Introduction

Alien plant invasion attracts increasing attention from ecologists [1-2], as it is posing profound negative impacts and challenges on ecosystem balance and human society's healthy development [3-4]. This event is mainly caused by the introduction of exotic species to a new habitat. However, how to exactly predict and control plant invasions always perplexes most researchers [5]. Normally, invasive plants are highly possible to meet their congeners in a novel environment since they have similar ecological niche demands.
and inherent biological properties [6]. Therefore, the growth and physiological performance comparison between invasive species and indigenous or non-native congeners (with weak invasiveness) in the same conditions will facilitate the exploration of underlying invasion mechanisms [7-8].

Nitrogen (N) is a macro-element essential for plant growth and development, the plant invasion is highly affected by environmental N conditions [9-10]. Generally, invasive plants tend to use the nutrient more efficiently than native plants when exposed to resources pulse [11]. Correspondingly, Davis [12] proposed the fluctuating resource availability hypothesis holding that high temporal variability in resource (e.g. N) can promote exotic plant invasions. Temporal variability of N supply exists universally across habitats. For example, plant uptakes for N critically rely on the soil moisture, and thus changes in precipitation inevitably increase the temporal variability of N availability [13]. Another example is that different land-use types possess various N input patterns, yielding increased variability as well [14]. Consequently, the rising climate changes and anthropogenic disturbances will predictably generate an increase in the temporal variability of N supply [15-16].

Plant internal N levels could influence the chlorophyll content, which is closely linked to the photosynthetic capacity [17]. It's universally believed that the chlorophyll fluorescence (ChlF) technique is a fast, non-invasive and precise probe for plant photosynthesis function [18-19]. The ChlF is sensitive to different biotic and abiotic stress and plant growth stage, thus it's been widely used in studies of plant photosynthetic apparatus response to environmental changes [20-22]. For example, Wu et al. [23] found that proper timing for N application for star cluster (Pentas lanceolata) can be achieved by analyzing ChlF combined with other techniques. Studies of breeding cultivars with resistance to pathogens will be shown by ChlF images [24]. Some experts also use fast ChlF O-J-I-P to demonstrate the heat tolerance in the invasive plant (Ageratina adenophora) populations and its high spread potential to tropical areas [25]. Hence, it's practicable to apply the ChlF technique for estimating the photosynthesis performance of invasive plants under various N supply patterns.

Native to the Sonoran Desert in America, Amaranthus palmeri S. Watson is an annual C₄ weed in the Amaranthaceae family [26]. A. palmeri is a troublesome weed because it can cause significant yield losses in several crops and vegetables because of the dominant competitiveness for resources [27-28]. Meanwhile, chemical control methods for this weed are becoming invalid nowadays as it has evolved resistance to various herbicides [29]. Currently, its spread range has expanded beyond America [30]. In China, A. palmeri was first found in Fanzhuangzi Village, Fengtai District, Beijing, in 1985 and was listed as the fourth group of invasive species by the State Environmental Protection Administration of China in 2016 [31]. A. palmeri mainly invades the areas in the North China Plain, which covers the main crops cultivation areas and is undergoing expansion to the south and southwest [32]. This weed thrives in the riverbanks, roadsides, open fields, and farmlands, where the N input is temporal heterogeneous [33-34]. Amaranthus tricolor L. is a domestic plant widely distributed in China, it can co-occur with A. palmeri in the same habitats [35-36]. The present study aimed to (1) analyze the difference in light transport, utilization, and dissipation between two Amaranthus species to the increased N supply variability; (2) determine the impact of N temporal variability on their growth performance and competitive ability; and (3) evaluate the invasion of A. palmeri in divergent N supply environments by combining the ChlF, growth and competition indexes.

**Experimental**

**Materials and Methods**

**Experiment Design**

Seeds of A. palmeri were collected from wild populations along the roadsides in Xiqing District, Tianjin, China (39°02’16”N, 117°4’21”E). Tianjin has a typical temperate and monsoonal climate characterized by hot and rainy summers, cold and dry winters. The annual mean temperature is about 24°C, the hottest month is July, and the monthly average temperature from June to August is 28°C. The average annual precipitation is 360-970 mm. Inflorescences bearing massive mature seeds in the healthy invasive plants among several populations were clipped and put in paper bags. The bags were transported to the lab immediately. Inflorescences were air-dried and hand-thrashed in the lab. Plump seeds were obtained according to Schutte’s method [37]. Seeds of native A. tricolor were purchased from Hebei Qingfeng Seed Co., LTD. The both two Amaranthus species seeds were separately stored in glass bottles and kept in dark at 4°C until use.

Seeds were germinated in the 9-cm Petri dish with moist filter paper placed in. The petri dish was put in an incubator with day and night (d/n) temperature was 30/25°C and 14 h light. Germinated seeds were then transplanted into a seedling-raising plate, the plate has 72 holes, each hole was filled with 60 g of a mixture of soil and sand at a 3:1 ratio (v/v). The soil was collected from the farmland in Xiqing District (116°57’34”N, 39°08’53”E). The propriety of the mixture contained 1.5% organic matter, 0.05% total nitrogen (TN), 1.29% total carbon (TC). The uniformly sized and vigorous seedlings of each Amaranthus species with 4-true-leaf were transplanted to a pot (25×20×20 cm).

Every pot had 3.8 kg the same mixture as the plate with a pH value of about 7.9. The experiment had two modes of competition treatment (monoculture and co-culture):
for monoculture, each pot contained four *A. palmeri* or *A. tricolor* seedlings, whereas for co-culture, two seedlings of *A. palmeri* and two seedlings of *A. tricolor* grown together in the same pot. After the preculture of two weeks, 100 mL NH$_4$NO$_3$ (GR, >99%; Fuchen Chemical Reagent Co., Ltd, Tianjin, China) solution was applied six times to create four different temporal N supply patterns: no N supply treatment (CK), constant low N supply treatment (LN), constant high N supply treatment (HN), pulsed N supply treatment (PN). The N supply variability increased from the control to the PN treatment. The LN treatment was totally supplied with 0.18g N per pot, and the HN and PN treatments received the same total N amounts as 0.36g per pot. The amount and time of N supply at each time were shown in Table 1. The experiment was performed in a greenhouse at Nankai University (Tianjin, China, 39°06′09″N, 117°10′02″E) from June 22 to August 28, 2020. Thirty-six pots were randomly placed in a greenhouse with a glass rooftop and wire netting walls, so it can let the sunlight and ambient air pass but prevent disturbances from birds and rains. All pots were watered with 100 mL distilled water once a day and moved biweekly over the duration of the experiment.

**Chlorophyll Fluorescence and Growth Performance Parameters Measurements**

The sixth fully developed leaf from the top was chosen to measure the ChlF parameters by Portable Handy FluorCam (Ecotech Ecological Technology Ltd. USA) at 9:00-12:00 h. We set the protocols of the FluorCam as Shutter = 0, Sensitivity = 20, AChlt2 = 100, AChlt1 = 100, Super = 70 according to results of preliminary experiments and previous research [38]. Before the measurement, the leaves were adapted to darkness for 25 min. The initial fluorescence (Fo) under dark adaptation was measured with a weak measuring radiation. The maximal fluorescence (Fm) under dark adaptation was measured by giving saturated pulse radiation. Saturation pulse radiation was turned on to measure the maximal fluorescence (Fm') under light adaptation. Turn off photochemical radiation and turn on far-red radiation to measure the minimum fluorescence (Fo') under light adaptation. The variable fluorescence (Fv) in the dark-adapted state was calculated as Fm-Fo, and the variable fluorescence (Fv') under light adaptation was calculated as Fm'-Fo'.

The potential photochemical efficiency (Fv/Fo) was calculated as (Fm-Fo)/Fm, these parameters reflect the potential activity of the PSII [39]. The maximum quantum yield of PSII photochemistry in dark, QYmax = Fv/Fm, reflects the maximum efficiency to transform the light absorbed by PSII [40]. The effective quantum yield of PSII photochemistry in the light, Fv/Fm' = (Fm'-Fm')/Fm', indicates the primary efficiency at which light absorbed by PSII is used for photochemistry, which is the PSII operating efficiency when all the PSII centers were open (primary quinone electron acceptor-Q$_A$ oxidized) [19]. The electron transport rate (ETR) was calculated as $\Phi$PSII × 0.84 × PAR × 0.5, where 0.84 is the average absorbance of leaves and 0.5 is the PAR (Photosynthetically Active Radiation) is equally absorbed by photosystems I and II [41]. ETR indicates the apparent photosynthetic electron transport rate which is leading to carbon fixation, it’s proportional to the photosynthetic activity [42].

Non-photochemical quenching (NPQ) was calculated as follows: NPQ = (Fm-Fm')/Fm', its value varies from 0 to infinity, this parameter means the process that excess light excitation energy in PSII is dissipated non-radiatively by heat [43]. The excitation pressure (1-qP) was obtained as 1-(Fm'-Fs)/(Fm'-Fo'), this index indicated the state of reduction of plastoquinone pool at the PSII receptor side and the balance between energy supply and utilization [44].

Plant height was measured as the distance from the soil surface to the shoot tips. Plants were then harvested and separated as shoots and roots, the total biomass was weighed after oven-dried at 80°C for 72 h. The relative biomass (RB) was determined by the following formula [45] to measure the competitive effects between invasive and native *Amaranthus* species under various N supply variability treatments. The RB was calculated as RB$_i$ = B$_i$ / B$_n$, where B$_i$ is the biomass production of species i when it grown in co-culture with species j, B$_n$ is the biomass production of species i when it grown in monoculture. RB>1

| Treatment | Date and content of nitrogen application per pot each time | Total nitrogen input content |
|-----------|----------------------------------------------------------|-------------------------------|
|           | 0 DAT | 12th DAT | 24th DAT | 36th DAT | 48th DAT | 60th DAT |
| CK        | 0 g   | 0 g      | 0 g      | 0 g      | 0 g      | 0 g      |
| LN        | 0.03 g | 0.03 g   | 0.03 g   | 0.03 g   | 0.03 g   | 0.03 g   | 0.18 g   |
| HN        | 0.06 g | 0.06 g   | 0.06 g   | 0.06 g   | 0.06 g   | 0.06 g   | 0.36 g   |
| PN        | 0.03 g | 0.09 g   | 0.03 g   | 0.09 g   | 0.03 g   | 0.09 g   | 0.36 g   |

DAT: days after treatment. CK, control; LN, plants growing constant low N supply pattern; HN, plants growing constant high N supply pattern; PN, plants growing pulsed high N supply pattern.
indicates that the species i outperformed species j under the competition situation. RB<1 indicates that species i underperformed species j under the competition situation [46]. A high value of RB indicates the strong competitive capacity of the species [47].

**Statistical Analysis**

The means of three replicates and their standard deviation (SD) were determined. Differences of two species under various N supply patterns were determined by the pairwise comparison analysis in Generalized Linear Model (GLM) at P<0.05. Each ChlF parameter and growth index was set as an independent variable, the N supply treatments and cultivation types together were set as a dependent variable. To analyze the relative biomass differences due to various N supply conditions, an analysis of Duncan at P<0.05 was also performed, with the N supply as the independent variable and the relative biomass as the dependent variable. All data were analyzed using SPSS 20.0 for windows (SPSS Inc.). A reference sequence that was a little higher than the optimal measured values of each parameter was achieved. Because the Fo and 1-qP were negative with the performance of plants, the reciprocal of the two parameters was adopted in the analysis process. Then the measured values of each parameter were set as a comparison sequence. After the dimensionless processing, the data were analyzed by the Gray Relation Analysis (GRA) in Excel 2003 (Microsoft Cor).

**Results and Discussion**

Differences in Fo, Fm, and Fv between *A. palmeri* and *A. tricolor* under Various N Supply Variability Patterns

The exogenous N supply variability increased the Fo, Fm, and Fv of plants in both cultivation types, but a decline in these parameters of the *A. palmeri* in co-culture was observed (Table 2). If the N was supplied at a constant low rate (LN treatment), the three ChlF parameters of the two *Amaranthus* species in monoculture all reached the peak values. However, in co-culture, the ChlF parameters of *A. palmeri* were at the highest when plants were grown under control; the Fo and Fm values of *A. tricolor* were at the highest when N supply was at a constant high rate (HN treatment), and the Fv reached the maximal value under the pulsed N supply condition (PN treatment). The Fo, Fm and Fv of plants grown in monoculture were all higher than it in co-culture respectively, except the Fv of natives under the greatest N supply variability (PN treatment). The three ChlF parameters of *A. palmeri* were both significantly higher in the monoculture than in co-culture when supplied with exogenous N. All the ChlF parameters of invaders were significantly higher than natives in co-culture under all N supply patterns with the exception of Fm under the constant low N supply treatment (Table 2).

|          | CK       | LN       | HN       | PN       |
|----------|----------|----------|----------|----------|
| **Fo**   |          |          |          |          |
| NM       | 185.25±34.69 c | 283.92±36.26 a | 225.91±35.60 b | 233.72±39.00 b |
| IM       | 146.42±21.22 d | 178.34±32.72 cd | 145.23±13.89 d | 161.04±25.55 cd |
| NC       | 119.29±15.38 de | 138.96±23.84 d | 216.99±29.97 bc | 140.00±24.27 d |
| IC       | 126.34±16.49 de | 97.70±8.94 e | 122.47±18.74 de | 88.13±14.05 e |
| **Fm**   |          |          |          |          |
| NM       | 273.30±40.24 c | 442.95±52.90 a | 352.16±57.51 b | 358.91±58.74 b |
| IM       | 257.88±37.75 e | 331.94±43.79 b | 257.07±24.89 e | 310.21±53.15 b |
| NC       | 205.93±25.24 f | 181.44±18.73 f | 328.79±36.77 b | 271.14±37.60 de |
| IC       | 232.84±27.86 ef | 162.18±19.20 f | 189.63±28.23 f | 173.41±25.91 f |
| **Fv**   |          |          |          |          |
| NM       | 88.05±5.58 cd | 159.03±23.11 a | 126.25±22.62 b | 125.19±20.22 b |
| IM       | 111.46±17.04 b | 153.60±13.46 a | 111.84±11.00 b | 149.17±28.24 ab |
| NC       | 86.64±11.86 cd | 42.48±5.55 f | 111.80±8.50 b | 131.14±15.66 b |
| IC       | 106.50±11.82 c | 64.49±10.27 e | 67.15±11.57 d | 85.27±14.14 d |

Data were shown mean±SD, $n=3$. Dissimilar lowercase letters indicate significant differences between various N supply treatments for the two *Amaranthus* species under divergent cultivation types (P<0.05). Fo, initial fluorescence; Fm, maximal fluorescence; Fv, variable fluorescence. NM, native *A. tricolor* was grown in monoculture; IM, invasive *A. palmeri* was grown in monoculture; NM, native *A. tricolor* was grown in co-culture; IM, invasive *A. palmeri* was grown in co-culture. CK, control; LN, plants growing constant low N supply pattern; HN, plants growing constant high N supply pattern; PN, plants growing pulsed high N supply pattern.
Different Light Energy Utilization Responses of two *Amaranthus* Species to Various N Supply Temporal Variability

The N supply slightly decreased the Fv/Fo, ETR, QYmax and Fv'/Fm' of the *A. tricolor* in monoculture, except the Fv/Fo and QYmax under the constant high N supply treatment (Fig. 1). The Fv/Fo and QYmax of *A. palmeri* in monoculture under the LN and PN treatments were all higher than that under the control and HN conditions, and the increase in Fv/Fo was significant (*P*<0.05). The N input decreased the Fv'/Fm' but significantly promoted the ETR of *A. palmeri* in monoculture (*P*<0.05; Fig. 1b, d). The ChlF parameters of *A. tricolor* under the LN treatments were all lower than control in co-culture, moreover the reduction in Fv/Fo and Fv'/Fm' was remarkable (*P*<0.05). By contrast, the greatest N supply variability sharply improved the ChlF of *A. tricolor* in co-culture (*P*<0.05; Fig. 1). The four ChlF parameters of *A. palmeri* in co-culture were significantly lower than the constant low and high N supply treatments than under the control and pulsed N supply treatments, the greatest N supply variability improved these indexes, and the increases in Fv/Fo and ETR were notable (*P*<0.05; Fig. 1). The four ChlF parameters of two species in co-culture and *A. palmeri* in monoculture were highest under the greatest N supply variability.

In monoculture, the ChlF parameters of *A. palmeri* were significantly higher than *A. tricolor* under all treatments except the ETR and QYmax under the HN treatment, while a dramatic opposite relationship about ETR was detected under control (*P*<0.05; Fig. 1). In co-culture, the Fv/Fo, ETR, QYmax, and Fv'/Fm' of *A. palmeri* were all significantly greater than *A. tricolor* under the control and LN treatments. Besides the differences of ETR under the PN treatment and Fv'/Fm' under the HN treatment were also pronounced (*P*<0.05; Fig. 1).

The Fv/Fo, ETR, QYmax, and Fv'/Fm' of *A. tricolor* were all greater in monoculture than in co-culture under the control, LN, and HN patterns, except the Fv/Fo under the control treatment and QYmax under the HN treatment (Fig 1). Furthermore, some of the improvements were significant, but a contrast comparison relationship of those ChlF parameters existed under the pulsed N supply treatment (*P*<0.05; Fig. 1). The ChlF indexes of *A. palmeri* in monoculture under the control and PN treatments were all lower than that in co-culture. However, if the N was supplied at a constant rate (LN and HN treatments), the relationships between the two cultivation types were sharply contrary. Meanwhile, the differences in ETR of *A. palmeri* between two cultivars under all the N supply patterns were significant (*P*<0.05; Fig. 1b).

![Graphs showing chlorophyll fluorescence parameters](image-url)

Fig. 1. The Fv/Fo, ETR, QYmax, and Fv'/Fm' of *A. tricolor* and *A. palmeri* growing in the two cultivation types under increased temporal N supply variability treatments. All bars in the figure show mean±SD, *n* = 3. Dissimilar lowercase letters indicate significant differences between various N supply treatments for the two *Amaranthus* species under divergent cultivation types (*P*<0.05). Fv/Fo, potential photochemical efficiency; ETR, electron transport rate; QYmax, the maximum quantum yield of PSII; Fv'/Fm', effective quantum yield of PSII. CK, control; LN, plants growing in constant low N supply pattern; HN, plants growing in constant high N supply pattern; PN, plants growing in pulsed high N supply pattern.
The maximum value of the color bar was 0.55 and the minimum value was 0, the corresponding color was orange and blue respectively, and the green represented a median value (Fig. 2). If the leaf had larger orange areas, it tended to have a higher QYmax which indicated the light energy conversion efficiency of PSII was greater. While the blue region revealed the reaction center of PSII in this part was damaged or even inactivated. The comparison results of different color sizes in the QYmax images were entirely consistent with the pattern demonstrated by Fig. 2.

Different Non-Photochemical Quenching and Light Reactions Damage in PSII of Two *Amaranthus* Species under Various N Temporal Supply Patterns

The NPQ of *A. tricolor* significantly improved by the N supply treatment, except it in monoculture under pulsed N supply treatment (Fig 3a). The N input also significantly promoted the NPQ of *A. palmeri* grown alone, but the NPQ value under HN treatment decreased (Fig 3a). The NPQ of *A. palmeri* in co-culture was significantly inhibited under the relative low N supply variability (LN and HN treatments), but the greatest variability increased the NPQ values. Exogenous N supply decreased the 1-qP of *A. tricolor* in co-culture and *A. palmeri* in monoculture but increased the values of the two species in monoculture and co-culture respectively except the *A. palmeri* under the greatest variability. It’s noted that the greatest N supply variability significantly declined the 1-qP of *A. palmeri* in two cultivation modes and *A. tricolor* in co-culture (*P*<0.05; Fig 3b).

The NPQ of plants in monoculture was both higher than that in co-culture under all the N treatments, but the comparison relationship was converse for *A. palmeri* under control and *A. tricolor* in the HN treatment (*P*<0.05). The 1-qP was higher in co-culture than in monoculture for both *Amaranthus* species except the *A. palmeri* under control. Differences in NPQ and 1-qP between two cultivation types under the constant low N supply treatment were both significant (*P*<0.05; Fig 3).

For plants in monoculture, the NPQ and 1-qP of *A. palmeri* were higher than *A. tricolor* under all conditions except the NPQ under the HN treatments and 1-qP under the PN treatments (Fig 3). Moreover, the advantages of *A. palmeri* over *A. tricolor* in NPQ under the control, LN, and HN treatments were notable (*P*<0.05). In co-culture, the NPQ of *A. palmeri* was greater under control but lower under the LN, HN, and PN treatments than *A. tricolor*. However, the relationship of 1-qP between two *Amaranthus* species under the control, LN, and HN treatments was contrary to the NPQ (*P*<0.05; Fig 3).

Distinct Growth Performance of Native *Amaranthus* Species and Invasive *Amaranthus* Species under Divergent N Variability

The plant height of the plants in both cultivation types increased under the exogenous N input treatments except *A. palmeri* under the LN treatment and *A. tricolor* under the PN treatment in co-culture. Remarkably, the increase of *A. palmeri* was sharp (*P*<0.05; Fig 4a). The two *Amaranthus* species accumulated more
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Fig. 3. The NPQ and 1-qP of *A. tricolor* and *A. palmeri* growing in the two cultivation types under increased temporal N supply variability treatments. All bars in the figure show mean±SD, n = 3. Dissimilar lowercase letters indicate significant differences between various N supply treatments for the two *Amaranthus* species under divergent cultivation types \( (P<0.05) \). NPQ, non-photochemical quenching; 1-qP, excitation pressure. CK, control; LN, plants growing in constant low N supply pattern; HN, plants growing in constant high N supply pattern; PN, plants growing in pulsed high N supply pattern.

Fig. 4. The plant height (cm) and total biomass (g/plant) of *A. tricolor* and *A. palmeri* growing in the two cultivation types under increased temporal N supply variability treatments. All bars in the figure show mean±SD, n = 3. Dissimilar lowercase letters indicate significant differences between various N supply treatments for the two *Amaranthus* species under divergent cultivation types \( (P<0.05) \).

CK, control; LN, plants growing in constant low N supply pattern; HN, plants growing in constant high N supply pattern; PN, plants growing in pulsed high N supply pattern.

bimass under various N supply patterns compared with control. And the improvements in total biomass of *A. tricolor* in monoculture and *A. palmeri* in co-culture were pronounced \( (P<0.05; \text{Fig. 4b}) \). The plants in co-culture reached maximal biomass under the pulsed N supply condition, while *A. tricolor* and *A. palmeri* in monoculture reached the optimal values under the constant high or low N supply treatments respectively.

The height of *A. tricolor* was higher in monoculture than in co-culture under all conditions and the divergences under the control, LN, and PN treatments were significant \( (P<0.05; \text{Fig. 4a}) \). For *A. palmeri*, the plant in monoculture was significantly shorter than that in co-culture with an opposite mode of it under the LN treatment \( (P<0.05) \). The biomass of *A. palmeri* in co-culture was significantly greater than it in monoculture, but that of *A. tricolor* in co-culture was significantly lower than it in monoculture \( (P<0.05; \text{Fig. 4b}) \). The height and total biomass were sharply greater of *A. palmeri* than of *A. tricolor* in the same cultivation types \( (P<0.05; \text{Fig. 4}) \).

Divergent Competition Response between Native *Amaranthus* Species and Invasive *Amaranthus* Species to Various N Supply Variability Conditions

The RB of *A. palmeri* was all greater than 1 and significantly higher than that of *A. tricolor*, the value even beyond 2 under the PN treatment \( (P<0.05; \text{Fig. 5}) \). There was no significant difference in RB of *A. tricolor* under the all N supply treatments compared with the control, but RB of individuals under the pulsed N supply treatment was much greater than that under the constant N supply treatments \( (P<0.05) \). And RB of *A. palmeri* under the greatest N supply variability was the highest among all the N supply patterns \( (P<0.05; \text{Fig. 5}) \).
Correlation of Fluorescence Parameters and Growth Performance of Two *Amaranthus* Species under Diverse N Supply

The GRA results showed that responses in the ChlF and growth indices of *A. palmeri* were much more positive to the N input treatments than *A. tricolor* in both two cultivation types, except *A. palmeri* in co-culture under the HN treatment which was at the low order (Table 3). The GRA results showed that gray correlative degree (GCD) and equal weight order (EWO) in the ChlF and growth of *A. palmeri* was much greater than *A. tricolor* under all N supply treatments in both two cultivation modes, an exception from this pattern was detected in *A. palmeri* only in co-culture under the HN treatment (Table 3). And the invasive species in monoculture had greater GCD and EWO than it in co-culture under the LN and HN treatments, however, native plants in co-culture had greater GCD and EWO under the HN and PN treatments than in monoculture. The GRA results also revealed that plants all maximized their GCD and EWO under the greatest N supply variability, but the *A. tricolor* in monoculture reached the peak values under the constant low N supply treatment (Table 3).

### Discussions

The chlorophyll fluorescence technique is an ideal probe reflecting the state of PSII, Fo, Fm, and Fv which are three basic parameters of ChlF [19]. The Fo represents the minimal or primary fluorescence when all functional centers of PSII are open, it would increase when plants are in a stressful environment [39]. The up-regulation of Fo in this study indicated the situation of *A. palmeri* in monoculture and *A. tricolor* in two cultivation types became unfavorable with exogenous N supply. This is probably because the interspecific and intraspecific competition turned out to be more aggressive as the growth and photosynthesis of plants were dramatically promoted by N input [48]. However, considering *A. tricolor* was a weak competitor causing little interspecific pressure to *A. palmeri*, the Fo of *A. palmeri* in co-culture under the N input conditions was lower compared with it under the control and other plants in this experiment (Table 2).

The Fv/Fo, QYmax, and Fv'/Fm' mean the potential activity, maximum quantum yield, and effective quantum yield of PSII respectively [38]. And the ETR indicates the activity of PSII and carbon fixation of plants [42, 49]. The variation in these ChlF parameters could reflect the change in the absorption.

| N supply patterns | CK     | LN     | HN     | PN     |
|------------------|--------|--------|--------|--------|
| GCD of *A. tricolor* in monoculture | 0.4711 | 0.5309 | 0.4956 | 0.4870 |
| EWO of *A. tricolor* in monoculture | 14     | 7      | 11     | 13     |
| GCD of *A. palmeri* in monoculture | 0.5376 | 0.5802 | 0.5256 | 0.5953 |
| EWO of *A. palmeri* in monoculture | 6      | 3      | 8      | 2      |
| GCD of *A. tricolor* in co-culture | 0.4665 | 0.4366 | 0.4986 | 0.5679 |
| EWO of *A. tricolor* in co-culture | 15     | 16     | 10     | 4      |
| GCD of *A. palmeri* in co-culture | 0.5531 | 0.5172 | 0.4884 | 0.6518 |
| EWO of *A. palmeri* in co-culture | 5      | 9      | 12     | 1      |

Gray correlative degree (GCD), equal weight order (EWO). CK, control; LN, plants growing in constant low N supply pattern; HN, plants growing in constant high N supply pattern; PN, plants growing in pulsed high N supply pattern.
transformation, and transport of the PSII for the light energy. A notable superiority in the Fv/Fo, QYmax, ETR, and Fv'/Fm' of A. palmeri beyond A. tricolor was detected under all the N supply variability patterns in monoculture, showing invaders had remarkable higher fluorescence characteristics and photosynthetic capacity that mainly result from the difference in plant species. Although the two *Amaranthus* species were *C*₄ plants with a higher N use efficiency [50], *A. palmeri* had an apparent greater sunlight utilization and electron transfer efficiency than natives especially under control, LN, and PN concentrations when in monoculture (Fig 1). This was the same as the research of Guo [51] about *A. palmeri* had a higher Fv/Fm (QYmax) than *Amaranthus retroflexus* and *Amaranthus rudis* at 35/30°C treatments. Whereas the pronounced advantage in the ChlF of *A. palmeri* over *A. tricolor* disappeared when they were grown together under high N supply patterns, and the index of *A. palmeri* decreased with the N input concentration increased (Fig 1). The possible reason was that *A. tricolor* was poor at resource competition, thus the constant high N supply treatments perhaps exceeded the maximal absorption of the *A. palmeri* causing the high N stress and reducing the ChlF of *A. palmeri* [52]. Most notably, both two species caught a much higher and similar ChlF under the PN treatment, this was consistent with the previous studies [53]. Perhaps because the greatest temporal variability of N supply fitted in with the N consumption demand of two *Amaranthus* species at different development stages, thus ChlF of the two species was promoted mostly under this pattern. And ChlF differences between them were narrowed as the promotion in ChlF of natives was unexpectedly higher than it of invaders.

The NPQ states one part of excess absorbed light energy which can not be transported by electrons but can be dissipated in the form of heat [43]. It’s one of the most important protection mechanisms helping plants to avoid photodamage caused by biotic and abiotic stresses [54]. The NPQ of *A. palmeri* in monoculture and *A. tricolor* increased under the increased N supply variability, but that of *A. palmeri* in co-culture decreased with N supply variability and was the lowest in each treatment group. Usually, the relations between NPQ and Fv/Fm' of healthy leaves were negative, if a decreasing Fv/Fm' met a declining NPQ then the structure and function of PSII were probably broken [39]. The NPQ variation demonstrated that invaders grown alone and natives had great NPQ efficiency to protect it from the damage to PSII under the increased N supply variability. However, invaders’ abilities to dissipate excessive excitation energy were sharply inhibited by N supply in co-culture, this may be related to the plant growth cycle [52]. The 1-qP (excitation pressure) is an estimate of the redox state of the electron acceptor (Qₐ) of PSII and thus reflects the balance between energy supply and utilization in PSII [55]. Vlčková et al. [56] found that the senescent plant up-regulated the 1-qP and excitation pressure could be an indicator of senescence. The 1-qP of invaders was greater than natives especially under the HN condition, but this relationship was converse under the PN treatment. Notably, the N supply in pulses dramatically decreased the 1-qP of natives in co-culture and invaders in both cultivation modes. Similarly, previous studies found excitation pressure mediated plant natural senescence and premature senescence caused by detrimental N status [57]. Thus, it’s believed that senescence led to the decrease in NPQ and the increase in 1-qP of *A. palmeri* under constant N supply conditions in co-culture, but the greatest N supply variability treatment delayed senescence and improved the ChlF indexes of two *Amaranthus* species.

The higher photosynthetic and CO₂ assimilation capacity of plants were motivated by elevated N supply, as it can increase the stromal and thylakoid proteins contents in chloroplasts [58]. Further, invasive plants attributed more resources to photosynthesis and hence utilized resources more efficiently and grow faster [9]. Stronger and healthy photosynthesis is essential to the growth improvement of green plants. Previous studies have demonstrated that invasive *Amaranthus* species always had greater nutrient competitive abilities, sunlight in particular, through the super plant height [59]. Thus, prominent advantages in plant height of *A. palmeri* than *A. tricolor* also can be found in this study regardless of cultivation types (Fig 4a). But invaders grew taller in co-culture than in monoculture which conflicts with the ChlF performance in the LN and HN treatments (Figs 1, 4). Khangura et al. [60] demonstrated a nonlinear relationship between the plant height and chlorophyll levels, so a low ChlF performance would not lead to a shorter *A. palmeri* height under the relative lower N supply variability. Previous studies also found that *A. palmeri* could produce much more leaves placed vertically to the ray of the sun than *A. tricolor*, improving invaders’ whole photosynthetic efficiency that’s able to grow longer stems [36, 61]. Therefore, the ChlF reduction at single leaf levels might have little influence on invaders’ growth under the lower N supply variability patterns, but the ChlF and dry matter accumulations of invaders improved notably under the pulsed N input treatment. The same results could be found in former studies [45]. The taller height and greater biomass contributed the superior relative biomass (RB) to *A. palmeri* than *A. tricolor* under all treatments, and RB values proposed the powerful competition abilities of invaders and were in line with former studies [35]. It should be pointed out that the higher RB of plants under the greatest N supply variability in co-culture may attribute to the significant improvement in ChlF performance yielding much biomass accumulation.

Following the analysis principle of correlative degree in gray system theory, the reference group is an ideal model responding to the increased N supply temporal variability most positively [62]. So, a higher GCD between the reference group and a treatment group represents this group was more actively sensitive.
to the N supply variability compared with the other groups. In this study, invaders reacted more vigorously to the exogenous N supply treatment. The results were in agreement with the findings of Wang et al [63]. Besides, A. palmeri under the HN treatment in coculture had low EWO. This was mainly due to the sharp decrease in ChlF parameters and features of equal weight order [64].

Conclusions

The objective of this study was to gain insights into the differences in ChlF and growth responses of invasive A. palmeri and native A. tricolor to the increased temporal N supply variability and evaluate the invasion of A. palmeri under such conditions. The results showed that A. palmeri possessed the notably higher Fv/Fo, ETR, QYmax, and Fv'/Fm', conferring the greater light energy transport and utilization efficiency to invaders in monoculture and under low N supply variability in coculture. Moreover, the greatest N supply variability (PN treatment) sharply improved the photosynthetic efficiency of the two Amaranthus species in co-culture and thus diminished the difference between them. The higher NPQ of invaders could facilitate a more efficient excess light energy dissipation in monoculture, but this photoprotection mechanism was more effectively operated by natives in co-culture. The N supply variability contributed to dramatically greater plant height and total biomass of A. palmeri, which can help invader to establish competitive dominance and boost its growth performance in turn. The GRA results suggested that ChlF and growth responses of A. palmeri were more active than A. tricolor and more positive to the PN treatment, thus the invasion of A. palmeri will be aggravated under N supply conditions especially with greater temporal variability.

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Conflict of Interest

The authors declare no conflict of interest.

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