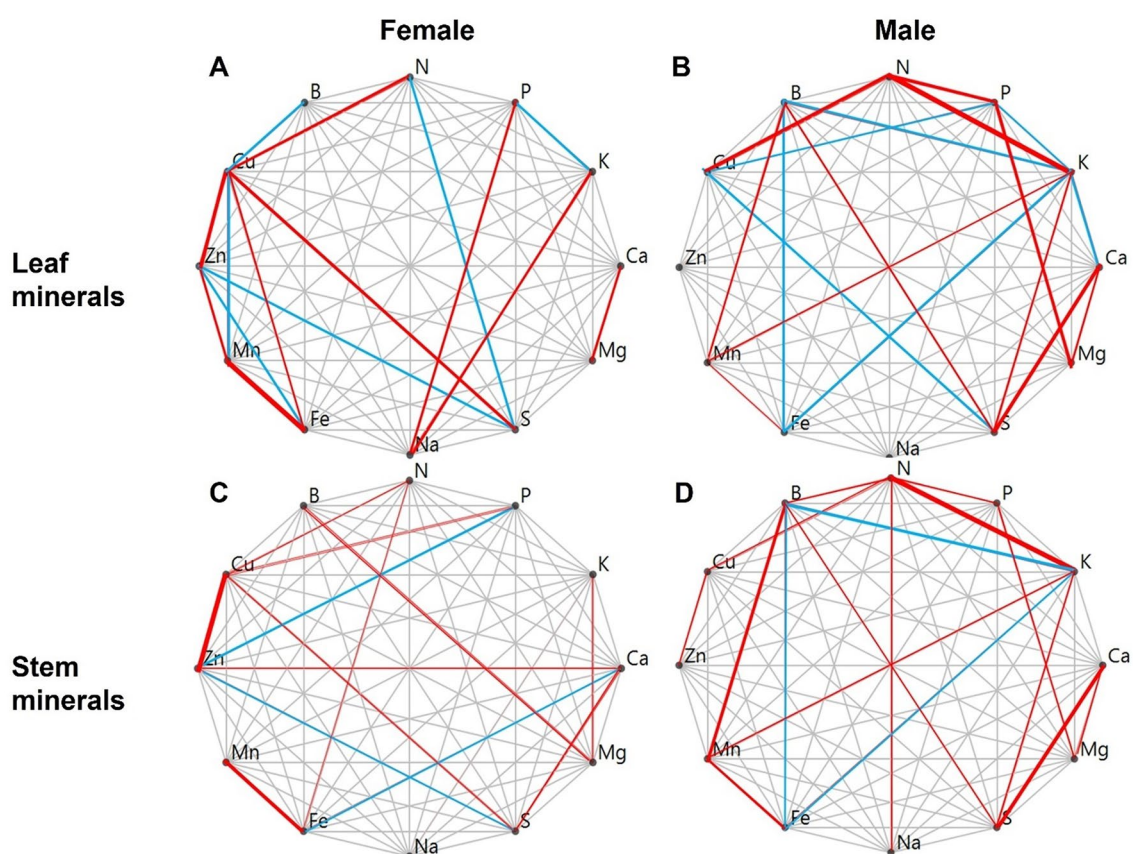

Figure 1. Mineral content of the leaves and the stems including inflorescences of the male and female *Amaranthus palmeri* plants. Red and blue lines connecting two minerals within each graph represent a positive or a negative partial correlation between these two minerals respectively. The thicker the line the higher the significance level (see Tables S2, and S3 for correlation coefficients).

only) and Cu–Zn, Ca–S content (in stems only) were recorded in both male and female *A. palmeri* plants (Fig. 1 and SI, Tables S2, S3).

In general, correlations between the micro-nutrients (i.e., Cu, Zn, Mn, S and Fe) were mostly observed in the leaves of female plants compared to that in the male plants (Fig. 1A, B). We note that the correlations between the leaf minerals were mostly observed for the macro-nutrients (i.e., N, P, K) and the cations such as Ca, and Mg (Fig. 1 and SI, Tables S2, S3). Interestingly, comparable positive and/or negative correlations between the

mineral contents in the stems and the leaves, such as for N–P, N–K, P–Mg, Ca–S, B–Mn, Mn–Fe, Fe–B, were observed in male plants only (Fig. 1B, D and SI, Tables S2, S3). In contrast, correlations between the leaf and the stem mineral content in the female plants did not show a clear pattern for most cases, with the exception for Cu–Zn and for Mn-Fe (Fig. 1A, C and SI, Tables S2, S3).

A negative correlation between P–K was observed in the leaves, but not in the stems, of *A. palmeri* female plants. Further, the positive correlation, observed between Cu–K, Zn–Ca and Cu–P content in the stems, was not found in the leaves of female plants (Fig. 1A, C and SI, Tables S2, S3).

Chlorophyll *a* and chlorophyll *b* content of male and female *Amaranthus palmeri* plants under abiotic stress. Nitrogen deficiency, when white light intensity was increased, caused a significant decrease in both chlorophyll *a* (P = 0.007) and chlorophyll *b* (P = 0.006) content, averaged across the sampling times; this result was independent of the sex of the *A. palmeri* plants (Fig. 2A,B and SI, Table S4). However, as compared to the male plants, the female plants had higher chlorophyll *a* and *b* content at low intensity (150 μmol photons m^{-2} s^{-1}) of white light. Under K deficiency, at 450 μmol photons m^{-2} s^{-1}, chlorophyll *a* and chlorophyll *b* increased significantly in the female plants compared to that in the male plants. (Fig. 2A,B and SI, Table S4). However, no difference in the chlorophyll content was recorded between the female and the male plants, grown under P deficiency at medium (450 μmol photons m^{-2} s^{-1}) white light intensity. The content of chlorophyll *a* and chlorophyll *b* in female compared to male plants, under PK deficiency and high white light intensity (1300 μmol photons m^{-2} s^{-1}) showed a significant decrease (P = 0.007 for chlorophyll *a*, and P = 0.006 for chlorophyll *b*; see Fig. 2A,B and SI, Table S4).

Effects of white light intensity on chlorophyll *a* and chlorophyll *b* in *Amaranthus palmeri* male and female plants. Female plants, grown at 150 μmol photons m^{-2} s^{-1} of white light, had higher chlorophyll
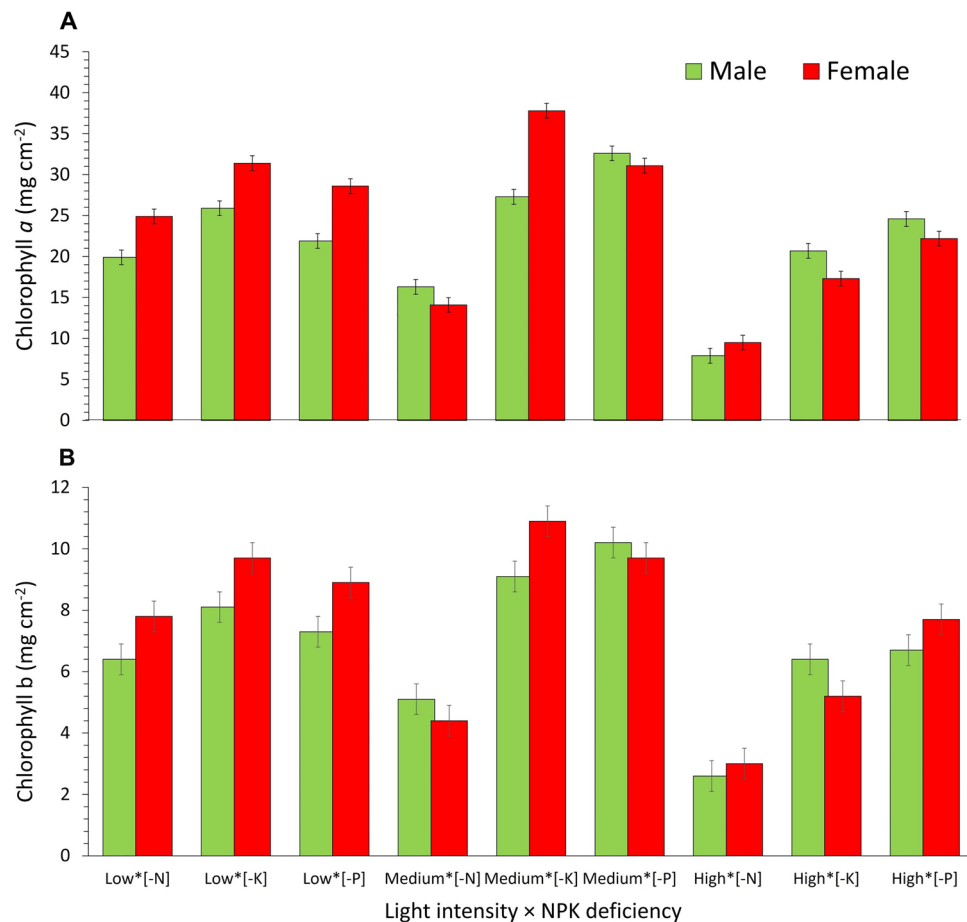

Figure 2. (**A**) Leaf chlorophyll *a* content, and (**B**) leaf chlorophyll *b* content of male and female *Amaranthus palmeri* plants as affected by white light intensity × NPK deficiency averaged across 5 sampling times throughout the experimental period; Values of chlorophyll *a* and chlorophyll *b* content for male and female *A. palmeri* plants on the Y-axis stand for the statistically defined interaction of white light intensity × NPK deficiency shown on the X-axis. Low, Medium, and High light intensities refer to 150, 450 and 1300 μmol photons m^{-2} s^{-1}, respectively; [–N], [–K], and [–P] refer to NPK deficiency. Vertical bars represent Least Significant Difference at *a* = 0.05.

α and chlorophyll *b* content at their earlier life stages (i.e., 14, 21 and 28 days after treatment initiation-DAT; see Fig. 3A,B and SI, Table S4). However, with increasing time (i.e., 35 and 42 DAT), chlorophyll α and chlorophyll *b* content, averaged across NPK deficiencies, was slightly higher, and statistically significant ($P < 0.0001$), in male compared to female plants (Fig. 3A,B), grown at 1300 μmol photons m^{-2} s^{-1} of white light. Gradual increases in white light intensity led to decreases in chlorophyll *a* and *b* content, especially at high light intensity, in both male and female *A. palmeri* plants. In addition, both *Amaranthus palmeri* genders showed no differences in total chlorophyll content, averaged across NPK deficiency, when they were grown under ambient light compared to these grown at high light intensity ($P < 0.0001$) (Table S5 and Fig. S1). Consequently, the chlorophyll *a/b* (Chl α/*b*) ratio was reduced ($P = 0.0002$) 28 DAT onwards in both male and female *A. palmeri* plants, compared to plants that were kept at lower intensities of white light (150 and 450 μmol photons m^{-2} s^{-1}) (Fig. 4 and SI, Table S4). Furthermore, the Chl α/*b* ratio at 38 and 45 DAT at high light intensity (1300 μmol photons m^{-2} s^{-1}) was slightly, although significantly greater ($P = 0.0002$) in the male compared to that in the female plants (Fig. 4 and SI, Table S4).

The total chlorophyll content in untreated *A. palmeri* plants throughout the experimental period showed no significant differences between the male and female plants (Fig. S1). Chlorophyll *a* content was 31.7 and 32.5 mg cm^{-2} ($P = 0.722$) and chlorophyll *b* was 9.97 and 10.25 mg cm^{-2} ($P = 0.712$) for male and female plants, respectively. In addition, no statistically significant interaction between the untreated male and female plants was observed ($P = 0.406$ and $P = 0.478$ for chlorophyll *a* and chlorophyll *b*, respectively) when the sampling time was treated as a fixed variable. Likewise, no significant difference between the untreated male and female *A. palmeri* plants was observed for Chl *a/b* ratio ($P = 0.351$) when the sampling time was treated as a fixed variable.

High white light intensity reduces the operating capacity of Photosystem II in the female plants of *Amaranthus palmeri*.

Chlorophyll fluorescence is a reliable tool to study and quantify photosynthesis and responses of plants to environmental changes and abiotic stresses as these in our research[32–34]. Steady state fluorescence (F'_S), used in our research, is the fluorescence emission from a light-adapted leaf induced by a non-saturating irradiation[35]. On the other hand, maximum fluorescence (F'_M) describes the state of PSII when all its reaction centers are closed or reduced, i.e., the electron transport carrier Q_A (the first bound plastoquinone). Analysis of chlorophyll fluorescence parameters (F'_S, F'_M) and Φ_{PSII} revealed an statistically sig-
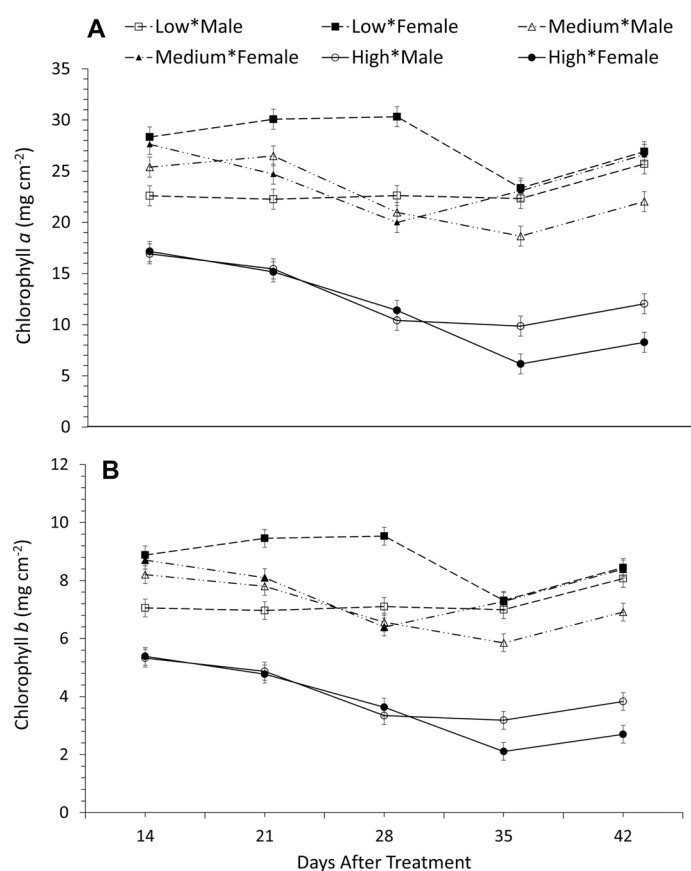

Figure 3. (**A**) Leaf chlorophyll α content, and (**B**) leaf chlorophyll *b* content in male and female *A. palmeri* plants at 3 different white light intensities as measured throughout the experimental period (DAT) and averaged across NPK deficiency treatments. Low, Medium, and High refer to 150, 450 and 1300 μmol photons m^{-2} s^{-1} (of light) respectively for male or female plants; vertical bars represent Least Significant Difference at $a = 0.05$.
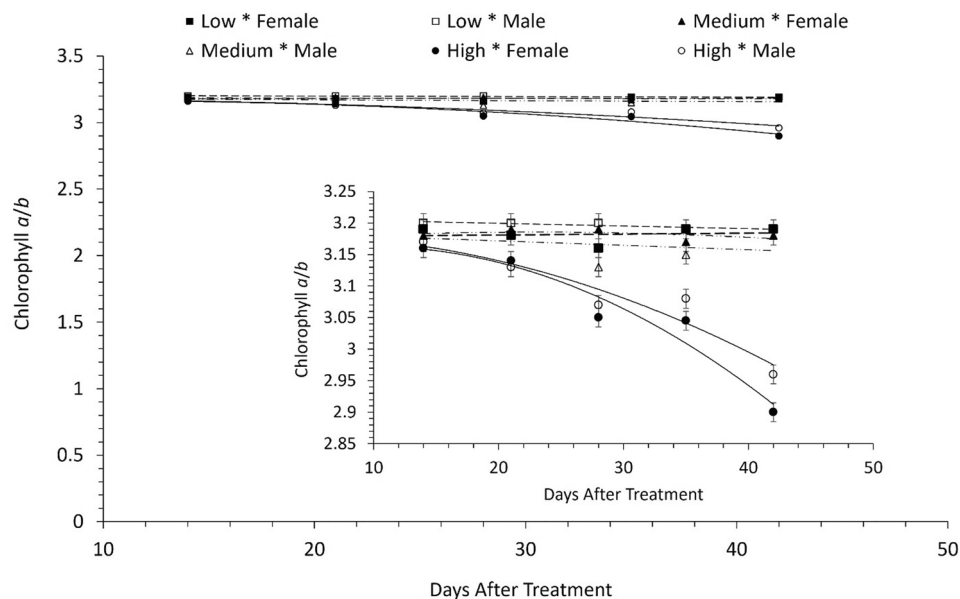
Figure 4. Effects of white light intensity on leaf chlorophyll *a*/b ratio as measured throughout the experimental period (DAT) for *Amaranthus palmeri* male and female plants and averaged across NPK deficiency treatments. The Y-axis, in the inset figure, has been rescaled to improve the readability of the figure. Squares, in the inset figure, represent the chlorophyll *a*/*b* (Chl *a*/*b*) ratio in male plants (open squares), and in female plants (closed squares) both under low light intensity (150 μmol photons $m^{-2} s^{-1}$) (see dashed line). Open triangles are for the data from the male plants, and closed triangles are for female plants, both under medium light intensity (450 μmol photons $m^{-2} s^{-1}$) (long dashed line with dots). The Chl *a*/*b* ratio under high light intensity (1300 μmol photons $m^{-2} s^{-1}$) (solid line) is shown for both the male (open circles) and the female (closed circles) plants. Vertical bars represent LSD = Least Significant Differences at *a* = 0.05.

nificant interaction between the white light intensity, the sex of *A. palmeri* and the sampling time (P < 0.0001) (SI, Table S6). We observed significant differences between the male and the female plants at high intensity (1300 μmol photons $m^{-2} s^{-1}$) of white light, 28 DAT onwards in both steady state chlorophyll fluorescence (F′$_S$) (P = 0.09) and maximum fluorescence (F′$_M$) (P = 0.03) (Fig. 5A,B and SI, Table S6).

Furthermore, male plants had a greater (22.5%) Φ$_{PSII}$ value (P = 0.04) at 1300 μmol photons $m^{-2} s^{-1}$, 35 and 42 DAT compared to female plants. However, both male and female plants showed lower Φ$_{PSII}$ values at higher light intensity (1300 μmol photons $m^{-2} s^{-1}$) in comparison to those that were kept at 150 and 450 μmol photons $m^{-2} s^{-1}$ of white light. More specifically, Φ$_{PSII}$ values were 44.5 and 66.1% lower for male and female plants respectively at high light intensity compared to Φ$_{PSII}$ values at low white light intensity. Similarly, the Φ$_{PSII}$ values at 1300 μmol photons $m^{-2} s^{-1}$ were 26 and 55.3% lower for male and female plants respectively at high light intensity compared to medium light intensity) (Fig. 6 and SI, Table S6).

Analysis of untreated controls of *A. palmeri* showed no differences between male and female plants for chlorophyll fluorescence parameter F′$_S$ (P = 0.613) or between *A. palmeri* sex × sampling time (P = 0.056) (SI, Fig. S2). Likewise, no differences were obtained for F′$_M$ between the male and the female plants (P = 0.992) or when these parameters were analyzed against the sampling time (P = 0.586) (SI, Fig. S2). F′$_S$ values for female and male *A. palmeri* plants, averaged across sampling times, were 189.8 and 213.4 (P = 0.613) whereas F′$_M$ measured were at 495.1 and 520.4 (P = 0.992). Likewise, no differences were obtained for Φ$_{PSII}$ between the male and female plants (P = 0.491), or when the values of Φ$_{PSII}$ of both male and female plants were analyzed against the sampling time (P = 0.109). Φ$_{PSII}$ of untreated *A. palmeri* male and female plants was 0.72 and 0.71 (P = 0.491) respectively.

Effects of NPK deficiency on F′$_M$ and Φ$_{PSII}$ parameters. Significant differences between male and female plants were observed in F′$_M$, and Φ$_{PSII}$ values when they were under mineral deficient conditions (SI, Table S6). F′$_M$ increased at 14 DAT onwards in both male and female plants independent of mineral deficiency (Table 2). However, the female plants had significantly higher F′$_M$ values, 5.5% higher F′$_M$, under N deficiency compared to those in the male plants 42 DAT. In addition, the F′$_M$ values for the male plants, when they were grown under N deficiency, were lower compared to the corresponding values under P and K deficiency, particularly 28 DAT onwards, but interestingly, this was not the case for the female plants (Table 2). Further, higher Φ$_{PSII}$ values, ranging between 7.3% and 10.5%, were obtained for the male compared to female plants 28 DAT onwards under P deficiency, and to lesser extent under K and N deficiency (Table 2).
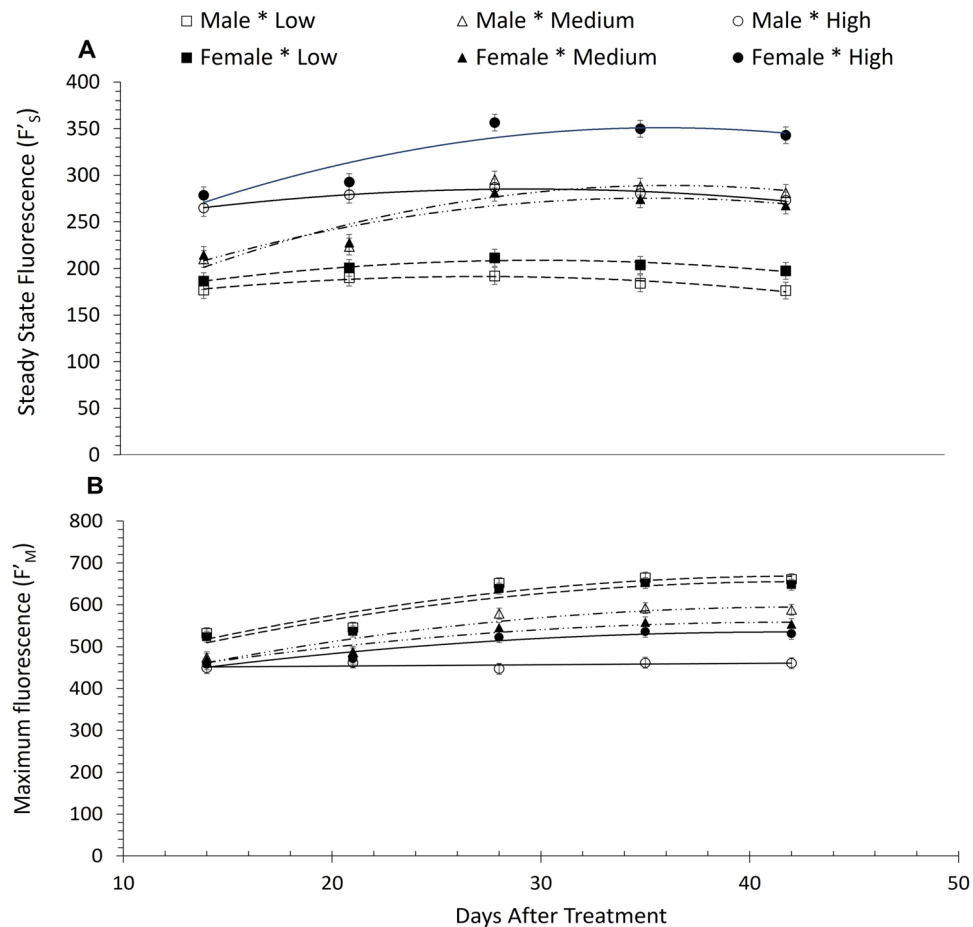
Figure 5. (**A**) Steady state chlorophyll *a* fluorescence (F′$_S$) for leaves; and (**B**) Maximum chlorophyll *a* fluorescence (F′$_M$) from the leaves of male and female *A. palmeri* plants, grown at 3 different white light intensities, measured throughout the experimental period (DAT) and averaged across NPK deficiency treatments. Open and closed squares: Male and female F′$_S$ and F′$_M$ respectively under low light intensity (150 μmol photons m^{-2} s^{-1}) (dashed line); open and closed triangles: Male and female F′$_S$ and F′$_M$ respectively under medium light intensity (450 μmol photons m^{-2} s^{-1}) (long dashed line with dots); open and closed circles: male and female F′$_S$ and F′$_M$ respectively under high white light intensity (1300 μmol photons m^{-2} s^{-1}) (solid line). Low, Medium, and High refer to 150, 450 and 1300 μmol photons m^{-2} s^{-1} respectively, used for growing male or female plants; vertical bars represent Least Significant Difference at $a = 0.05$.

Discussion

Secondary sexual dimorphism is the differentiation of traits between the male and the female plants, which is not directly linked to gamete production[16]. In this research, we examined the secondary sexual dimorphism related to functional characteristics, such as the mineral content, the dynamics of changes in chlorophyll content, and chlorophyll fluorescence parameters, related to photosynthesis, in both male and female plants of *A. palmeri*.

Changes in the content of minerals in plant organs are related to dry matter accumulation[36]. Female plants accumulate higher amount of minerals in the stems (including inflorescences) than in the leaves in contrast to the male plants which accumulate relatively equal amounts in both leaves and stems (Table 1). The accumulation of mineral elements, especially N, P, K, and Mg in the stem and inflorescences, is particularly important for seed filling[36,37]. Further, the extent of mineral accumulation in plant organs depends upon the interrelationship between the "sink-size" e.g., the biomass produced or the number of seeds with the "source-size" e.g., leaf area or specific leaf area[38], but also whether the plants and seeds are inadequately supplied with mineral nutrients[39], as shown in this paper. The NPK deficiency was found to be the most important factor affecting the mineral content in the stems (including inflorescences) and in the leaves of male and female *A. palmeri*; this is especially the case for the stems (including inflorescences) of female plants (Table 1 and SI, Table S1). Therefore, it is reasonable to suggest that the higher accumulation of minerals in the stems (and inflorescences) compared to the leaves in the female plants are related to the reproductive demand for resources, a sex-specific characteristic in dioecious species, as already noted by Nowak-Dyjeta et al.[40]. The female plants are more efficient in gathering or using certain resources when compared to the male plants[41]. Natural selection in dioecious species, that initiated the evolution of sexual dimorphism[42], allows *A. palmeri* female plants to reproduce successfully under resource limiting environments by exploiting the resources more efficiently than those in the male plants. No effect of
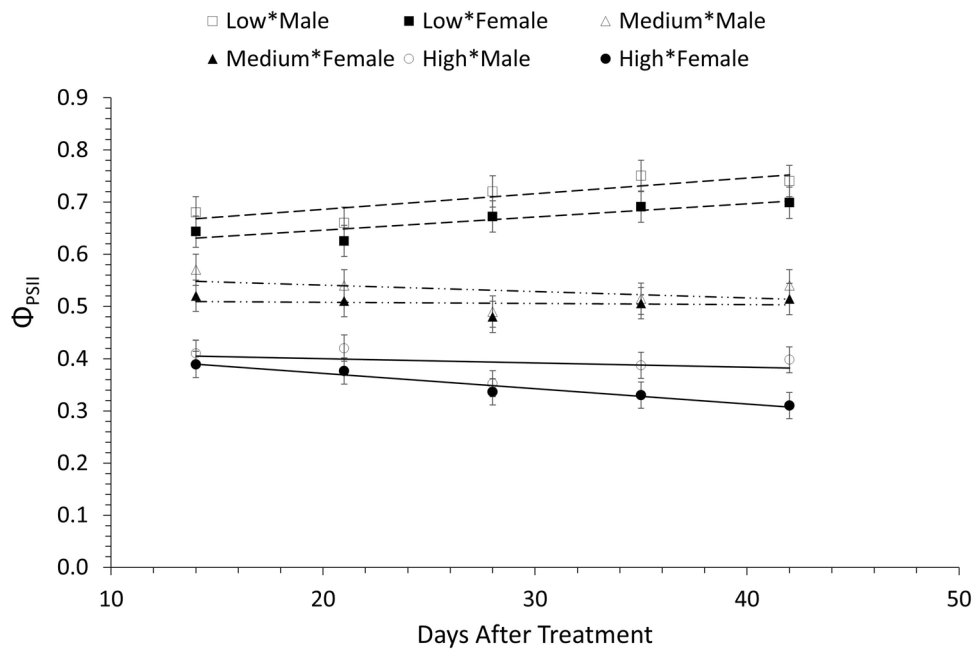
Figure 6. Photochemical operation efficiency of PSII (Φ_{PSII}) in male and female *A. palmeri* plants, grown at 3 different white light intensities measured throughout the experimental period (DAT) and averaged across NPK deficiency treatments. Open and closed squares: Φ_{PSII} of male and female plants, grown under low light intensity (150 µmol photons m^{-2} s^{-1}) (dashed line); open and closed triangles: Φ_{PSII} (of male and female plants) under medium light intensity (450 µmol photons m^{-2} s^{-1}) (long dashed line with dots); open and closed circles: F'$_S$ and F'$_M$ (of male and female plants) grown under high white light intensity (1300 µmol photons m^{-2} s^{-1}) (solid line). Vertical bars represent Least Significant Difference at $a = 0.05$.

DAT	Female			Male		
	[–N]	[–P]	[–K]	[–N]	[–P]	[–K]
F'$_M$						
14	475.7^{kl}	507.0^{i-k}	470.5^{kl}	473.2^{kl}	457.8^l	462.0^l
21	488.7^{j-l}	520.7^{h-j}	481.8^{kl}	533.7^{g-i}	544.0^{e-i}	477.4^{kl}
28	569.4^{a-g}	581.9^{a-e}	554.7^{b-h}	538.0^{f-i}	564.4^{a-g}	575.5^{a-f}
35	583.2^{a-d}	595.1^a	567.4^{a-g}	551.7^{d-h}	577.5^{a-e}	589.0^{abc}
42	579.0^{a-e}	590.4^{ab}	563.3^{a-g}	547.98^{g-h}	573.5^{a-f}	585.4^{a-d}
Φ_{PSII}						
14	0.51^{e-h}	0.49^{g-i}	0.55^{a-c}	0.57^{ab}	0.54^{a-e}	0.51^{e-h}
21	0.52^{c-h}	0.49^{hi}	0.53^{b-g}	0.54^{a-e}	0.55^{a-e}	0.49^{g-i}
28	0.52^{c-h}	0.51^{e-h}	0.46^i	0.54^{a-e}	0.55^{a-e}	0.49^{hi}
35	0.54^{a-e}	0.51^{c-h}	0.49^{hi}	0.54^{b-f}	0.57^{ab}	0.52^{c-h}
42	0.55^{a-d}	0.52^{c-h}	0.50^{f-i}	0.51^{d-h}	0.58^a	0.53^{c-h}

Table 2. Effects of NPK deficiency on *A. palmeri* sex on values of F'$_M$ and Φ_{PSII} throughout the experimental period. Values for each response variable separately with the same letter are not different at $a = 0.05$. Letters were consolidated to facilitate the readability of the table. *DAT* days after treatment.

light intensity on stem or leaf mineral content of *A. palmeri* sex was observed in our research (SI, Table S1). Currently, a controversy exists about the effects of light intensity on the nutrient content of plant organs. Li et al.[43], for example, have shown that low white light supply significantly decreases the content of leaf N in soybean [*Glycine max* (L.) Merr] plants, whereas Bouma[44] has shown that when plants were grown at low white light intensities, P content was higher in all parts of the subterranean clover (*Trifolium subterraneum* L.) plant. Therefore, it is reasonable to suggest that the mineral content allocation and accumulation in plant organs, exposed to a range of light intensity, is a species-specific storage strategy to cope with light stress. Korres et al.[14] have shown that female plants of *A. palmeri* respond to shading by stem elongation, whereas the male plants respond by increasing specific leaf area.

In this current paper, we have clearly observed sex-specific intra- and intersexual correlations between leaf and stem mineral content (Fig. 1 and SI, Tables S2, S3). The synergistic interaction between N and K, two important minerals in regulating leaf photosynthesis, has been well documented in other plants[45]; however, such association is absent in *A. palmeri* female plants (Fig. 1A,C and SI, Table S2). In contrast, Ca and Mg, two important elements for determining the structural rigidity of the cell wall[46] and chlorophyll production[47], respectively, have been shown here to be positively correlated in leaves of both *A. palmeri* sexes, but only in the stems of the male plants (Fig. 1D, and SI, Tables S2, S3). The positive correlation between Ca and Mg indicates a synergistic interaction between these minerals in both male and female plants. Lopez-Lefebre et al.[48] showed that Ca exerts a positive effect on N assimilation by activating enzymes responsible for N assimilation. However, no such relationship was observed in our current research (Fig. 1 and SI, Tables S2, S3) suggesting a species specificity of Ca-N association. A negative correlation was observed between K and Ca in the leaves of the male, but not in the female plants (Fig. 1A,B and SI, Tables S2, S3) indicating an antagonistic interaction between these two minerals in male plants. Iron, an essential mineral for photosynthesis and chlorophyll synthesis[49], is another mineral that is shown here to be associated negatively with K in both leaves and stems of male plants (Fig. 1B,D and SI, Tables S2, S3). Wu et al.[49] reported that potassium affects iron translocation, whereas Bolle-Jones[50] suggested that iron may impede the translocation of potassium.

Nitrogen is an essential component of chlorophyll[31], the content of which decreased in both male and female plants under nitrogen deficiency and when these plants were grown at high light intensity. Our results confirm the findings of Ding et al.[37] and Li et al.[43] who reported decreases in chlorophyll *a* and *b* content under N deficiency. Research on the effects of abiotic stress on sexually related secondary traits, including chlorophyll content, has been, in the past, conducted mostly as mono-factorial experimental designs. However, plants are exposed to multidimensional environmental changes that occur concurrently, as shown in our paper. Therefore, it is rational to suggest that increased light intensity which can potentially damage or even destroy chlorophyll *a* and *b*[51,52] in conjunction with N deficiency, has possibly caused decreases in the content of both chlorophyll *a* and chlorophyll *b* in both sexes. On the contrary, the effects of P and K deficiencies have been shown to be less deleterious on the content of chlorophyll *a* and *b* compared to that by N deficiency; chlorophyll *a*, for example, shows a fluctuating response between male and female plants under P and K deficiencies and different white light intensities, indicating the importance of N deficiency on chlorophyll content. Nevertheless, at high light intensity (i.e., 1300 μmol photons m^{-2} s^{-1}) and PK deficiencies, the male plants had greater chlorophyll *a* content compared to the female plants (Fig. 2A). A similar effect was observed for chlorophyll *b* under high light intensity (i.e., 1300 μmol photons m^{-2} s^{-1}) and K deficiency (Fig. 2B). On the contrary, female plants exhibited greater chlorophyll *a* and *b* content at low and medium light intensities (150 and 450 μmol photons m^{-2} s^{-1}) and N, P or K deficiency (Fig. 2A,B).

Feng et al.[52] found that light intensities in the range of 100–500 μmol photons m^{-2} s^{-1} resulted in gradual increases in both chlorophyll *a* and *b* content in soybeans. However, the light intensity used by Feng et al.[52] was much lower compared to the light intensity of 1300 μmol photons m^{-2} s^{-1} used in our research here. On the other hand, Marshall and Proctor[53] found that light intensity of 1000 μmol photons m^{-2} s^{-1} led to low chlorophyll content but high Chl *a*/b ratio in bryophytes, whereas, in barley leaves, Zivcak et al.[54] observed the opposite between low and high irradiance ranging from 100 to 1200 μmol photons m^{-2} s^{-1}. Nevertheless, decreases in the amount of chlorophyll, observed under conditions of stress such as high light intensity[51], have been attributed either to disruption in chlorophyll synthesis or increased degradation of chlorophyll due to higher activity of the enzyme chlorophyllase or due to oxidative damage with consequent destruction of chloroplasts[55,56]. In our work, higher content of chlorophyll *a* and chlorophyll *b* was observed in the male plants, at high light intensity (i.e., 1300 μmol photons m^{-2} s^{-1}) at 35 and 42 DAT compared to that in the female plants, which showed greater chlorophyll *a* and *b* content at low light intensities up to 28 DAT (Fig. 3A,B). It is important to note that both *Amaranthus palmeri* genders did not show any differences in total chlorophyll content when allowed to grow under ambient white light, lower or even medium white light intensities throughout the experimental period (Fig. S1). These effects can be attributed to ontogenetic differences between *A. palmeri* sexes. Korres et al.[14], for example, have reported a greater leaf area and specific leaf area values in the male than in the female plants at high light (i.e., 1300 μmol photons m^{-2} s^{-1}) intensity, and under NPK mineral deficiency. Therefore, it is reasonable to suggest that increases of chlorophyll content in the male plants at high light intensity (i.e., 1300 μmol photons m^{-2} s^{-1}) towards maturity reflects intersexual differences in secondary sex-specific ontogenetic traits such as leaf area and specific leaf area. Chl *a* affects the photosynthetic activity in plants[57,58]; thus, the tendency towards a higher content of chlorophyll *a* and *b* in the male plants at high light intensity (Fig. 3A,B) might partially explain their increased photosynthetic capacity mentioned below. Shade tolerance, an adaptive strategy exhibited by heliophytic photosynthetic plants in response to low light levels[59] (Valladares and Niinemets, 2008), is typically characterized by decrease of chlorophyll *a*/*b* ratio, a key parameter to judge the shade tolerance of a particular species, and other morpho-physiological traits[59–61]. Reduction of the Chl *a*/*b* ratio under low light indicates an increase in the relative abundance of Chl *b*[62]. However, Johnson et al.[63] and Murchie and Horton[64] showed only a weak association between Chl *a*/*b* ratio and shade tolerance. In addition, Kitajima[65], and Beneragama and Goto[66] reported an increase of Chl *a*/*b* ratio under low light in response to low light conditions. Similar results were obtained in this research as the Chl *a*/*b* ratio under low light intensity (150 and 450 μmol photons m^{-2} s^{-1}) was higher for both *Amaranthus palmeri* sexes when they were exposed to high irradiance level (i.e., 1300 μmol photons m^{-2} s^{-1}). Consequently, changes in Chl *a*/*b* ratio is a characteristic of species themselves and not an "absolute" indicator of shade tolerance of species in general. Further, Grieco et al.[67] have used the Chl *a*/*b* ratio as a parameter to monitor the acclimation processes of *Arabidopsis thaliana*. According to Anderson and Andersson[68], differences in this ratio reflect modifications in the stoichiometry of the photosynthetic pigment protein complexes. Therefore, lower Chl *a*/*b* ratio in female plants at 42 DAT (Fig. 4) might reflect modifications of stoichiometry between Chl *a* and Chl *b* as an adaptation mechanism, to compensate for decreases in their

photosynthetic capacity[69,70] under high light intensities[51]. Spundova et al.[71], on the other hand, have correlated decreases in Chl *a/b* ratio to the damage of the PS II light-harvesting complex, as a counteractive mechanism for photooxidative damage and consequent losses in energy utilization efficiency[72]. Decreases in energy utilization efficiency are known to result in the accumulation of excess excitation energy in the photosynthetic chain[73] and ultimately in the over-production of reactive oxygen species (ROS)[74].

Chlorophyll fluorescence is a reliable tool to study photosynthetic efficiency and responses of plants to abiotic stresses such as mineral deficiencies or light restricted environments[32–34]. Female plants of *A. palmeri* show significantly higher steady state fluorescence (F'_S) values under high light intensity, especially 28 DAT onwards, compared to the male plants, indicating a lower photosynthetic performance of the former, given the competitive nature between the two processes i.e., chlorophyll fluorescence and photosynthetic efficiency[75]. On the other hand, the higher values of maximum fluorescence (F'_M) in female *A. palmeri* plants, under high light intensity, as time progresses (i.e., 28 DAT onwards) (Fig. 5A,B) are possibly caused by decreases in the content of Chl *a*, and Chl *b*, and in the Chl *a/b* ratio, mentioned above, especially at the later stages of the life cycle of the plant. Chlorophyll *a* and Chlorophyll *b* content reduction is related to decreased PSII capacity due to decreased electron transport[32]. Φ_{PSII}, the quantum yield of PSII in the light-adapted state, is a quick indicator to determine the proportion of absorbed light by the light harvesting complex, and, thus, the efficiency of PSII[76], hence to the photosynthetic efficiency under different light intensities[77]. Several studies have shown a direct or indirect relationship between Φ_{PSII} and chlorophyll content in leaves[35,51,77,78]. In this research, Φ_{PSII} was found to be positively correlated with chlorophyll content in both male and female *A. palmeri* plants ($R^2 = 0.534$ and 0.468 for male and female plants respectively) (Fig. 7). Further, a similar trend was observed between Φ_{PSII} and chlorophyll *a* and *b* in both *A. palmeri* male and female plants. The orientation of the transition dipole properties and the aggregation status of chlorophyll molecules in chloroplasts might have been affected due to abiotic stress; here, both *A. palmeri* male and female plants were equally exposed, resulting in almost equal disruption of the energy transfer coherence, hence on the PSII operation efficiency.

Consequently, the lower Φ_{PSII} values, especially at the mature life stages of the female plants under high white light intensity (Fig. 6) seems to be caused by a low PSII operating capacity, as suggested by the low Chl *a/b* ratio[79] and observed at high white-light intensity in this research. Surprisingly, the electron transport rate (ETR) and total chlorophyll content are negatively correlated (Fig. 8), especially in female plants, with $R^2 = 0.456$ vs $R^2 = 0.274$ for female and male plants respectively (same trends were observed between ETR and chlorophyll *a* and chlorophyll *b*). This is an indication of a negative energy feedback that denotes a photoinhibition symptom, despite the positive correlation between Φ_{PSII} and chlorophyll content, which causes reduction in the operating efficiency of *A. palmeri* PSII[80], especially in the female plants. Further research is needed to find whether photoinhibition results from photodamage or it is a regulatory and protective adjustment to abiotic stress related to mineral deficiency and white light environment. It is also imperative, ultimately, to quantify these effects in relation to the mechanisms of non-photochemical quenching, including thermal dissipation and photosynthesis state transitions. Finally, investigating the dynamics between chlorophyll content and PSII operation efficiency will expand our knowledge in up- and down-stream regulatory processes in photosynthesis.

The lower Φ_{PSII} values of the female *A. palmeri* plants, compared to that of the male plants under P and to lesser extent under K and N deficiencies (Table 2) indicate the lower PS II operating efficiency of the former. Korres et al.[14] reported a lower leaf area and specific leaf area in female compared to male *A. palmeri* plants, under NPK deficiency and high light intensity treatment, which was accompanied by the presence of "albino" leaves (SI, Fig. S3), an indication of lower chlorophyll content. Further, our results on *A. palmeri* plants agree with the
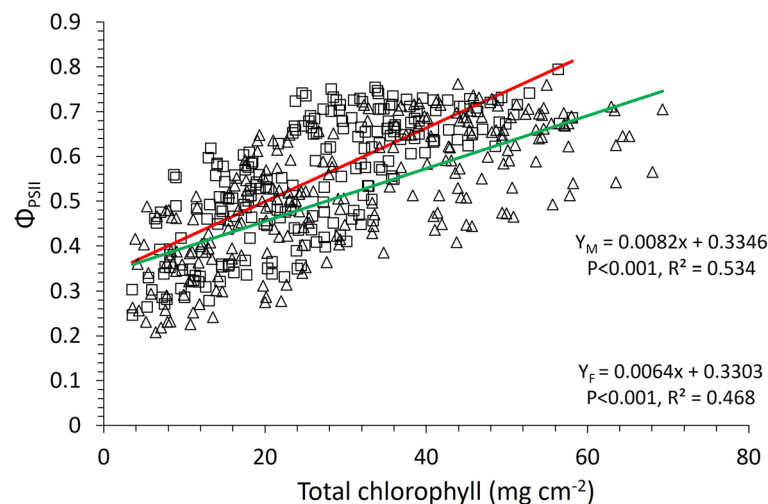

Figure 7. Correlation between the operating efficiency of PSII (Φ_{PSII}) and the total leaf chlorophyll content in male (open squares) and female (open triangles) *A. palmeri* plants. Y_M and Y_F in the equations denote Φ_{PSII} for male and female *A. palmeri* plants, respectively, and *x* denotes the total leaf chlorophyll content. A red colored curve is fitted for data from male plants, and a green colored curve is for data from female plants.
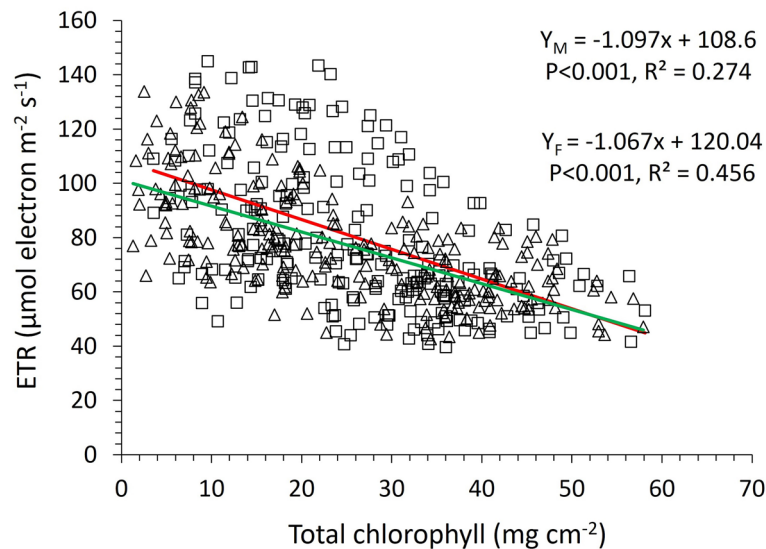
Figure 8. Correlation between electron transport rate (ETR) and total leaf chlorophyll content in male (open squares) and female (open triangles) *A. palmeri* plants. Y_M and Y_F in the equations denote ETR for male and female *A. palmeri* plants respectively, and *x* stands for the total leaf chlorophyll content. A red colored curve was fitted for data (from male plants) and a green colored curve was fitted for data from female plants.

results on nutrient deficient *Populus* plants, where the photosynthetic efficiency of male plants was higher than that of the female plants[81]. Thus, our results on the differences in male and female *A. palmeri* plants are of general importance to overall plant biology. Furthermore, our research has revealed new information on the ontogenetic and physiological secondary traits that make *A. palmeri* one of the most successful competitors in nature.

We suggest that the dioecious nature of the species has weaknesses based on which we can adjust our weed management approaches by manipulating the microclimate, e.g., light/dark conditions or mineral availability on a field scale, without relying solely on the herbicides for its control. In addition, the exogenous or endogenous stressful photodynamic treatments with the aim of decreasing the amount of chlorophyll through natural photosensitizers, for example porphyrins, merits further investigation. Rebeiz et al.[82] reported that dicotyledonous weeds including *Amaranthus retroflexus*, another species of the Amaranthaceae family, are susceptible to photodynamic treatments, especially when used in monocotyledonous crops such as maize (*Zea mays* L.), where *A. palmeri* is a major problem. However, it has been hypothesized that reduction of chlorophyll content, in plants that overinvest in chlorophyll content such as soybean, can lead to an improved leaf and canopy photosynthesis[83]. A critical point that should be noted here is that the slight, although statistically significant, differences recorded for the chlorophyll a and chlorophyll b content and the Chl *a/b* ratio between the male and the female *Amaranthus palmeri* plants, under high white light intensity, were measured using a handheld SPAD-502 device. Chlorophyll content determination using spectrophotometry after chlorophyll extraction, using an organic solvent, could possibly have given a different value for this variable. Lichtenhaler and Babani[84] investigating the differences between C4 grass species (i.e., *Miscanthus floridulus*, *M. sinensis*, *Saccharum officinarum* and *Zea mays*) grown under ambient light conditions and C3 plants (i.e., *Fagus sylvatica*, *Quercus robur*, and *Ginkgo biloba*), but not dioecious broadleaf weeds grown under abiotic stress as in our case, using a two-wavelength spectrophotometer after chlorophyll extraction with organic solvents, reported Chl *a/b* values between 3.4 and 4.5. However, a strong relationship has been reported between SPAD method and in vitro chlorophyll determination. Based on these observations future research on this topic is expected to enhance our understanding on the photochemical events in C4 plants.

Materials and methods

Plant material, experimental design, and treatment arrangements.
Amaranthus palmeri seeds were collected from a field at the University of Arkansas, Fayetteville, AR. Cuttings from 50 different F1-generation plants of each sex, were placed under NPK deficient conditions and different white light intensities. Each NPK deficiency treatment contained only 10% N, P, or K of the standard N (91.4 g NH_4NO_3 L^{-1}), P (40.3 g $NaH_2PO_4 \times 2H_2O$ L^{-1}) or K (71.4 g K_2SO_4 L^{-1}) stock solutions[85]. For low, medium, and high light, we used 150, 450 and 1300 μmol photons m^{-2} s^{-1} of white light respectively. The experimental design was based on a randomized complete block arrangement with white light intensity (one growth chamber for each white light intensity) as blocking treatment and N, or P, or K deficiency (henceforth NPK deficiency) for both male and female *A. palmeri* cuttings as randomized treatments within each block. More specifically, for each white light-intensity, each of the 20 *A. palmeri* cuttings (10 cuttings from the male parents and 10 cuttings from the female parents) were exposed to NPK deficient environment. Also, 10 plants from each *A. palmeri* sex, receiving full nutrition, were left to grow under greenhouse conditions, and used as controls. The above set of treatments was conducted

twice. A detailed description of plant material preparation, experimental design and treatments is provided as Supplementary Information (see SI, Materials and Methods S1).

Determination of mineral content in the stems and leaves of *A. palmeri* male and female plants.
At final harvest time (i.e., before the dormant stage), leaves and stems (the latter including inflorescence) taken from five randomly selected plants were dried in a forced-draft oven at 70 °C for 48 h and mechanically ground prior to the determination of their mineral content.

The determination of plant leaf and stem mineral content was made after digestion with a mixture of HNO_3/peroxide digest using hot block "digest" and inductively coupled Agron plasma (ICP) emission spectrometry, as described by Jones and Case[86]. Nitrogen content was determined by combustion, as described by Campbell[87]. To facilitate statistical analysis, mineral content was expressed in micrograms per g of leaf (or stem) dry weight.

Chlorophyll content.
Chlorophyll *a* and *b* of both male and female *A. palmeri* plants was assessed by taking five measurements from five fully expanded, light-exposed leaves in the upper half of the main stem (the same leaves were used for chlorophyll fluorescence measurements; see below) from five randomly selected plants per treatment, using a handheld SPAD-502 (Minolta Camera, Osaka, Japan). Using a leaf of 0.06 cm^2 area, SPAD-502 was used to calculate an index in "SPAD units", with an accuracy of ± 1.0 SPAD units. Chlorophyll *a*, chlorophyll *b* and total chlorophyll, in mg cm^{-2}, based on the "SPAD units", were estimated using the equations developed by Richardson et al.[88] and Porra et al.[89].

$$\text{Chlorophyll } a = 1.56 \times 10^{-6} + 3.33 \times 10^{-4}\text{SPAD} + 9.03 \times 10^{-6}\text{SPAD}^2 \tag{1}$$

$$\text{Chlorophyll } b = 5.46 \times 10^{-4} + 6.89 \times 10^{-5}\text{SPAD} + 3.37 \times 10^{-6}\text{SPAD}^2 \tag{2}$$

$$\text{Total chlorophyll} = 5.52 \times 10^{-4} + 4.04 \times 10^{-4}\text{SPAD} + 1.25 \times 10^{-5}\text{SPAD}^2 \tag{3}$$

where, SPAD = SPAD units. The chlorophyll content was only determined via SPAD measurements, which has a curvilinear relationship to the chlorophyll content of leaves as shown in Eqs. (1–3). Chlorophyll content determination spectrophotometrically after the extraction of chlorophylls was not applied in this research since the experiment was not designed for destructive sampling.

Chlorophyll fluorescence.
The photochemical efficiency (Φ_{PSII}) of Photosystem II (PSII) complex in the light-adapted state was estimated via chlorophyll fluorescence, as described by Baker[79]; here we used OS5p modulated fluorometer (Opti-Science, Tyngsboro, MA) for these measurements. For each treatment, five light-adapted, fully expanded young leaves, from the upper half of the main stem of 5 randomly selected *A. palmeri* plants, were chosen. These measurements were made weekly for both the experimental runs from the leaves of the same randomly chosen plants at the beginning of the experiment. Data was recorded 5 h after the initiation of the daily 14 h white light intensity treatment, i.e., in the photoperiod cycle, when the leaves were in a fully light-adapted state. Using exciting light of 1800 μmol photons m^{-2} s^{-1}, we measured changes in chlorophyll fluorescence: F′q or $\Delta F = F'_M - F'_S$, where F′$_S$ is the steady state fluorescence in the light-adapted state, and F′$_M$ is the maximum fluorescence induced by a saturating light pulse immediately after F′$_S$; note that F′q is a measure of the photochemical quenching of chlorophyll fluorescence by open PSII centers[77]. In light-adapted leaves at high actinic light history, with all PSII reaction centers closed measurement of F′$_M$ is underestimated even with the highest amount of saturation light[90]. In addition, saturating pulses of ~ 2000 μmol photons m^{-2} s^{-1}, while sufficient for measuring dark adapted F$_M$, may be too low to provide a true F′$_M$ under high actinic illumination. In our research we have used a method[91] which involves the use of a single multiphase saturation flash of 7000 μmol photons m^{-2} s^{-1} for 0.3 s, with linearly decreasing light intensity by 20% over another 0.5 s; during this period chlorophyll fluorescence values were recorded. With this method, accurate values of F′$_M$ were recorded[90]. The operating (i.e., photochemical) efficiency of PSII in light-adapted state of leaves (Φ_{PSII} or $\Delta F/F'_M$ ratio) and the electron transport rate were also estimated. Particularly, an estimate of electron transport rate was calculated based on Eq. (4)[92].

$$ETR = \frac{F'_M - F'_S}{F'_M} \times PAR \times 0.84 \times 0.5 \tag{4}$$

where, ETR = electron transport rate; F′$_M$-maximum chlorophyll *a* fluorescence; F′$_S$ = steady state chlorophyll fluorescence; PAR-photosynthetically active radiation at the leaf surface. The constant 0.5 corresponds to the excitation energy being divided equally between photosystems I and II, and 0.84 is the leaf absorbance coefficient for C4 plants[93,94].

Data analysis.
Analysis of variance was performed to obtain information on the effects of *A. palmeri* sex, light intensity, NPK deficiency and their statistically significant interactions on the stems (including inflorescences) and the leaves; for this, we measured the mineral content, chlorophyll *a* and chlorophyll *b* content, chlorophyll *a/b* ratio, as well as chlorophyll fluorescence parameters, namely the steady state fluorescence (F′$_S$), maximum fluorescence (F′$_M$), whereas the operating efficiency of PSII (Φ_{PSII}) and the electron transport rate were estimated from these parameters. Before the analysis of stem (incl. inflorescences) and leaf mineral content, mineral concentrations were expressed as a fraction of stem and leaf dry weights (i.e., ppm or μg/g leaf dry weight) to standardize the units of mineral concentrations. The concentration of a mineral in a plant organ can

affect the dynamics of another mineral in the same organ[38]. Therefore, an analysis of partial correlations between the leaf and the stem mineral content between *A. palmeri* sex was performed to examine whether the male and the female plants are different in their mineral trade-off strategies. Partial correlation analysis excludes the effects of confounding variables that are numerically related to the variables of interest. In addition, regressions were fitted between chlorophyll *a*, chlorophyll b, chlorophyll *a/b* ratio and fluorescence parameters F'_S, F'_M and the estimated Φ_{PSII} against the sampling time throughout the experimental period using white light intensity as a grouping factor. Correlations were also fitted between total chlorophyll and the estimates of Φ_{PSII} and ETR. JMP Pro v. 16.0 (SAS Institute, Cary, NC), and SigmaPlot v. 14.0 (Systat Software, San Jose, CA) were used for statistical analyses and curve-fitting regressions. Means were separated by Least Significant Difference (LSD) test at $a = 0.05$ significance level.

Ethics.
Plant material used in this research comply with relevant institutional, national and international guidelines and legislation.

Data availability
Data produced during this study are presented in the manuscript, however they are available from the corresponding author on reasonable request.

References
1. Webster, T. M. & Nichols, R. L. Changes in the prevalence of weed species in the major agronomic crops of the southern United States: 1994/1995 to 2008/2009. *Weed Sci.* **60**, 145–157 (2012).
2. Mesgaran, M. B., Matzrafi, M. & Ohadi, S. Sex dimorphism in dioecious Palmer amaranth (*Amaranthus palmeri*) in response to water stress. *Planta* **254**, 17 (2021).
3. Korres, N. E., Norsworthy, J. K. & Mauromoustakos, A. Effects of Palmer amaranth (*Amaranthus palmeri*) establishment time and distance from the crop row on biological and phenological characteristics of the weed: Implications on soybean yield. *Weed Sci.* **67**, 126–135 (2019).
4. Korres, N. E., Norsworthy, J. K., Mauromoustakos, A. & Williams, M. M. Jr., Soybean density and Palmer amaranth (*Amaranthus palmeri*) establishment time. Effects on weed biology, crop yield and production cost. *Weed Sci.* **68**, 467–475 (2020).
5. Heap, I. The International Survey of Herbicide Resistant Weeds. Available at: http://weedscience.org (2023).
6. Korres, N. E., Norsworthy, J. K., Brye, K. R., Vaughn, S. J. Jr. & Mauromoustakos, A. Relationships between soil properties and the occurrence of the most agronomically important weed species in the field margins of eastern Arkansas—implications for weed management in field margins. *Weed Res.* **57**, 159–171 (2017).
7. Ziska, L. H., Blumenthal, D. M. & Franks, S. J. Understanding the nexus of rising CO₂, climate change, and evolution in weed biology. *Inv. Plant Sci. Man.* **12**, 79–88 (2019).
8. Korres, N. E. & Dayan F. E. Climate change effects on crop and weeds. The need for climate-smart adaptation paradigm. *Outlooks Pest Man.* **31**:210–215 (2020).
9. Fitzpatrick, C. R., Gehant, L., Kotanen, P. M. & Johnson, M. T. J. Phylogenetic relatedness, phenotypic similarity and plant-soil feedbacks. *J. Ecol.* **105**, 786–800 (2017).
10. Adelman, B. E. On the conditioning of plants: A review of experimental evidence. *Pers. Behav. Sci.* **41**, 431–446 (2018).
11. Franssen, A. S., Skinner, D. Z., Al-Khatib, K., Horak, M. J. & Kulakow, P. A. Interspecific hybridization and gene flow of ALS resistance in *Amaranthus* species. *Weed Sci.* **49**, 598–606 (2001).
12. Montgomery, J. S., Giacomini, D. A., Weigel, D. & Tranel, P. J. Male-specific Y chromosomal regions in waterhemp (*Amaranthus tuberculatus*) and Palmer amaranth (*Amaranthus palmeri*). *New Phytol.* **229**, 3522–3533 (2021).
13. Korres, N. E. & Norsworthy, J. K. Palmer amaranth demographic and biological characteristics in wide-row soybean. *Weed Sci.* **65**, 491–503 (2017).
14. Korres, N. E., Norsworthy, J. K., FitzSimons, T., Roberts, T. L. & Oosterhuis, D. M. Differential response of Palmer amaranth (*Amaranthus palmeri*) sex to abiotic stress. *Weed Sci.* **65**, 213–227 (2017).
15. Sultan, S. E. An emerging focus on plant ecological development. *New Phytol.* **166**, 1–8 (2005).
16. Dudley, L. S. Ecological correlates of secondary sexual dimorphism in *Salix glauca* (Salicaceae). *Am. J. Bot.* **93**, 1775–1783 (2006).
17. Obeso, J. R. The costs of reproduction in plants. *New Phytol.* **155**, 321–348 (2002).
18. Sanchez-Vilas, J. & Retuerto, R. Response of the sexes of the sub-dioecious plant *Honckenya peploides* to minerals under different salt spray conditions. *Ecol. Res.* **27**, 163–171 (2012).
19. Zhang, S., Jiang, H., Peng, S., Korpelainen, H. & Li, C. Sex-related differences in morphological, physiological, and ultrastructural responses of *Populus cathayana* to chilling. *J. Exp. Bot.* **62**, 675–686 (2011).
20. Montesinos, D., Villar-Salvador, P., Garcia-Fayos, P. & Verdu, M. Sexes in *Juniperus thurifera* have different functional responses to variations in mineral availability. *New Phytol.* **193**, 705–712 (2012).
21. Webster, T. M. & Grey, T. L. Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) morphology, growth, and seed production in Georgia. *Weed Sci.* **63**, 264–272 (2015).
22. Delph, L. F., Gehring, J. L., Arntz, A. M., Levri, M. & Frey, F. M. Genetic correlations with floral display lead to sexual dimorphism in the cost of reproduction. *Am. Nat.* **166**, S31–S41 (2005).
23. Morales, M., Pinto-Marijuan, M. & Munné-Bosch, S. Seasonal, sex- and plant size-related effects on photoinhibition and photoprotection in the dioecious Mediterranean dwarf palm *Chamaerops humilis*. *Front. Plant Sci.* **7**, 1116 (2016).
24. Solbrig, O. T. Studies on the population biology of the genus Viola. II. The effect of plant size on fitness in *Viola soraria*. *Evolution* **35**, 1080–1093 (1981).
25. Rowland, D. & Johnson, N. Sexual demographics of riparian populations of *Populus deltoides*: Can mortality be predicted from a change in reproductive status?. *Can. J. Bot.* **79**, 702–710 (2001).
26. Delph, L. F. Sexual dimorphism in life history. In *Gender and sexual dimorphism in flowering plants*, M. Geber, T. E. Dawson, L. F. Delph, Eds. pp. 149–173 (Springer-Verlag, 1999).
27. Charnov, E. L. *The theory of sex allocation. Monographs in Population Biology* 18. p. 357 (Princeton University Press, 1982).
28. Gagliano, M., Vyazovskiy, V. V., Borbely, A. A., Grimonprez, M. & Depczynsk, M. Learning by association in plants. *Sci. Rep.* **6**, 38427 (2016).
29. Ramegowdaa, V. & Senthil-Kumar, M. The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. *J. Plant Phys.* **176**, 47–54 (2015).

30. Reddy, K. J. Mineral stress. In *Physiology and Molecular Biology of Stress Tolerance in Plants*. M. S. Rao, A. S. Raghavendra, K. J. Reddy KJ, Eds. Pp. 187–219 (Springer, 2006).
31. Brainard, D. C., Bellinder, R. R. & DiTommaso, A. Effects of canopy shade on the morphology, phenology, and seed characteristics of Powell amaranth (*Amaranthus powellii*). *Weed Sci.* **53**, 175–186 (2005).
32. Murchie, E. H. & Lawson, T. Chlorophyll fluorescence analysis: A guide to good practice and understanding some new applications. *J. Exp. Bot.* **64**, 3983–3998 (2013).
33. Kalaji, H. M. *et al.* Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Phys. Plan.* **38**, 102 (2016).
34. Stirbet, A., Lazar, D., Kromdijk, J. & Govindjee, G. Chlorophyll *a* fluorescence induction: Can just a one-second measurement be used to quantify abiotic stress responses?. *Photosynthetica* **56**, 86–104 (2018).
35. Lichtenthaler, H. K., Buschmann, C. & Knapp, M. How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer. *Photosynthetica* **43**, 379–393 (2005).
36. Hocking, P. J. & Pate, J. S. Mobilization of minerals to developing seeds of legumes. *Ann. Bot.* **41**, 1259–1278 (1976).
37. Ding, K. *et al.* Effects of nitrogen deficiency on photosynthetic traits of maize hybrids released in different years. *Ann. Bot.* **96**, 925–930 (2005).
38. Marschner, H. General introduction to the mineral nutrition of plants. In Inorganic plant nutrition, A. Lauchli, R. L. Bieleski, Eds. pp. 5–49 (Springer-Verlag, 1983).
39. Loneragan, J. F., Snowball, K. & Robson A. D. Remobilization of nutrients and its significance in plant nutrition. In Transport and Transfer Processes in Plants, I. F. Wardlaw, J. B. Passioura, Eds. Pp. 463–471 (Academic Press, 1976)..
40. Nowak-Dyjeta, K., Giertych, M. J., Thomas, P. & Iszkuło, G. Males and females of *Juniperus communis* L. and *Taxus baccata* L. show different seasonal patterns of nitrogen and carbon content in needles. *Acta Physiol. Plant.* **39**, 191 (2017).
41. Lei, Y. *et al.* Reproductive investments driven by sex and altitude in sympatric Populus and Salix trees. *Tree Phys.* **37**, 1503–1514 (2017).
42. Brock, M. T. *et al.* Allocation to male vs female floral function varies by currency and responds differentially to density and moisture stress. *Heredity* **119**, 349–359 (2017).
43. Li, Y. T. *et al.* Dynamic light caused less photosynthetic suppression, rather than more, under nitrogen deficit conditions than under sufficient nitrogen supply conditions in soybean. *BMC Plant Biol.* **20**, 339–342 (2020).
44. Bouma, D. Diagnosis of mineral deficiencies using plant tests. In *Inorganic Plant Nutrition,* A. Lauchli, R. L. Bieleski, Eds. Pp. 120–141 (Springer-Verlag, 1983).
45. Hou, W. *et al.* Interactive effects of nitrogen and potassium on photosynthesis and photosynthetic nitrogen allocation of rice leaves. *BMC Plant Biol.* **19**, 302 (2019).
46. Anonymous, C. A central regulator of plant growth and development: Historical perspective essay. *Plant Cell* **17**, 2142–2155 (2005).
47. Hao, X. & Papadopoulos, A. P. Effects of calcium and magnesium on plant growth, biomass partitioning, and fruit yield of winter greenhouse tomato. *Hort. Sci.* **39**, 512–515 (2004).
48. Lopez-Lefebre, L. R. *et al.* Effects of calcium on mineral uptake and growth of tobacco. *J. Sci. Food Agric.* **81**, 1334–1338 (2001).
49. Wu, L. B., Holtkamp, F., Wairich, A. & Frei, M. Potassium ion channel gene OsAKT1 affects iron translocation in rice plants exposed to iron toxicity. *Front. Plant Sci.* **10**, 579 (2019).
50. Bolle-Jones, E. W. The interrelationships of iron and potassium in the potato plant. *Plant Soil* **6**, 129–173 (1955).
51. Glime, J. M. Photosynthesis: Photoinhibition. In Bryophyte Ecology. Vol. 1. Physiological Ecology. Chapters 9–4 and 11–2, J. M. Glime, Ed. (Michigan Technological University and the International Association of Bryologists 2017), pp. 1–16. Available at: http://digitalcommons.mtu.edu/bryophyte-ecology (2022).
52. Feng, L. *et al.* The influence of light intensity and leaf movement on photosynthesis characteristics and carbon balance of soybean. *Front. Plant Sci.* **9**, 1952 (2019).
53. Marschall, M. & Proctor, M. C. V. Are bryophytes shade plants? Photosynthetic light responses and proportions of chlorophyll a, chlorophyll b and total carotenoids. *Ann. Bot.* **94**, 593–603 (2004).
54. Zivcak, M., Brestic, M., Kalaji, H. M. & Govindjee, G. Photosynthetic responses of sun- and shade-grown barley leaves to high light: is the lower PSII connectivity in shade leaves associated with protection against excess of light?. *Photosyn. Res.* **119**, 339–354 (2014).
55. Hortensteiner, S. Chlorophyll breakdown in higher plants and algae. *Cel. Mol. Life Sci.* **56**, 330–347 (1999).
56. Zhang, D. W. *et al.* Light intensity affects chlorophyll synthesis during greening process by metabolite signal from mitochondrial alternative oxidase in Arabidopsis. *Plant Cell Environ.* **39**, 12–25 (2016).
57. Sestak, Z. Limitations for finding linear relationships between chlorophyll content and photosynthetic activity. *Biol. Plan.* **8**, 336–346 (1966).
58. Papageorgiou, G. C. & Govindjee G. Chlorophyll *a* fluorescence: A signature of photosynthesis. *Adv. Photosynth. Respir.* **19**, 818 (2004).
59. Valladares, F. & Niinemets, U. Shade tolerance, a key plant feature of complex nature and consequences. *Ann. Rev. Ecol. Evol. System.* **39**, 237–257 (2008).
60. Lichtenthaler, H. K. & Babani F. Light adaptation and senescence of the photosynthetic apparatus. Changes in pigment composition, chlorophyll fluorescence and photosynthetic activity. In *Chlorophyll Fluorescence: A Signature of Photosynthesis*, Papageorgiou, G. C. & Govindjee G., Eds. pp. 713–736 (Kluwer Academic Publishers, 2004).
61. Givnish, T. J. Adaptation to sun and shade: A whole-plant perspective. *Aus. J. Plant Physiol.* **15**, 63–92 (1988).
62. Dale, M. P. & Causton, D. R. Use of the chlorophyll a/b ratio as a bioassay for the light environment of a plant. *Funct. Ecol.* **6**, 190–196 (1992).
63. Johnson, G. N., Scholes, J. D., Horton, P. & Young, A. J. Relationships between carotenoid composition and growth habit in British plant species. *Plant Cell Environ.* **16**, 681–686 (1993).
64. Murchie, E. H. & Horton, P. Contrasting patterns of photosynthetic acclimation to the light environment are dependent on the differential expression of the responses to altered irradiance and spectral quality. *Plant Cell Environ.* **21**, 139–148 (1998).
65. Kitajima, K. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 subtropical species. *Oecologia* **98**, 419–428 (1994).
66. Beneragama, C. K. & Goto, K. Chlorophyll a: b ratio increases under low-light in shade-tolerant *Euglena gracilis*. *Tropic. Agric. Res.* **22**, 12–25 (2010).
67. Grieco, M., Tikkanen, M., Paakkarinen, V., Kangasjarvi, S. & Aro, E. M. Steady-state phosphorylation of light-harvesting complex II proteins preserves photosystem I under fluctuating white light. *Plant Phys.* **160**, 1896–1910 (2012).
68. Anderson, J. M. & Andersson, B. The dynamic photosynthetic membrane and regulation of solar-energy conversion. *Trends Biochem. Sci.* **13**, 351–355 (1988).
69. Friedland, N. Friedland, N., Negi, S., Vinogradova-Shah, T., Wu, G., Ma, L., Flynn, S., Kumssa, T., Lee, C. H.& R. Sayre, R. T. Fine-tuning the photosynthetic light harvesting apparatus for improved photosynthetic efficiency and biomass yield. *Sci. Rep.* **9**, 13028 (2019).
70. Saito, K., Mitsuhashi K. & Ishikita H. Dependence of the chlorophyll wavelength on the orientation of a charged group: Why does the accessory chlorophyll have a low site energy in photosystem II? *J. Photoch. Photobiol. A: Chem.* **402**, 112799 (2020).

71. Spundova, M. *et al.* Ultra-structural and functional changes in the chloroplasts of detached barley leaves senescing under dark and light conditions. *J. Plant Phys.* **160**, 1051–1058 (2003).
72. Dinc, E., Ceppi, M. G., Toth, S. Z., Bottka, S. & Schansker, G. The chl a fluorescence intensity is remarkably insensitive to changes in the chlorophyll content of the leaf as long as the chl a/b ratio remains unaffected. *Biochim. Biophys. Acta* **1817**, 770–779 (2012).
73. Ramel, F. *et al.* Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 5535–5540 (2012).
74. Foyer, C. H. Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. *Environ. Exp. Bot.* **154**, 134–142 (2018).
75. Govindjee, G. Chlorophyll *a* fluorescence: A bit of basics and history. In *Chlorophyll Fluorescence: A Signature of Photosynthesis*, Papageorgiou, G. C. & Govindjee G., Eds., pp. 1–42 (Kluwer Academic Publishers, 2004).
76. Genty, B., Goulas, Y., Dimon, B., Peltier, G., Briantais, J. M. & Moya I., Modulation of efficiency of primary conversion in leaves, mechanisms involved at PS2. In *Research in photosynthesis, Vol. IV*, N. Murata Ed.: *Proc. of IXth Int. Cong. Photosynthesis*. Nagoya, Japan, 30 August-4 September 4, pp. 603–610 (1992).
77. Baker, N. R. Chlorophyll fluorescence: A probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* **59**, 89–113 (2008).
78. Kumagai, E., Araki, T. & Kubota F. Correlation of chlorophyll meter readings with gas exchange and chlorophyll fluorescence in flag leaves of rice (*Oryza sativa* L.). *Plants Plant Prod. Sci.* **12**, 50–53 (2009).
79. Bertamini M. & Nedunchezhian N. Photosynthetic functioning of individual grapevine leaves (*Vitis vinifera* L. cv. Pinot noir) during ontogeny in the field. *Vitis* **42**, 13–17 (2003).
80. Roach, T. & Krieger-Liszkay, A. Regulation of photosynthetic electron transport and photoinhibition. *Curr. Prot. Pept. Sci.* **15**, 351–362 (2014).
81. Zhang, S., Jiang, H., Zhao, H., Korpelainen, H. & Li, C. Sexually different physiological responses of *Populus cathayana* to nitrogen and phosphorus deficiencies. *Tree Phys.* **34**, 343–354 (2014).
82. Rebeiz, C. A., Montazer-Zouhoor, A., Hopen, H. J. & Wu, S. M. Photodynamic herbicides: 1 Concept and phenomenology. *Enz. Microb. Technol.* **6**, 390–396 (1984).
83. Ort, D. R., Zhu, X. & Melis, A. Optimizing antenna size to maximize photosynthetic efficiency. *Plant Physiol.* **155**, 79–85 (2011).
84. Lichtenthaler, H. K. & Babani, F. Level of photosynthetic pigments and ratios of chlorophyll a/b and chlorophylls to carotenoids (a+b)/(x+c) in C4-plants as compared to C3-plants. *Photosynthetica* **60**, 3–9 (2022).
85. Yoshida, S., Forno, D. A., Cock, J. H. & Gomez, K. A. Laboratory manual for physiological studies of rice. 3rd ed, pp. 61–65 (International Rice Research Institute, 1976).
86. Jones, J. B. & Case V. W. Sampling, handling, and analyzing plant tissue samples. In Soil Testing and Plant Analysis. 3rd ed. R.L. Westerman, Ed. SSSA Book Ser. 3. SSSA (1990), pp. 389–428.
87. Campbell, C. R. Determination of total nitrogen in plant tissue by combustion. In Plant analysis reference procedures for the southern U.S. Coop., Ser. Bull. 368. C. O. Plank, Ed. Univ. of Georgia (1992), pp. 20–22.
88. Richardson, A. D., Duigan, S. P. & Berlyn, G. P. An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytol.* **153**, 185–194 (2002).
89. Porra, R., Thompson, W. & Kriedemann, P. Determination of accurate extinctions coefficients and simultaneous equations for assaying chlorophylls *a* and b extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta* **975**, 384–394 (1989).
90. Loriaux, S. D. *et al.* Closing in on maximum yield of chlorophyll fluorescence using a single multiphase flash of sub-saturating intensity. *Plant Cell Environ.* **36**, 1755–1770 (2013).
91. McClain, A. M. & Sharkey, T. D. Building a better equation for electron transport estimated from Chl fluorescence: Accounting for nonphotosynthetic light absorption. *New Phytol.* **225**, 604–608 (2020).
92. Motohashi, R. & Myouga F. Chlorophyll fluorescence measurements in Arabidopsis plants using a pulse-amplitude modulated (PAM) fluorometer. *Bio-protocol* 5:e1464 (2015). http://www.bio-protocol.org/e1464 (2022).
93. Oberhuber, W., Dai, Z. & Edwards, G. F. Light dependence of quantum yields of photosystem II and CO2 fixation in C3 and C4 plants. *Photosynth. Res.* **35**, 265–274 (1993).
94. Cazzaniga, S., Osto, L. D., Kong, S. G., Wada, M. & Bassi, R. Interaction between avoidance of photon absorption, excess energy dissipation and zeaxanthin synthesis against photo oxidative stress in Arabidopsis. *Plant J.* **76**, 568–579 (2013).

Acknowledgements

Author contributions

N.E.K. conceptualized and designed the research; N.E.K. and T.F. performed the experiment; N.E.K. analyzed the data and wrote the manuscript with contribution from G.G.; N.E.K. and J.K.N. secured resources and supervised research; N.E.K., J.K.N., T.F., T.L.R., D.M.O., and G.G. reviewed and edited the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-023-40453-6.

Correspondence and requests for materials should be addressed to N.E.K.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.