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Genotypic Diversity Improves Photosynthetic Traits of *Hydrocotyle vulgaris* and Alters Soil Organic Matter and N₂O Emissions of Wetland Microecosystems

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Abstract: In plant communities, genotypic diversity can impact the plant community structure and ecosystem functions, but related research has focused on native plants. Therefore, whether genotypic diversity affects the growth of invasive plants and then changes the wetland microecosystem remains unresolved. In this study, six different genotypes of *Hydrocotyle vulgaris*, a common invasive plant in China, were selected to construct populations with three different genotypic diversity levels (one, three, and six genotype combinations, respectively) to explore the effects of different genotypic diversity levels on the growth and physiological traits of *H. vulgaris*, and soil nutrients and greenhouse gas emissions of the wetland microecosystem under flooding conditions. We found that genotypic diversity improved the leaf area, root to shoot ratio and photosynthetic physiological traits of *H. vulgaris*, especially under flooding. Moreover, genotypic diversity increased soil organic matter (SOM) contents in the wetland microecosystem, while it reduced the cumulative nitrous oxide emissions under flooding conditions. Overall, genotype diversity improved photosynthetic traits of *H. vulgaris*, further increased SOM, and reduced the N₂O emissions of the wetland microecosystem. The results of this study can provide a theoretical basis for exploring how genotypic diversity levels affect the invasiveness of invasive plants and ecosystems in wetland microecosystems.

Keywords: *Hydrocotyle vulgaris*; genotypic diversity; growth traits; flooding condition; invasive plant

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1. Introduction

Plant invasion can pose a significant threat to the native biodiversity and the stability and functioning of ecosystems. Thus, invasive mechanisms have long been a research focus for ecologists [1–4].

Recent research has reported that plant invasiveness is determined by many factors, such as the nutrient environment and the composition of the native plant community. It is also hypothesized that fluctuating resources and the evolution of increased competitive ability (EICA) can potentially contribute to invasiveness [5–9]. However, recent advances in molecular genetic technology provide new approaches to explore the mechanism of plant invasion from a genetic perspective [10–13].

Previous studies have shown that when moved to new environments, exotic plants can rapidly evolve or change under the natural environmental selection pressure to better adapt to the new ecosystem [14,15]. Therefore, genetic diversity plays an imperative role in exotic plant invasion, and high levels of genetic diversity promote the successful

colonization of new environments by exotic plants [16–18]. Genotypic diversity is a crucial component of genetic diversity, with positive effects on the growth and colonization of invasive plants [19–21]. Plant invasion can alter the characteristics of invaded ecosystems, such as the soil nutrient levels [22,23] and greenhouse gas emissions [24,25]; therefore, genotypic diversity of invasive plants may affect the characteristics of invaded ecosystems. For example, ecosystem invasion by *Solidago canadensis* positively affects the richness and abundance of soil animals in the invaded region [24].

In addition to genotypic diversity, environmental factors, such as soil nutrients and climate, play significant roles in plant invasion [26,27]. Among them, invasive plants often encounter flooding in wetland ecosystems [28,29]. The effect of flooding is determined by its extent and duration [30,31]. Previous studies have shown that proper flooding could benefit the invasion of *Alternanthera philoxeroides* [32]; the species has stronger photosynthetic ability and higher tolerance to flooding than other species in the community. Therefore, flooding gives *A. philoxeroides* a competitive advantage [33]. However, long term or high water-level flooding conditions can inhibit the growth and invasion of *Spartina alterniflora* [34,35]. Notably, low water levels could favor the invasion of terrestrial invasive plants, while high water levels may inhibit their expansion [30,36].

Although several studies have evaluated the importance of genotypic diversity or environmental factors on plant invasion, the combined effect of the two on the invasive plant and the invaded ecosystem is less researched. *Hydrocotyle vulgaris* is considered an invasive species in China, owing to its high phenotypic plasticity, rapid clonal reproduction, strong adaptability, and competitive exclusion of native species [37–40]. This plant often grows on the waterside under the forest canopy, where it is continually exposed to flooding conditions [40]. Using *H. vulgaris* as the plant material, this study conducted a greenhouse experiment to test the effects of genotypic diversity on the growth and physiological traits of invasive plants and the invaded ecosystem under flooding conditions. Specifically, we addressed the following questions: (1) Does genotypic diversity affect the growth and photosynthetic physiological traits of *H. vulgaris*? (2) Does the genotypic diversity of *H. vulgaris* affect greenhouse gas emissions and soil nutrients in the wetland microecosystem? (3) Do the impacts of genotypic diversity vary with different levels of flooding treatment?

2. Materials and Methods

2.1. Plant Material

Hydrocotyle vulgaris L. (Araliaceae) is a perennial clone herb native to Europe and northwest Africa [41]. The species has substantial phenotypic plasticity and can survive in both wet or dry habitats [37,42]. The species was introduced to China as an ornamental plant in the 1990s due to its unique umbrella shape [39]. The species can reproduce both sexually and asexually; therefore, it has spread extensively to some wetlands in China, such as Hangzhou Bay [39]. This species is now considered invasive in China [40,43,44].

Twenty *H. vulgaris* populations were selected from the wetlands of Hangzhou, Zhejiang Province, China, and a distance of at least 500 m was observed between adjacent populations. The *H. vulgaris* ramets used in this experiment were collected from these populations. The genotypes of all the ramets were confirmed by the amplified fragment length polymorphism (AFLP) method, and please refer to our previous research [45] for a detailed description of the AFLP method (Appendix A). The AFLP genotyping was performed according to the original protocol of Vos et al. [46], with several modifications. We randomly selected six genotypes from these *H. vulgaris*. Six genotypes were used for the following experiments. These plants were cultured in a greenhouse of Beijing SFK Technology Co., Ltd., Beijing, China.

2.2. Experimental Design

The experiment included two flooding levels \times three genotypic levels \times six replicates. The two flooding levels were control (no flooding) and flooding. The water levels of the flooding treatments were 20 cm above the container soil surface. In the control treatment, the soil was watered three times a week to ensure a maximum water holding capacity of 60–70% during the experiment. The three genotypic levels were 1-genotype (six plants per genotype), 3-genotype (two plants per genotype), and 6-genotype (one plant per genotype). Six plants were planted per pot (29.5 cm top diameter, 25.5 cm bottom diameter, 35 cm deep) and then filled with 10 cm of deep substrate (1:1:1 volume ratio of peat, vermiculite, and quartz sand). This soil mixture contained the following nutrients: $2.662 \pm 0.05 \text{ g}\cdot\text{kg}^{-1}$ total carbon (C), $0.739 \pm 0.02 \text{ g}\cdot\text{kg}^{-1}$ total nitrogen (N) and $1.402 \pm 0.07 \text{ g}\cdot\text{kg}^{-1}$ total phosphorus (P). Each selected ramet had a node with adventitious roots and one leaf, and all the ramets had similar heights (10–15 cm in petiole length). See Figure 1 for details.

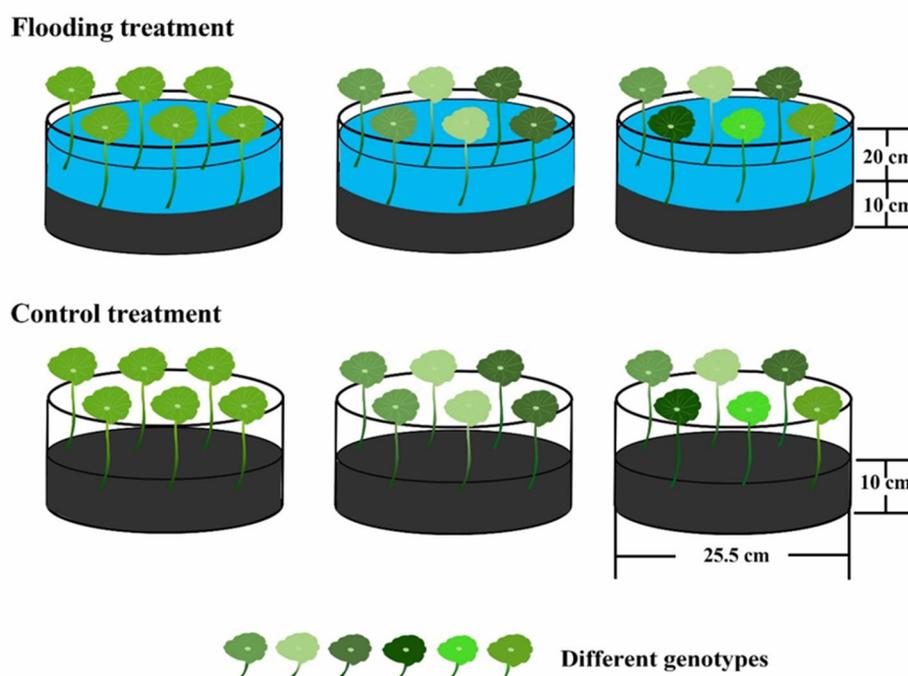


Figure 1. Experimental design.

The experiment began on 15 July 2015. During the experimental period, the average temperature and humidity of the greenhouse were maintained at $28.35 \text{ }^\circ\text{C}$ and 87.3%, respectively. The experimental period was 2 months. No nutrients were added, and weeds and algae were removed regularly during the experiment.

2.3. Harvest and Measurements

To determine the photosynthetic parameters of *H. vulgaris*, three similarly sized leaves of each ramet were randomly selected on 21 September 2015 to determine the net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular carbon dioxide (CO_2) concentration (C_i), and the transpiration rate (T_s). The measurements were taken between 07:00 a.m. and 08:00 a.m. using a portable photosynthesis system LI-6400 (LI-COR, Lincoln, NE, USA).

On 25 September 2015, all plants were harvested, and the plants in each container were sorted into four parts: petioles, stem, leaves, and roots. Subsequently, the petiole length, internode length, and the number of ramets were recorded separately. The leaf area was measured by the WinFOLIA (Pro2004a, Regent Instruments, Québec, QC, Can-

ada) broadleaf analysis system. Then, each part of the four parts was dried separately at 70 °C for 72 h, the biomass of each was measured, and then the root to shoot ratio was calculated.

We measured the greenhouse gas emissions of carbon dioxide (CO₂), nitrous oxide (N₂O), and methane (CH₄) by static chamber-gas chromatographic techniques during the experiment. The static chamber captured gas samples from all containers on 4 August, 16 August, 2 September, and 15 September. All gas samples were collected between 09:00 a.m. and 10:00 a.m. For measurements, the chambers first enclosed the container and were sealed for 60 min, and then greenhouse gas emissions from the container were captured. Gas samples (40 mL) were taken at 0, 15, 30, and 45 min with a syringe and analyzed within 24 h using modified gas chromatography (GC Agilent 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with flame ionization detectors and electron capture detectors.

Fresh soil samples were collected from the containers (100 g each) and air-dried for physicochemical property analyses. The soil samples were then sieved (mesh size of 2 mm) to remove stones, roots, and large organic residue. The soil organic matter [47], total nitrogen [14] and total phosphorus (TP) content were determined via the K₂Cr₂O₇ oxidation-reduction titration method [48,49], the semi-micro-Kjeldahl protocol, and the Mo-Sb colorimetric method [48], respectively.

2.4. Data Analysis

2.4.1. The Net Diversity, Complementarity, and Selection Effects

The net diversity, complementarity, and selection effects of the treatments on the growth characteristics of *H. vulgaris* were calculated as follows [50]:

$$\Delta Y = Y_o - Y_E = \sum RY_{o,i} M_i - \sum RY_{Ei} M_i = \sum \Delta RY_i M_i \quad (1)$$

ΔY is a measure of the net diversity effect. The net diversity effect (ΔY) represents the deviation of the observed yield (Y_o) from the total expected yield (Y_E) in the mixture [50]. RY is the observed genotype performance of species (genotype) i in the multiple genotype combinations [51]. $RY_{o,i} = Y_{o,i}/M_i$ represents the relative value of species (genotype) i in the mixture. $Y_{o,i}$ represents the observed value of species (genotype) i in the mixture. M_i represents the observed value of species (genotype) i in a mono genotype. $RY_{Ei} = Y_{Ei}/M_i$ is the planted proportion of species (genotype) i in the multiple genotype combinations. $Y_{Ei} = N_i/M_i$ is the expected value of species (genotype) i in the mixture. Thus, the net diversity effect can be calculated directly [51].

The net diversity effects can be divided into complementary and selective effects:

$$\Delta Y = N\Delta RY, M + \Delta N_{cov} (\Delta RY, M) \quad (2)$$

$N_{cov} (\Delta RY, M)$ are measures of the complementarity and selection effects, respectively. $\Delta RY_i = \Delta RY_{o,i} - \Delta RY_{Ei}$ is the deviation from the expected value of species (genotype) i in the multiple genotype combinations. N is the number of species (genotype) in the multiple genotype combinations. Thus, the selection effects can be calculated directly, while the complementarity affects the deviation of the selection effect from the net diversity effects [51,52].

2.4.2. Greenhouse Gas Emissions

The emission fluxes of the greenhouse gases (CO₂, N₂O, and CH₄) were calculated by the following formula:

$$F = \frac{M}{V_o} \cdot \frac{dc}{dt} \cdot \frac{T_o}{T} \cdot \frac{P}{P_o} \cdot H \quad (3)$$

F represents greenhouse gas emission fluxes (mg·m⁻²·h⁻¹); M represents the molar mass of the gas; V_o represents the molar volume of the gas under standard conditions;

dc/dt represents the slope of the curve of gas concentration versus time, P_0 and T_0 are the air pressure (kPa) and temperature (K) under standard conditions, respectively. P and T represent the air pressure (kPa) and temperature (K) in situ. H represents the height of the gas-sampling box (m) [43,53]. The cumulative emission fluxes of the greenhouse gases were calculated by the following formula:

$$CE = \sum_{i=1}^n \left(\frac{F_i + F_{i+1}}{2} \right) \times (t_{i+1} - t_i) \times 24 \quad (4)$$

CE represents the cumulative emission fluxes ($\text{mg}\cdot\text{m}^{-2}$); F_i represents the gas emission flux ($\text{mg}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$); $t_{(i+1)} - t_i$ represents the time interval between two adjacent measurement dates [54,55].

2.4.3. Statistical Analysis

Data statistical analyses were performed by SPSS 22.0 (SPSS Inc., Chicago, IL, USA). We performed two-way ANOVA to test the effects of flooding (presence or absence) and genotypic diversity (1-, 3- and 6-genotype) of *H. vulgaris* on (i) the growth performance (internode length, petiole length, number of ramets, leaf area, total biomass, leaf biomass, stem biomass, petiole biomass, root biomass, and root to shoot ratio) of *H. vulgaris*; (ii) photosynthetic physiological characteristics (P_n , G_s , C_i , and T_s) of *H. vulgaris*; (iii) greenhouse gas (CO_2 , N_2O , and CH_4) emissions, and soil nutrients (the contents of SOM, TN, and TP) in the wetland microecosystem. Then, we used a Spearman's rank correlation test to assess the correlation between the growth of *H. vulgaris* and the wetland microecosystem and this analysis was performed with R \times 64 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). Before the analysis, we determined the normality and homogeneity of all variables by a Shapiro–Wilk test and Levene's test.

3. Results

3.1. Effects of Genotypic Diversity and Flooding on the Invasiveness of *H. vulgaris*

3.1.1. Growth Traits

The genotypic diversity significantly affected the leaf area and root to shoot ratio (Table 1, $p < 0.05$), but flooding affected such an effect (Table 1, genotypic diversity \times flooding: $p < 0.05$). The genotypic diversity increased leaf area and root to shoot ratio under the flooding treatment, but they were not affected by genotypic diversity under the control treatment (Figure 2D,J). Moreover, flooding significantly increased the internode length, petiole length, leaf area and root biomass (Table 1, $p < 0.05$; Figure 2A,B,D,I), but decreased the number of ramets and internode biomass (Table 1, $p < 0.05$; Figure 2C,G).

Table 1. ANOVAs for effects of genotypic diversity and flooding on the growth and physiological traits of *H. vulgaris*, greenhouse gas emissions and soil nutrient in wetland microecosystems.

| Indexes | Genotypic Diversity (G) | | Flooding (F) | | G × F | |
|--|-------------------------|------------------|--------------|------------------|-------------|--------------|
| | <i>F</i> | <i>p</i> | <i>F</i> | <i>p</i> | <i>F</i> | <i>p</i> |
| Growth traits | | | | | | |
| Internode length | 0.84 | 0.437 | 8.84 | 0.004 | 0.71 | 0.496 |
| Petiole length | 0.39 | 0.677 | 0.89 | 0.034 | 0.50 | 0.609 |
| Number of ramets | 0.56 | 0.574 | 2.83 | <0.001 | 0.96 | 0.388 |
| Leaf area | 8.41 | 0.005 | 3.56 | 0.034 | 4.80 | 0.011 |
| Total biomass | 5.48 | 0.060 | 0.11 | 0.740 | 1.21 | 0.304 |
| Leaf biomass | 4.78 | 0.110 | 0.03 | 0.846 | 1.02 | 0.366 |
| Internode biomass | 5.68 | 0.055 | 8.22 | 0.006 | 1.18 | 0.314 |
| Petiole biomass | 3.58 | 0.096 | 1.89 | 0.174 | 2.47 | 0.092 |
| Root biomass | 1.37 | 0.261 | 45.46 | <0.001 | 1.55 | 0.220 |
| Root to shoot ratio | 2.43 | 0.033 | 90.41 | 0.097 | 8.01 | 0.001 |
| Photosynthetic physiological traits | | | | | | |
| Net photosynthetic rate | 30.31 | <0.001 | 75.87 | <0.001 | 6.70 | 0.002 |
| Stomatal conductance | 30.46 | 0.001 | 80.51 | <0.001 | 5.51 | 0.005 |
| Intercellular CO ₂ concentration | 4.73 | 0.011 | 36.17 | <0.001 | 4.03 | 0.020 |
| Transpiration rate | 24.23 | <0.001 | 8.50 | 0.004 | 1.71 | 0.185 |
| Greenhouse gas emissions | | | | | | |
| Carbon dioxide cumulative emissions (CO ₂) | 0.12 | 0.886 | 0.44 | 0.509 | 2.43 | 0.097 |
| Methane flux cumulative emissions (CH ₄) | 0.71 | 0.499 | 1.15 | 0.289 | 0.27 | 0.767 |
| Nitrous oxide cumulative emissions (N ₂ O) | 3.27 | 0.044 | 11.89 | 0.001 | 2.47 | 0.093 |
| Soil properties | | | | | | |
| Soil organic matter (SOM) | 15.97 | 0.000 | 4.58 | 0.036 | 3.29 | 0.043 |
| Soil total nitrogen (TN) | 1.25 | 0.295 | 9.03 | 0.004 | 0.84 | 0.435 |
| Soil total phosphorus (TP) | 1.81 | 0.172 | 4.20 | 0.044 | 1.10 | 0.338 |

Significant positive values of *F* and *p* are shown in bold.

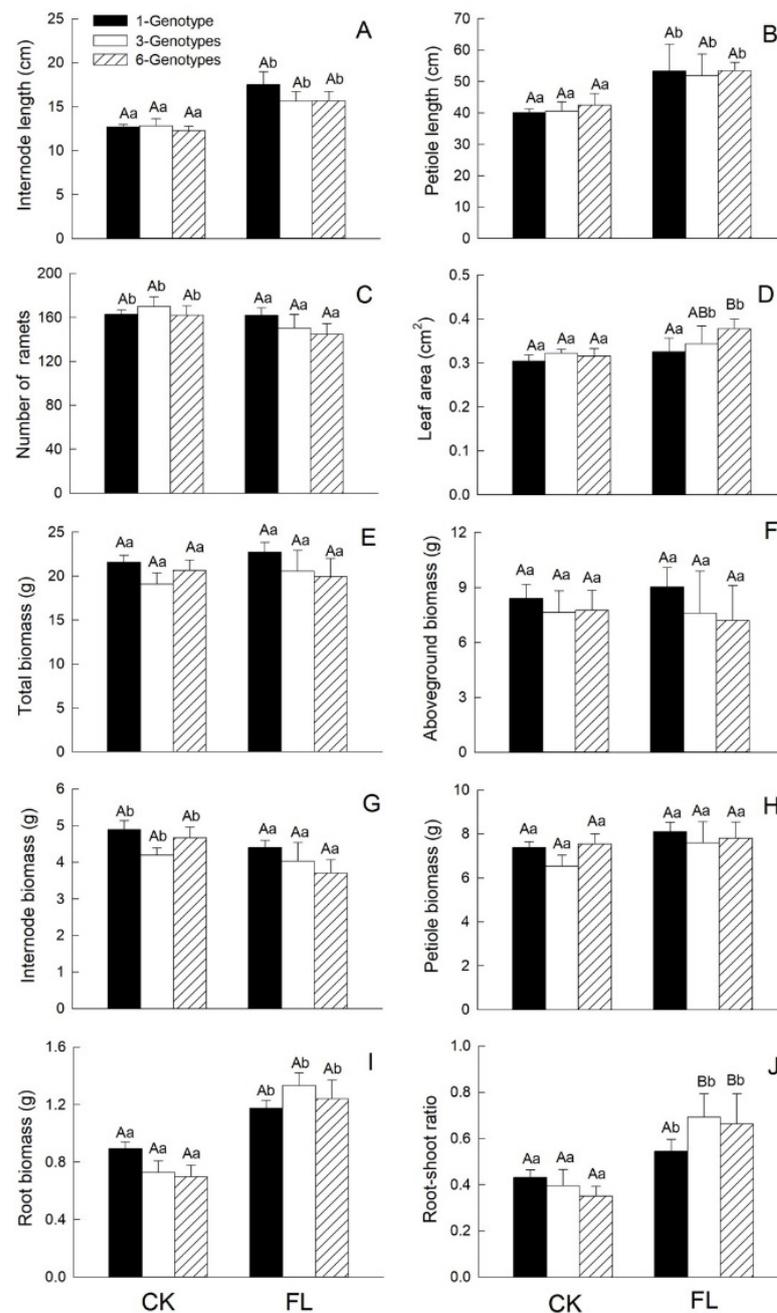


Figure 2. Effects of genotypic diversity and flooding on growth traits (A) Internode length, (B) Petiole length, (C) Number of ramets, (D) Leaf area, (E) Total biomass, (F) Leaf biomass, (G) Internode biomass, (H) Petiole biomass, (I) Root biomass and (J) Root to shoot ratio of *H. vulgaris* (mean \pm SE). The control and flooding treatment were named as CK and FL respectively. Different capital letters indicate significant differences among genotypic diversity treatments, and lower-case letters indicate significant differences among flooding treatments ($p < 0.05$).

3.1.2. Photosynthetic Physiological Traits

There were significant effects of genotypic diversity and flooding on all photosynthetic physiological traits of *H. vulgaris* (Table 1, $p < 0.05$), and flooding increased all photosynthetic physiological traits (Figure 3). Genotypic diversity significantly increased the Pn, Gs and Ts, especially under flooding (Table 1, genotypic diversity \times flooding: $p < 0.05$), but there was no significant difference between the 3- and 6-genotypes (Figure 3A,B,D). Moreover, the Ci increased with increasing genotypic diversity under flooding treatment, but the Ci was not affected by genotypic diversity under the control treatment (Figure 3C).

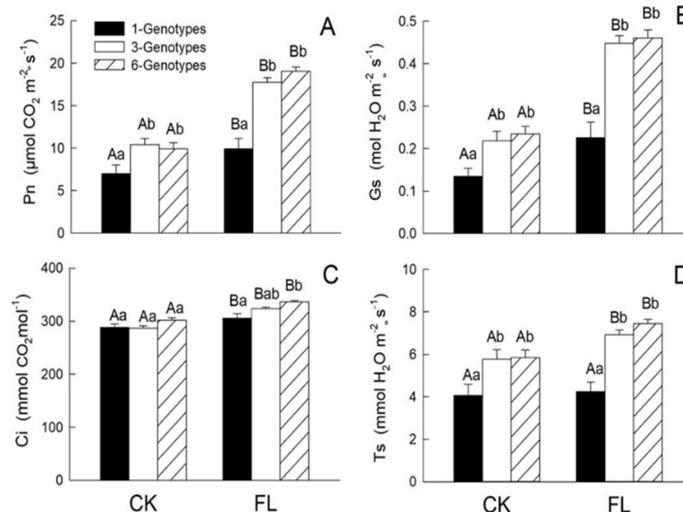


Figure 3. Effects of genotypic diversity and flooding on photosynthetic physiology traits (A) Pn, (B) Gs, (C) Ci and (D) Ts of *H. vulgaris* (mean \pm SE). The control and flooding treatments were named as CK and FL, respectively. Different capital letters indicate significant differences among genotypic diversity treatments, and lower-case letters indicate significant differences among flooding treatments ($p < 0.05$).

3.1.3. The Net Diversity, Complementarity, and Selection Effects

Genotype diversity significantly affects complementarity effect and selection effect of all growth indexes except petiole length; flooding and their interaction also have significant effects on complementarity effect and selection effect of most growth indexes (Table 2, $p < 0.05$). Moreover, we found that net diversity values of growth indexes are negative if net diversity values are significant. Complementary effect also found similar results, except leaf area and number of ramets. On the contrary, the significant selective effect values are positive (Figure 4). In addition, more net diversity effects values, and complementary effect values and selective effect values are significant under flooding (Figure 4).

Table 2. ANOVAs for effects of genotypic diversity and flooding on complementarity effect and selection effect of growth traits of *H. vulgaris*.

| | Genotypic Diversity (G) | | Flooding (F) | | G \times F | |
|-----------------------------|-------------------------|--------|--------------|--------|--------------|--------|
| | F | p | F | p | F | p |
| Complementary effect | | | | | | |
| Internode length | 9.57 | 0.006 | 21.94 | <0.001 | 5.52 | 0.029 |
| Petiole length | 3.22 | 0.088 | 12.13 | 0.002 | 4.363 | 0.050 |
| Leaf area | 11.06 | 0.003 | 116.08 | <0.001 | 50.76 | <0.001 |
| Number of plants | 25.84 | <0.001 | 41.20 | <0.001 | 16.24 | 0.001 |
| Internode biomass | 30.15 | <0.001 | 2.78 | 0.112 | 23.31 | <0.001 |
| Petiole biomass | 23.54 | <0.001 | 74.33 | <0.001 | 55.08 | <0.001 |
| Root biomass | 59.57 | <0.001 | 0.01 | 0.909 | 17.92 | <0.001 |

| | | | | | | |
|-------------------------|--------------|------------------|--------------|------------------|--------------|------------------|
| Leaf biomass | 31.75 | <0.001 | 35.92 | <0.001 | 20.42 | <0.001 |
| Total biomass | 34.58 | <0.001 | 39.60 | <0.001 | 36.94 | <0.001 |
| Selection effect | | | | | | |
| Internode length | 8.68 | 0.008 | 15.29 | 0.001 | 5.35 | 0.031 |
| Petiole length | 2.71 | 0.116 | 8.11 | 0.01 | 3.61 | 0.072 |
| Leaf area | 9.78 | 0.005 | 69.67 | <0.001 | 36.67 | <0.001 |
| Number of plants | 15.75 | 0.001 | 22.34 | <0.001 | 11.11 | 0.003 |
| Internode biomass | 16.13 | 0.001 | 1.81 | 0.194 | 9.16 | 0.007 |
| Petiole biomass | 16.56 | 0.001 | 41.44 | <0.001 | 33.22 | <0.001 |
| Root biomass | 30.11 | <0.001 | 0.08 | 0.783 | 7.40 | 0.013 |
| Leaf biomass | 22.43 | <0.001 | 20.15 | <0.001 | 13.26 | 0.002 |
| Total biomass | 22.46 | <0.001 | 21.38 | <0.001 | 20.66 | <0.001 |

Significant positive values of *F* and *p* are shown in bold.

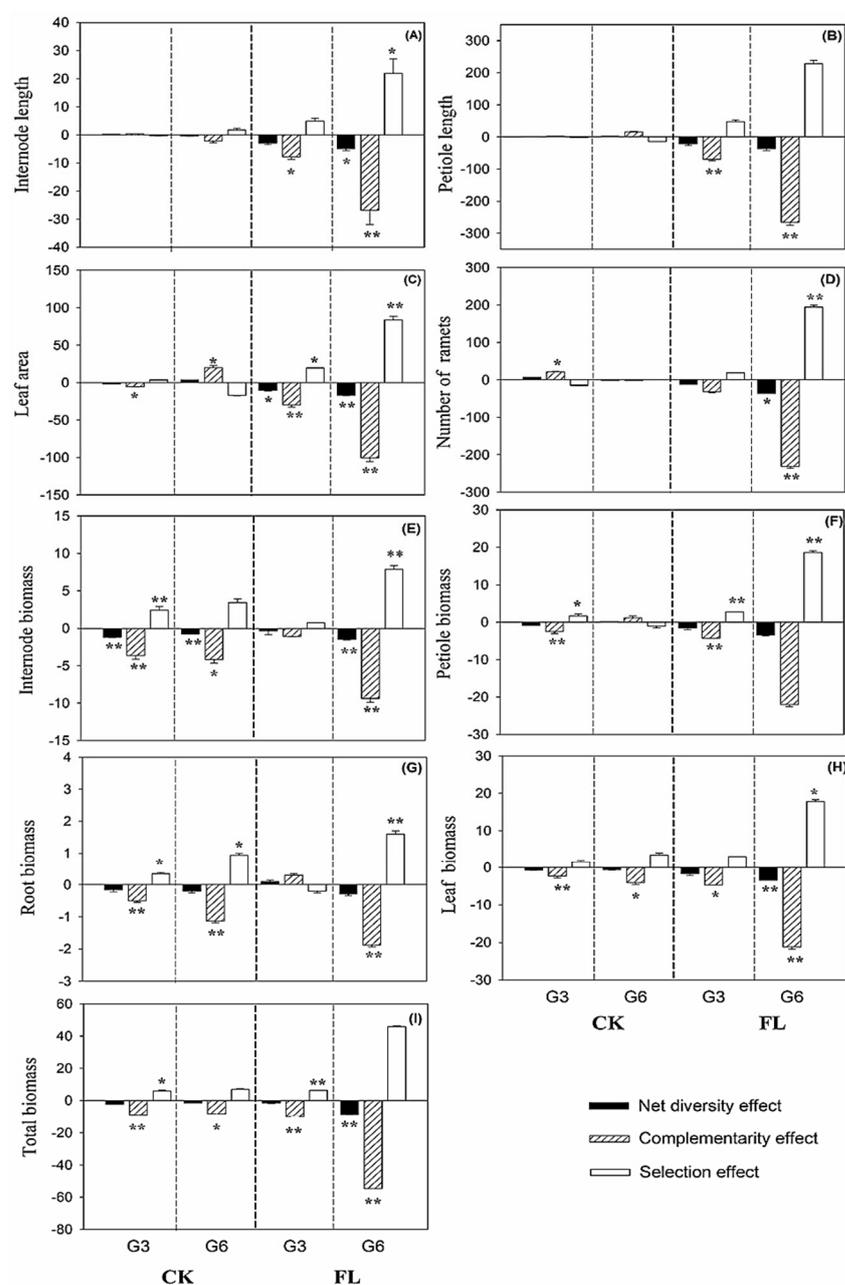


Figure 4. Genotypic diversity net effect value, complementarity and selection effect value of growth traits (A) Internode length, (B) Petiole length, (C) Leaf area, (D) Number of plants, (E) In-

ternode biomass, (F) Petiole biomass, (G) Root biomass, (H) Leaf biomass and (I) Total biomass of *H. vulgaris*. Asterisks *, and **, indicate significance at $p \leq 0.05$, and $p \leq 0.01$, respectively.

3.2. Effects of Genotypic Diversity and Flooding on the Wetland Microecosystem

3.2.1. Soil Properties

The genotypic diversity of *H. vulgaris* significantly affected the SOM content (Table 1, $p < 0.05$; Figure 5A), but this effect was affected by the flooding (Table 1 genotypic diversity \times flooding: $p < 0.05$). The 3- and 6-genotypes more significantly increased the SOM content under the control treatment than the flooding treatment, but there was no significant difference between the 3- and 6-genotypes (Figure 5A). In addition, flooding significantly increased the SOM and TN contents but decreased the TP content (Table 1, $p < 0.05$; Figure 5).

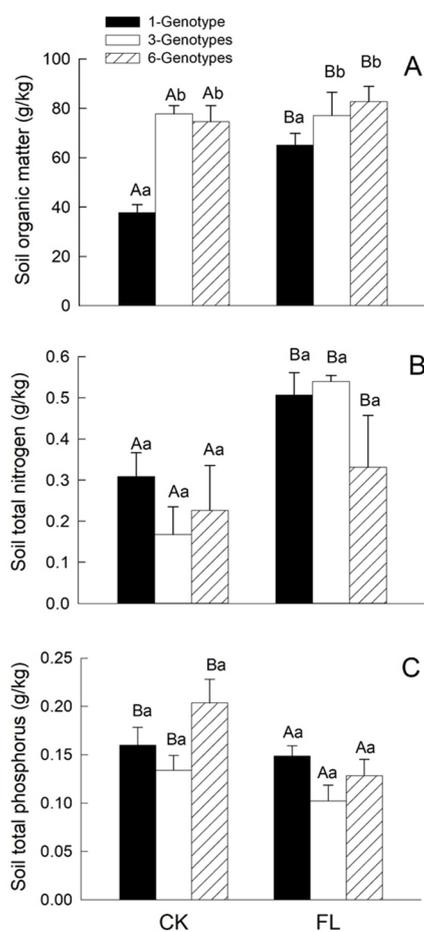


Figure 5. Effects of genotypic diversity and flooding on soil nutrients (A) Soil organic matter, (B) Soil total nitrogen and (C) Soil total phosphorus of the wetland microecosystem (mean \pm SE). The control and flooding treatments were named as CK and FL, respectively. Different capital letters indicate significant differences among genotypic diversity treatments, and lower-case letters indicate significant differences among flooding treatments ($p < 0.05$).

3.2.2. Greenhouse Gas Emissions

The genotypic diversity of *H. vulgaris* and flooding have significant effects on the emission rates of N_2O (Table 1, $p < 0.05$). The genotypic diversity of *H. vulgaris* reduced the emission rates of N_2O , especially under flooding treatment (Figure 6C). However, genotypic diversity and flooding had no significant effect on CO_2 and CH_4 emissions (Table 1, $p < 0.05$). The CO_2 and CH_4 cumulative emissions were negative, indicating that it was lower than zero (Figure 6A,B).

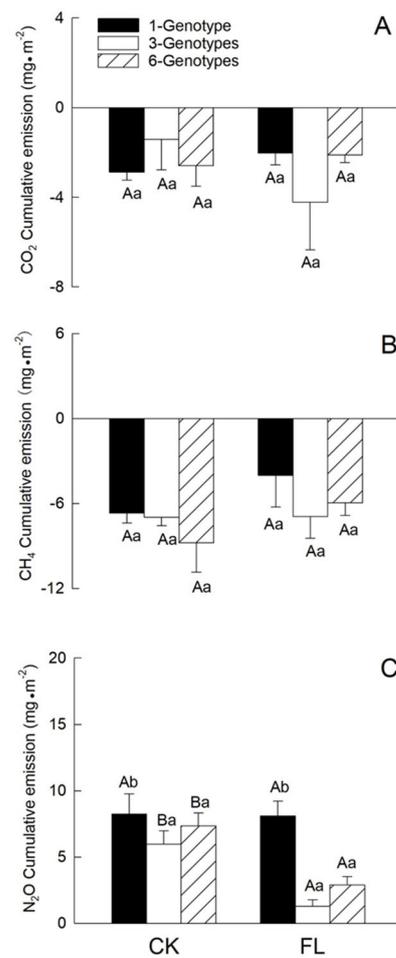


Figure 6. Effects of genotypic diversity and flood on the accumulated emission flux of greenhouse gases (A) CO₂, (B) CH₄ and (C) N₂O from the wetland microecosystem (mean ± SE). The control and flooding treatments were named as CK and FL, respectively. Different capital letters indicate significant differences among genotypic diversity treatments, and lower-case letters indicate significant differences among flooding treatments ($p < 0.05$).

3.3. Relationships between the Invasiveness of *H. vulgaris* and the Wetland Microecosystem

The SOM was significantly positively related to the root to shoot ratio ($R^2 = 0.084$, $p = 0.013$, Figure 7A) of *H. vulgaris*. In contrast, N₂O emissions were significantly negatively related to Ts ($R^2 = 0.084$, $p = 0.018$, Figure 7B) and Pn ($R^2 = 0.12$, $p = 0.005$, Figure 7C) of *H. vulgaris*.

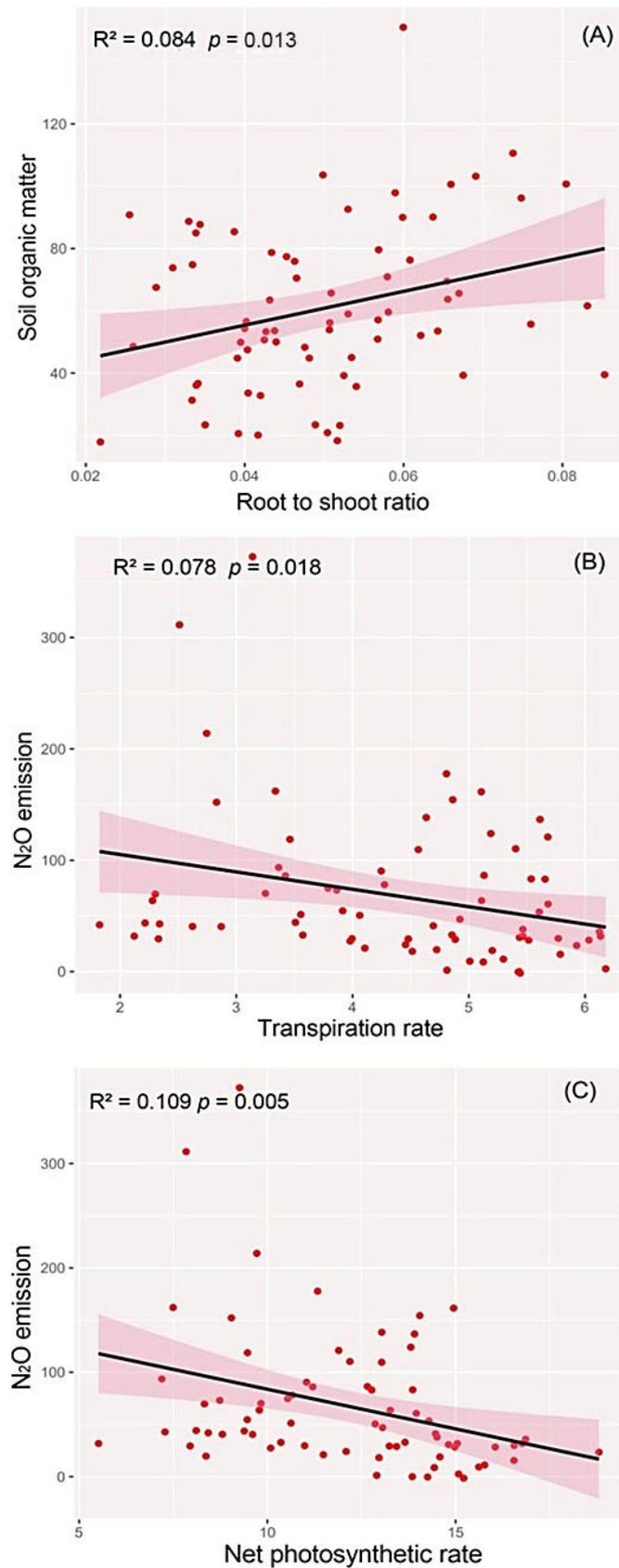


Figure 7. Relationships among the (A) Soil organic matter and Root to shoot ratio, (B) N_2O and T_s , and (C) N_2O and P_n during the experiment. The scattered points represent data measured in each microcosm.

4. Discussion

4.1. Effects of Genotypic Diversity and Flooding on *H. vulgaris* Growth

Genotype diversity is often reported to promote the invasion of invasive plants [21,56,57]. We indeed found that genotype diversity increased all photosynthetic physiological traits of *H. vulgaris*, which was conducive to the successful invasion of *H. vulgaris*. Because light is an essential environmental factor for plant growth, and better photosynthetic physiological characteristics increase the survival competitiveness of the invasive plants [58]. However, as for growth indicators, genotype diversity only affected the leaf area and root to shoot ratio, while it had no significant effect on other growth traits of *H. vulgaris*. One probable explanation for this result is associated with the experimental period. Wang et al. [21] have revealed that the positive effect of genotype diversity on growth traits of the invasive plant (*Spartina alterniflora*) was observed after one year. In addition, the limited container space aggravates the competition among different genotypes of *H. vulgaris*, which is considered to be a more likely reason [59]. Under 1-genotype treatment, that is, the selected genotype was planted alone, the total biomass, above-, and under-biomass of *H. vulgaris*, was better than those traits when three or six genotypes were planted together. In contrast, the photosynthetic traits, leaf area and root–shoot ratio of *H. vulgaris* was better under multi-genotype combination. This showed that genotypic diversity improves light-capturing and may change biomass allocation patterns of *H. vulgaris*. *H. vulgaris* seems to distribute more energy to underground biomass to obtain water/nutrients. This is a common way for plants to gain advantages in competition according to previous studies [60]. This indicates that genotype diversity may aggravate the intraspecific competition of *H. vulgaris* in limited space, especially under flooding, thus forcing *H. vulgaris* to improve its competitiveness by improving photosynthesis and obtaining more underground nutrients. Moreover, significant genotype diversity net effects are basically negative effects, and the significant negative complementary effects mainly play a leading role in the negative net diversity effect. The significantly negative complementary effects indicate that there is overlap in the use of space by different plants [61] so the competition between different plants has intensified [62], which also proves that the negative effect on growth traits of *H. vulgaris* by genotype diversity is more likely to be caused by competition. Therefore, we are considering conducting a long-term field experiment to reduce the impact of limited space and experimental period.

Besides, flooding could be considered a form of disturbance, and disturbance has reportedly increased the selection effect among species or genotypes [63–65]. Thus, we observed that more selection effect values were significant under flooding. This was beneficial to improving the utilization rate of genotype to resources, thereby improving plant productivity [66]. Therefore, previous studies have reported the positive effects of genotype diversity on plant growth under different disturbances [56,67,68] and our findings are consistent with this. The genotypic diversity improved the growth and physiological traits of *H. vulgaris* more obviously under flooding. In addition, flooding had a positive effect on the all-growth traits and photosynthetic physiological traits of *H. vulgaris*, except for number of ramets and the internode biomass. This implies that wet-type habitats caused by flooding might be more suitable for *H. vulgaris* growth. Similarly, Yang [69] reported that the growth of *H. vulgaris* observed in wet habitats was better. Furthermore, flooding could promote the formation of aerenchyma tissues in the stem to transport and diffuse oxygen, so it might increase the *H. vulgaris* stem cell gap, thereby reducing the internode biomass [39].

4.2. Effects of Genotypic Diversity and Flooding on the Wetland Microecosystem

Generally, many invasive plants achieve successful invasion by changing the soil nutrients in the new environment [70–72]. Our research also found a similar result, that is, genotype diversity increased the SOM in the wetland microecosystem. Furthermore, root–shoot ratio was positively correlated with the SOM in this study, which indicated that the increase in SOM is closely related to the root. This may be because the increase in genotypic diversity in this study intensifies the competition among *H. vulgaris* in limited space, which may affect the changes of root exudates, such as allelochemicals [73–75], and ultimately affect SOM. Other studies investigating the impact of invasion plant on soil also found similarly strong evidence that invasive plants increase SOM [70,76–78]. In contrast, the genotypic diversity of *H. vulgaris* did not affect the TN and TP contents in the soil. This might be attributed to the fact that the influence of plant invasion on soil nutrients is affected by invasive plant species [22,79] and soil properties of wetland microecosystems [80,81]. The original values of TN ($0.739 \pm 0.02 \text{ g}\cdot\text{kg}^{-1}$) and TP ($1.402 \pm 0.07 \text{ g}\cdot\text{kg}^{-1}$) in the soil used in this study were high, so the growth and invasion of *H. vulgaris* might not cause changes in TN and TP contents. Moreover, our study found that flooding had significant positive effects on the SOM and the TN contents, while negatively affecting TP. This result could be because the soil was in a reduced state under flooding conditions, so the mineralization rate of organic matter in the soil was slow and low [82]. Consequently, the soil organic matter and TN contents accumulate [83]. However, flooding increases the soil pH, thereby reducing phosphorus release in soil and then decreasing the TP content in the soil [84].

As for greenhouse gas emissions, genotypic diversity significantly reduced the N₂O emissions, specially under flooding treatment. This decrease may be attributed to the improvement of photosynthetic traits of plants by genotype diversity, especially under flooding and the negative correlation between N₂O emissions and photosynthetic traits (i.e., Ts and Gs) of *H. vulgaris* observed in this study. Previous studies have also reported that improvements in plant photosynthesis can decrease N₂O emissions [85–87], which may be due to the negative correlation between O₂ emissions and N₂O emissions [88,89]. In addition, this study found that flooding also reduced N₂O emissions. Hydrological changes in wetlands are often reported to affect N₂O emissions [90,91]. Research by Cheng et al. have also proved that N₂O emissions from wetlands decreased under flooding because water may hinder gas diffusion [92]. By contrast, the genotypic diversity and flooding had no significant effect on the CO₂ and CH₄ emissions in this study. The CO₂ and CH₄ emissions were negative, which indicates that the CO₂ and CH₄ emission processes resulted in net absorption. This finding may be because this experiment used a transparent static chamber (light transmittance $25.9 \pm 1.5\%$). A small amount of light could pass through, so plants still carried out photosynthesis. Photosynthesis would affect the production and emission of CH₄ and CO₂ [93,94]. This fact indicates that the photosynthetic rate of *H. vulgaris* is still relatively high under low light environments, which is a significant feature (high photosynthetic rate and resource-use efficiency) of invasive plants [95,96].

5. Conclusions

In this study, the genotype diversity improved photosynthetic physiological traits of *H. vulgaris*, as well as the leaf area and root–shoot ratio under flooding. As for the wetland microecosystem, the genotype diversity of *H. vulgaris* reduced N₂O emissions and increased SOM content. Furthermore, flooding also reduced N₂O emissions and improved *H. vulgaris* growth in this study. Overall, genotype diversity improved the photosynthetic traits of *H. vulgaris*, and further increased SOM and reduced N₂O emissions in the wetland microecosystem, but genotype diversity is not beneficial to *H. vulgaris* growth in limited space. These findings are helpful for us to gain a deeper understanding of the role of genotypic diversity on invasion of the exotic plant *H. vulgaris*. Because

the effect of genotype diversity on the growth and invasion of *H. vulgaris* is affected by space and time, future research could carry out long-term field experiments to better understand the effect and mechanism of genotype diversity.

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Appendix A. AFLP Protocols

For AFLP, the restriction was performed in a 20 µL reaction containing 200 ng DNA, 4 U *EcoRI* (New England Biolabs, NEB), 4 U *MseI* (NEB), 1× Cutsmart buffer (50 mM NaCl, 10 mM MgCl₂, 10 mM Tris-HCl, 1 mM DDT), and H₂O. After incubated in a DNA engine dyad peltier thermal cycler (BIO-RAD Inc., CA, USA) at 37 °C for 4 h, the reaction was denatured at 65 °C for 20 min. Then, the 20 µL ligation reaction containing 10 µL restriction product, 0.75 µM *EcoRI*-adaptor, 7.5 µM *MseI*-adaptor, 160 U T4 DNA ligase (NEB), 1× T4 DNA ligase buffer, and H₂O was performed at 4 °C for 16 h and denatured at 65 °C for 10 min. After that, we performed pre-amplification in a 20 µL PCR reaction containing 2 µL ligation product, 1 U Taq polymerase (Promega), 1× PCR buffer, 2.0 mM MgCl₂, 0.2 mM each dNTP (Promega), 0.375 µM of each pre-selective amplification primer (E-0 and M-0) and H₂O. The pre-amplification PCR was performed on the thermal cycler at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 56 °C for 1 min, 72 °C for 1 min, and then extending at 72 °C for 7 min. The pre-amplification product was diluted 10-fold by sterile water as the template of selective amplification reaction. The 20 µL selective amplification reaction contained 2 µL diluted pre-selective PCR product, 1 U Taq polymerase, 1× PCR buffer, 1.5 mM MgCl₂, 0.2 mM each dNTP, 0.25 µM of each selective primer (E-ANN primers were FAM-labelled) and H₂O. The selective amplification was performed at 94 °C for 5 min, followed by 13 cycles of 94 °C for 30 s, 65 °C for 30 s (decreasing by 0.7 °C each cycle), 72 °C for 1 min, 23 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min and finishing with 72 °C for 7 min.

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