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RESEARCH ARTICLE

Light condition experienced by parent plants influences the response of offspring to light via both parental effects and soil legacy effects

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Abstract

1. Environmental conditions experienced by parent plants can influence offspring performance through parental effects induced by DNA methylation. The offspring can also be influenced by environmental conditions experienced by their parents via soil legacy effects due to plant-mediated changes in the composition of soil microbes. These two effects are likely to act simultaneously, but empirical evidence for combined effects is limited.
2. We conducted a two-phase experiment with five genotypes of a clonal plant *Hydrocotyle vulgaris*. In the first phase, we grew parent plants of each genotype under two light conditions (ambient vs. shade) and two DNA demethylation treatments [treated with water vs. 5-azacytidine (5-azaC)]. We then collected soils and clonal offspring for each genotype from each of these four treatments and measured soil (a)biotic properties. In the second phase, we grew the offspring from each of the four treatments in the four different soils, under the two light conditions.
3. When grown under ambient light condition and in soil from ambient parents, offspring produced by ambient parents grew larger than offspring produced by shaded parents when the parents were treated with water. This difference was smaller when the parents were treated with 5-azaC, and disappeared when the offspring were grown in soil from shaded parents. The growth difference was also observed when the offspring were grown under shaded condition and in soil from shaded parents. However, this difference was greater when the parents were treated with 5-azaC, and disappeared when the offspring were grown in soil from ambient parents. Moreover, offspring growth was associated with fungal composition and total phosphorus of the soil in which the parents had grown.
4. Our results show, for the first time, that light condition experienced by parents can influence offspring responses to light through both parental effects and soil legacies. The parental effects were mediated by changes in DNA methylation and the soil legacies were due to plant-mediated changes in a combination of soil biotic and abiotic properties. These impacts may eventually influence the ecological and evolutionary trajectories of clonal plant populations.

KEYWORDS

clonal plant, DNA demethylation, legacy effect, paternal effect, plant–soil (below-ground) interactions, soil fungi, transgenerational effect

1 | INTRODUCTION

Environmental conditions experienced by parent plants can potentially influence their offspring performance indirectly through both parental effects (Dong et al., 2018; Hopwood et al., 2014; Huber et al., 2021; Monaghan, 2008; Pigeon et al., 2019) and soil legacy effects (Bever, 1994; Bever, 2003; Bezemer et al., 2006; Legay et al., 2018; Pugnaire et al., 2019; van der Putten et al., 2013). Parental effects are crucial for population dynamics and evolution as they can induce substantial heritable variation, and thus determine the ability of a population to adapt to rapidly changing environments (Badyaev & Uller, 2009; Latzel & Klimešová, 2010; Zhang et al., 2013). Soil legacy effects, that is, (a)biotic changes in the soil induced by the parent plant that can influence the performance of offspring of the plant or other plants that grow later in the soil, can also influence plant growth and ultimately the dynamics and structure of plant communities (Bardgett & van der Putten, 2014; van der Putten et al., 2016). Parental effects and soil legacy effects resulting from environment changes are most likely to act simultaneously in mediating plant performance and plant community dynamics (De Long et al., 2021). However, parental effects and soil legacy effects are typically tested independently, and we lack the knowledge on how they may interact to influence plant performance (but see De Long et al., 2019, 2021).

Environmental conditions experienced by parent plants can influence their performance, but also the (a)biotic properties of the soil in which they grow either directly, or via the plant, for example, via environmentally induced changes in root exudation patterns (Bezemer et al., 2010; De Deyn et al., 2004; van de Vooorde et al., 2011). These changes in soil properties (i.e. soil legacies), in turn, can affect the performance of plants that grow later in these soils (Bever, 2003; Crawford & Hawkes, 2020; De Long et al., 2019; van der Putten et al., 2013). Soil legacy effects can influence plant performance negatively through accumulation of pathogenic microbes (van der Putten et al., 2016) or nutrient depletion (Berendse, 1994), and positively through build-up of symbiotic mutualists (Klironomos, 2002; van der Putten et al., 2016) or nutrient enhancement (Berendse, 1990; Chapman et al., 2006; Wardle et al., 1999). Many studies have shown that soil legacy effects induced by changes in parent environmental conditions can influence offspring performance (Crawford & Hawkes, 2020; De Long et al., 2019; Friman et al., 2021; Kaisermann et al., 2017). However, whether soil legacy effects can influence parental effects has rarely been tested.

Offspring performance is frequently modified by parental effects (Hopwood et al., 2014; Pigeon et al., 2019; Taborsky, 2006; van de Pol et al., 2006). The parental effects on offspring performance can be transmitted via a provisioning effect, that is, when offspring

generated by parents grown in favourable conditions are of higher quality with initially greater body size and hence they exhibit more resource reserves than those generated by parents grown in unfavourable conditions (Hopwood et al., 2014; Shi & Bates, 2013). This difference in provisioning can result in a divergence in especially the early establishment and growth of the offspring. DNA methylation, a relatively well understood epigenetic mechanism, is also involved in the parental effects (Bossdorf et al., 2008; Cuerda-Gil & Slotkin, 2016; Gallego-Bartolomé et al., 2018; Herman & Sultan, 2011; Herrera et al., 2014; Münzbergová et al., 2019; Puy et al., 2021; Richards et al., 2017). DNA methylation can regulate gene expression via addition of methyl groups to cytosine residues, which are heritable and can mediate the responses of the offspring to stressful environments ranging from abiotic stresses such as drought, salinity, light and nutrient shortage (Baker et al., 2019; Boyko & Kovalchuk, 2011; Herman & Sultan, 2016) to biotic stresses such as competition and herbivory (Dong et al., 2019; Puy et al., 2021). Therefore, parental effects induced by DNA methylation are likely to play an important role in offspring responses to soil legacy effects resulting from environmental changes.

Both parental effects and soil legacy effects on offspring performance may vary depending on the environment experienced by the offspring (Baker et al., 2019; Crawford & Hawkes, 2020; De Long et al., 2019; Dong et al., 2018). The 'environmental-matching' hypothesis proposes that offspring exhibit a greater fitness only when they grow in conditions similar to those experienced by the parent plants (Baker et al., 2019; Beckerman et al., 2003; González et al., 2017; Monaghan, 2008). Alternatively, the 'environmental saturation' hypothesis predicts that the parental effect has little impact on offspring fitness when they grow under either extremely favourable or extremely unfavourable conditions (Engqvist & Reinhold, 2016; Hopwood et al., 2014; Pigeon et al., 2019). Similarly, the current environment experienced by the offspring plant can also greatly influence the effects of soil legacies on the succeeding plant (e.g. Crawford & Hawkes, 2020; De Long et al., 2019; Heinze et al., 2020). However, very few studies have tested how parental effects, soil legacy effects and the current environment conditions may interact to influence plant performance.

We conducted a two-phase experiment to examine how light conditions experienced by the parent plant of a clonal herb *Hydrocotyle vulgaris* influenced the responses of their clonal offspring to light through parental effects and soil legacy effects. In this study, we focus on light for two reasons. First, as different phenotypes are required for plant establishment and development under different light conditions, any light-induced phenotypic changes transmitted to offspring could potentially influence their performance (Baker et al., 2018, 2019). Second, light can greatly influence the allocation

of photosynthetic products of plants, and can change root exudates and rhizodeposition, which may eventually result in altered microbial communities in the soil, creating a soil legacy (Lopes et al., 2021; Ma et al., 2018). In contrast to other studies that used non-clonal plants and seeds (De Long et al., 2019, 2021), we used clonal plants and clonal offspring for two reasons. First, this ensures that the confounding effects associated with genetic differences (e.g. using seeds) are completely excluded. Second, as clonal offspring are generally distributed around their parent plants, they are likely to be influenced more strongly by soil legacy effects than sexual offspring.

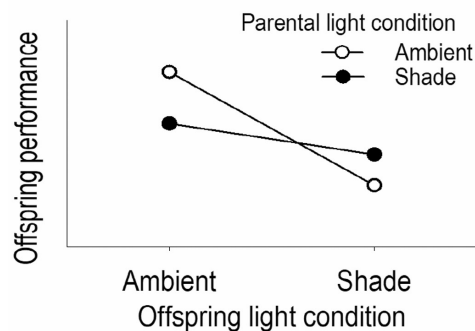
In the first phase, we grew parent plants under two light conditions (ambient vs. shaded) and two DNA demethylation treatments [with or without application of the demethylation agent 5-azacytidine (5-azaC)] and collected soils and clonal offspring from each of these four treatments. In the second phase, we grew clonal offspring from each of these four origins in the soils from the four origins, under the two light conditions. We applied 5-azaC to a subset of the parent plants to test whether DNA methylation was involved in parental effects, and measured soil abiotic and biotic properties to test the roles of soil microbes and nutrients in soil legacy effects. Specifically, we tested the following hypotheses: (1) offspring produced by parents under ambient light conditions grow better than those produced by shaded parents when the offspring grow in ambient light conditions; while offspring produced by shaded parents grow better than those produced by parents exposed to ambient light when the offspring grow in shade conditions. But overall offspring grow worse in shade than in ambient light conditions (Figure 1a). (2) If these effects occur through epigenetics, the application of a DNA demethylation agent will remove or reduce the effect of parental light conditions on offspring responses to light (Figure 1b). (3) If these effects occur through soil legacy effects, all offspring, irrespective of what their parent was experiencing, will respond to the soil, that is, all offspring will do better in soil from parents exposed to ambient light than in soil from parents exposed to shade conditions, or vice versa (Figure 1c).

2 | MATERIALS AND METHODS

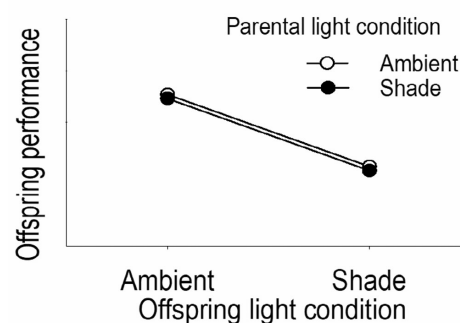
2.1 | Plant species

Hydrocotyle vulgaris L. (Araliaceae) is a perennial clonal herb native to Europe and North America. It was introduced into China as a garden species in 1990s, but now it can be frequently found in a broad range of habitats, such as valleys, dunes, wetlands, marshes and even deep water (Dong et al., 2015). This species produces only creeping stems, and each stem node has the potential to develop into a ramet consisting of a leaf and some adventitious roots (Dong et al., 2015). This species exhibits rapid clonal reproduction and high morphological plasticity (Dong, 1995; Dong et al., 2015). In China, sexual reproduction of *H. vulgaris* is very low so that populations in the wild consist of only one genotype or are dominated by one genotype (Wang et al., 2020).

(a) Parental light condition affects offspring responses to light



(b) Parental light condition affects offspring after removal of epigenetic effects



(c) Parental light condition affects offspring via soil legacy

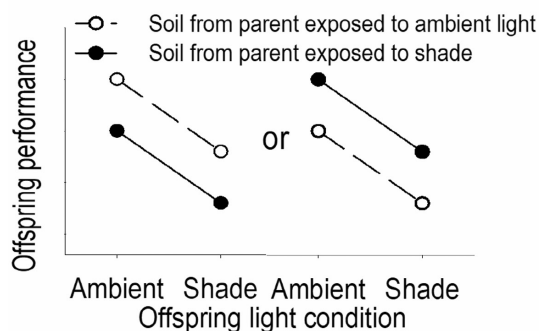


FIGURE 1 Schematic conceptual representation of how light condition that the parents experienced will influence responses of their offspring to light. We expect that (a) offspring produced by parents exposed to ambient light will perform better than those produced by shaded parents, when the offspring grow under ambient light conditions; while offspring produced by shaded parents perform better than those produced by parents exposed to ambient light when the offspring grow in shade conditions. Overall, offspring will perform worse under shade than under ambient light conditions. If these effects happen through epigenetics, we expect that (b) the application of a DNA demethylation agent will remove or reduce the parental light effect. If these effects occur through soil legacy effects, we expect that (c) all offspring, irrespective of the light conditions their parent was experiencing, will respond to the soil, that is, all offspring will do better in soil from parent exposed to ambient light or shade conditions.

In 2016, 128 ramets of *H. vulgaris* were collected from 10 populations in five Provinces in China, and a total of 20 genotypes were identified using AFLP. Then, ramets of the different genotypes were vegetatively propagated in separate containers (85 cm in diameter), and the ramets that were obtained were used as stock plants. We randomly selected five genotypes and used them for the two-phase experiment described below.

2.2 | Parent generation experiment

2.2.1 | Soil preparation and plant transplantation

In the parent generation experiment, we first collected soil from a barren hill. The field soil was air-dried, sieved (2-cm mesh) and mixed with river sand at a 1:1 volume ratio ('bulk soil' hereafter). Then, we filled 80 pots (5 L) with 7.0 kg of the bulk soil, which was evenly mixed with 20 g (4 g/L) of slow-release fertilizer (N:P:K = 14:14:14; Osmocote; Scotts).

On 3 May 2020, we collected 16 ramets of *H. vulgaris* from the stock plants of each of the five genotypes. Each ramet had a node with some adventitious roots, a petiole of 2 cm long, a proximal and a distal internode of 1 cm long. All 80 ramets were initially similar in size. We planted these ramets individually in the 80 pots and watered them every day.

2.2.2 | Experimental design

On 17 May 2020, the 16 ramets of each genotype were assigned to one of four treatments, that is, two light treatments (ambient vs. shade) crossed with two DNA demethylation treatments (control vs. 5-azaC; Figure 2). Each treatment was replicated four times, resulting in a total of 80 pots (2 light treatments $\times$ 2 DNA demethylation treatments $\times$ 5 genotypes $\times$ 4 replicates). For the shade treatment, the ramet in each pot was covered with a green film (type 4460, 60 green, LEE filters, Rosco Cinegel), while for the ambient light treatment, the ramets were not covered with the film. The film reduced the photosynthetically active radiation to 30% and red to far-red ratio to 0.4 (Table S1). For the 5-azaC treatment, the leaf surface of the ramet in each pot was sprayed with 2 ml of 100 μ mol/L 5-azaC (Macklin, Shanghai Macklin Biochemical Co., Ltd.) with 2 ml of surfactant (Twenn-80, Shanghai Qingxi Chemical Technology Co., Ltd.) every other day; ramets in control pots were sprayed with 2 ml of water with 2 ml of surfactant every other day (Puy et al., 2018). The demethylation agent 5-azaC inhibits DNA methyltransferase, thus blocking methyl groups into DNA, and has been widely used to determine the role of epigenetic variation in ecology and evolution (e.g. Bosdorf et al., 2008; González et al., 2018; Münzbergová et al., 2019; Puy et al., 2021). During the application of 5-azaC, we did not cover the soil as a lot of newly produced ramets (62–152 on average; Figure S1B) were randomly distributed in the pot. However, we sprayed the leaves carefully by checking that no solution dropped

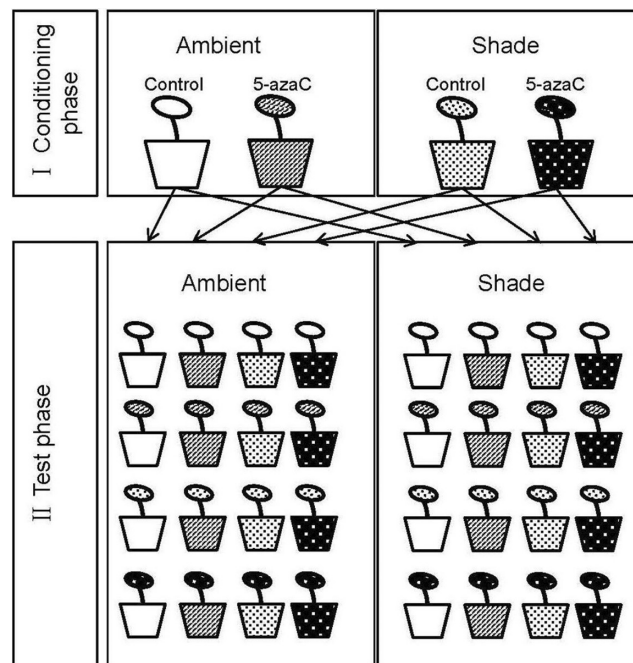


FIGURE 2 Schematic representation of the experimental design. In the conditioning phase, for each genotype, a single stolon node of *Hydrocotyle vulgaris* was grown in a pot subjected to one of four treatments, that is, two light treatments (Ambient vs. Shade) crossed with two DNA demethylation treatments [Control vs. 5-azacitidine (5-azaC); leaves of newly produced ramets were sprayed with either water or 5-azaC]. For each treatment and each genotype, we collected the offspring ramets and the soils; there were four offspring types and four parent soils. In the test phase, for each genotype, we grew each of the four offspring types in each of the parent soils, under the two light conditions. A subset of pots was used for determination of plant growth at the end of the conditioning phase. These are not shown, but were exposed to the same treatments in the conditioning phase.

down on the soil. Our soil analysis also confirmed that the application of 5-azaC did not influence soil fungal community composition (Figure 3a) or soil chemistry (Figure 3c). The DNA demethylation treatment was started on 1 June 2020, and terminated at the end of the conditioning phase on 12 August 2020. During the parent generation experiment, the mean temperature and relative humidity in the greenhouse were 29.7°C and 81.0%, respectively.

2.2.3 | Harvest of plant and soil

At harvest, we randomly selected two replicate pots for each genotype and each treatment (40 pots in total) to determine plant performance. The number of ramets in each pot was recorded and total biomass was determined after drying the plants at 70°C for 48 hr. The total biomass, number of ramets and plant size (biomass per ramet) of *H. vulgaris* parents are shown in Figure S1.

Plants and soils in the two remaining replicate pots (40 pots in total) were also harvested and used in the test phase of the experiment. For each treatment and each genotype, we collected

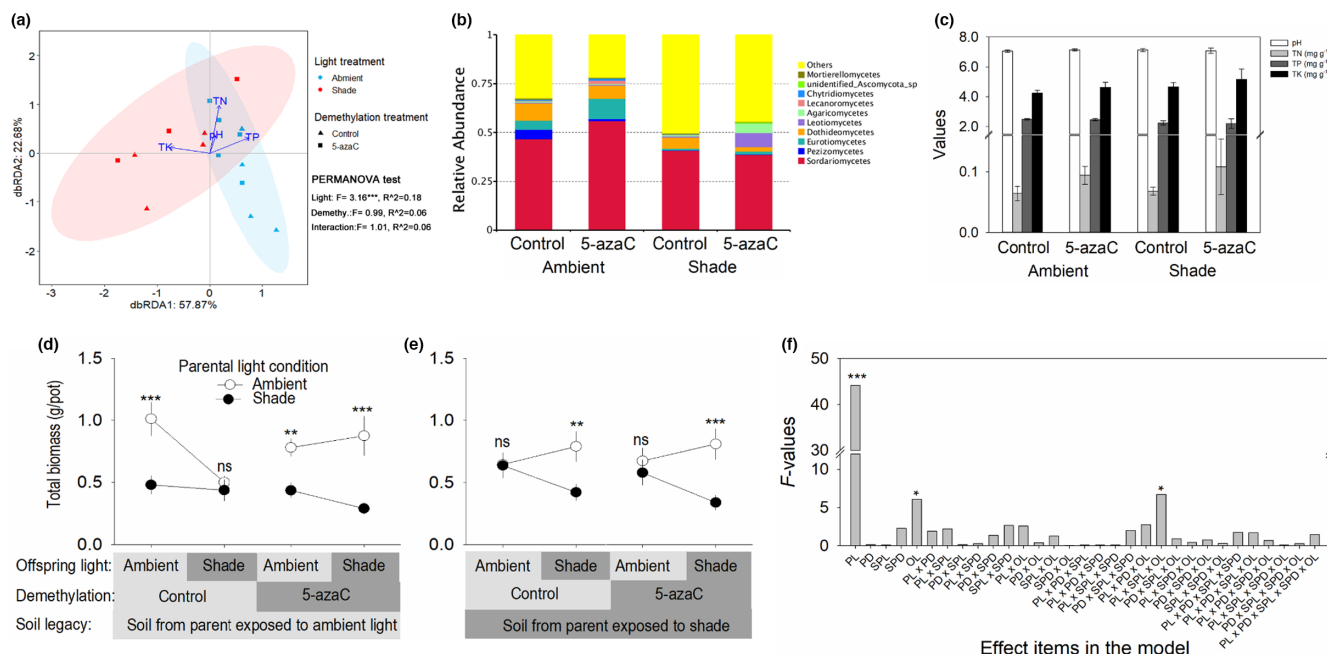


FIGURE 3 Distance-based redundancy analysis (db-RDA) plot based on Bray–Curtis dissimilarity showing correlations between soil fungal community composition, treatment effects and soil chemical variables (a), relative abundance of the dominant (top 10) fungi classes (b) and soil chemistry (c) under different treatments at the end of the parent generation experiment. Total biomass of clonal offspring of *Hydrocotyle vulgaris* (d and e) in response to light as mediated by parental light conditions (ambient vs. shaded parents), parental demethylation status [control vs. 5-azacitidine (5-azaC) parents] and soil legacy of parental light conditions (soil from parent exposed to ambient light vs. shade conditions) in the offspring generation experiment, and *F*-values for each effect items obtained from a linear mixed-effects model test for effects of parental light (PL) condition, parental demethylation (PD) status, soil legacy of parental light (SPL) condition, soil legacy of parental demethylation (SPD) status, offspring light (OL) condition and their interactions on total biomass of clonal offspring of *H. vulgaris* (f). In (a), soil from parent exposed to ambient light and shade conditions are presented with blue and red symbols, respectively; soil from parent exposed to control and 5-azaC treatment are presented with triangles and circles, respectively. In (b), mean values are presented; see Table S5 for statistic results. In (c), mean values (± 1 SE) are presented; see Table S4 for statistic results. In (d) and (e), mean values (± 1 SE) are presented; the asterisk indicates significant difference between offspring produced by ambient versus shaded parents based on a planned contrast: ns $p \geq 0.05$, ** $p < 0.01$, *** $p < 0.001$. In (f), the larger the *F*-value, the more variation in the data is explained by the item; asterisks above the bar indicate significance level of the effect term in the model: * $p < 0.05$, *** $p < 0.001$, otherwise $p > 0.05$.

the offspring ramets (each consisting of a stem node with some adventitious roots, a petiole of 2 cm long, a proximal and a distal internode of 1 cm long) and the soil from the two pots. The soil from each treatment and each genotype was homogeneously mixed with sterilized bulk soil (autoclaved at 121°C for 120 min) at a 1:1 volume ratio, and used to fill 16 pots (1 L) each with 0.8 kg. The filled pots were used in the offspring generation experiment described below.

2.3 | Offspring generation experiment

2.3.1 | Experimental design

In the offspring generation experiment, for each genotype, we grew the offspring ramets from each of the four origins (i.e. the offspring produced by the parent ramets subjected to the following treatments: (a) ambient + control, (b) ambient + 5-azaC, (c) shade + control and (d) shade + 5-azaC) in the soil from each of the four origins (i.e. the soils trained by the parent ramets

subjected to the following treatments (a) ambient + control, (b) ambient + 5-azaC, (c) shade + control and (d) shade + 5-azaC), under the two light conditions (ambient vs. shade). The ambient light and shade treatments were manipulated with the same procedure as applied in the parent generation experiment (see Figure 2 for a graphic explanation of the experimental design). Therefore, there were 4 offspring types $\times$ 4 parent soils $\times$ 2 light conditions for the offspring $\times$ 5 genotypes $\times$ 2 replicates for each genotype = 320 pots in total. Water was supplied to each pot every day. The offspring generation experiment was maintained for 11 weeks (from 13 August to 29 October 2020). During this period, the mean temperature and relative humidity in the greenhouse were 26.6°C and 87.8%, respectively.

2.3.2 | Plant harvest

At harvest, we first recorded the number of ramets and measured total length of the creeping stems in each pot. Then, we collected the third, fourth and fifth mature internodes and leaves in each pot,

and measured length of the internodes and lamina area of the leaves. We also measured the biomass by oven-drying the samples at 70°C for 48 hr. Based on these data, we calculated mean internode length, specific internode length (SIL, mean internode length/mean internode biomass), mean leaf area, specific leaf area (SLA, mean leaf area/mean leaf biomass). The remaining plants in each pot were separated into leaves, creeping stems and roots, oven-dried at 70°C for 48 hr and weighed to obtain biomass.

2.4 | Soil sampling, chemical measurements, DNA extraction, PCR amplification, sequencing and bioinformatics

2.4.1 | Soil sampling

On 12 August 2020, at the end of the parent generation experiment, for each treatment and each genotype, a subsample (200 g) of the homogenized soil was collected (20 samples in total), stored at -20°C and used later to measure soil chemical properties [pH, total nitrogen (TN), total phosphorus (TP) and total potassium (TK)] and soil microbial community composition. However, due to contamination, three soil samples were excluded from the analysis of soil microbial community composition ($N = 17$); a further soil sample was destroyed when transportation and thus was excluded from the analysis of soil chemical properties ($N = 16$).

2.4.2 | Soil chemical measurements

Soil TN, TP and TK were determined by adding 5 ml of H_2SO_4 and 0.5 ml of $HClO_4$ to soil samples (0.5 g), digesting it at 360°C for 35 min, filtering the solution at room temperature (20°C) and measured with Auto Analyser 3. Soil pH- H_2O was determined by adding 10.0 ml demi-water to soil samples (volume 5.0 ml), shaking for 5 min and measuring 2 hr later.

2.4.3 | DNA extraction, PCR amplification, sequencing and bioinformatics

DNA extraction, PCR amplification, sequencing and bioinformatics were conducted by MetWare Biotechnology Co., Ltd. (www.metware.cn, Wuhan, China). Microbial genomic DNA was extracted from 0.5 g soil using the cetyltrimethylammonium ammonium bromide method. The concentration and purity of the extracted DNA were quantified with agarose gel electrophoresis. Based on the concentration, DNA was diluted to 1 ng/ μ l using sterilized water. The ITS1 region of the fungal rDNA gene was amplified with primers ITS5-1737F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS2-2043R (5'-GCTGCGTTCTTCATCGATGC-3'). All PCRs were carried out with Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs), Fast Start High Fidelity Enzyme Blend and

template DNA. After amplification, the PCA product was used for agarose gel (2%) detection. Then equimolar amounts of the PCR product were pooled and purified with a Gel Extraction kit (Qiagen). The libraries were generated with the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina), and sequenced on the Illumina HiSeq platform (NovaSeq 6000, Illumina Inc.). The raw sequences were trimmed and merged, quality-filtered based on the Qiime process (V1.9.1; http://qiime.org/scripts/split_libraries_fastq.html) and chimeras were removed. After that, high-quality sequences were clustered into Operational Taxonomic Units (OTUs) at a 97% similarity according to the Uparse process (v7.0.1001, <http://www.drive5.com/uparse/>). The sequences were assigned to taxonomic groups using the blast method (http://qiime.org/scripts/assign_taxonomy.html) and the Unite database (v8.2, <https://unite.ut.ee>) for fungal. To account for differences in sequencing depths, sequence numbers of each sample were rarified to 60,000 reads. The rarefaction curve showed that all samples reached saturation. The rank abundance curve showed that all the sequencing efforts covered the full extent of the majority of the fungal diversity (Figure S2).

2.5 | Data analysis

2.5.1 | Analysis of plant performance in the offspring generation experiment

We used linear mixed-effects models fitted with the nlme package (version 3.1-145; Pinheiro et al., 2018) to examine the following factors: parental light condition (ambient vs. shaded parents), parental demethylation status (control vs. 5-azaC parents), soil legacy of parental light condition (soil conditioned by ambient vs. shaded parents), soil legacy of parental demethylation status (soil conditioned by control vs. 5-azaC parents), offspring light condition (ambient vs. shaded offspring). In the model, these factors and their interactions were treated as fixed effects, and genotype identity was included as a random factor to account for phylogenetic non-independence of genotypes; the response variables were the growth (total biomass, leaf biomass, creeping stem biomass, root biomass, number of ramets and total creeping stem length) and morphology (mean internode length, mean leaf area, SIL and SLA) of the *H. vulgaris* offspring. However, as we only have five genotypes, using genotype identity as a random factor may inflate the proportion of variance ascribed to the fixed effects. We therefore also ran linear models where we included genotype identity as a fixed effect. The results of the two models were similar (see Tables S2 and S3). In the two models, a planned contrast was made using the MULTCOMP package (version 1.4-15; Hothorn et al., 2008) to compare the performance of the offspring generated by ambient versus shaded parents; planned contrasts were corrected using the method of Benjamini and Hochberg (1995).

We also examined the allometric relationships for biomass and morphology of different organs versus plant size of the offspring in both ambient and shade light conditions using linear regressions. In this analysis, all data were log-transformed and offspring light condition and its interaction with plant size were also included in the

regression models; a significant interaction effect indicates different regression exponents in the ambient and shade light conditions. A consistent relationship in the ambient and shade light conditions indicates that there was a plant size effect only; while a divergent relationship in the ambient and shade light conditions indicates a signal of treatment effect.

2.5.2 | Analysis of soil fungal community composition and soil chemical characteristics at the end of the parent generation experiment

Differences in soil fungal community composition (based on OTU composition) between the different conditioned soils were visualized using a distance-based redundancy analysis (db-RDA) plot. The relationships between soil fungal community composition and soil chemical properties were added as vectors in the db-RDA plot; significance of these relationships were examined with an ANOVA-like permutation test (999 permutations) using the *capscale* function in the *VEGAN* package (version 2.5-6; Oksanen et al., 2019). In this analysis, soil chemical properties were treated as the matrix of explanatory variables, and the Bray–Curtis dissimilarities among fungal OTUs were used as response variables. We also performed PERMANOVA using the *adonis* function in the *VEGAN* package to test whether soil fungal community structure could be explained by light treatment, demethylation treatment and their interaction at the end of the parent generation experiment; significance was based on a permutation test (999 permutations). The relative abundance of the fungal taxa (phylum, class and genus) was examined using linear mixed-effects models with light condition (ambient vs. shaded), DNA demethylation status (control vs. 5-azaC) and their interaction as fixed factors, and genotype identity as a random factor. We used MetaStat analysis to examine whether there were significant differences among fungal communities at each taxonomic level among the treatment groups. We also used linear discriminant analysis (LDA) effect size (LEfSe) for the discovery and interpretation of differentially abundant taxa (biological markers) with LDA scores higher than 4 (i.e. the taxa that are significantly different between groups).

Soil chemical characteristics (pH, TN, TP and TK) were examined using linear mixed-effects models with light condition, DNA demethylation status and their interaction as fixed factors, and genotype identity as a random factor. We also performed a Pearson's correlation test to examine the correlation between different soil chemical characteristics.

2.5.3 | Analysis of relationships between plant performance in the offspring generation experiment and soil (a)biotic characteristics, parental plant size at the end of the parent generation experiment

The relationship between plant performance (growth and morphology) in the offspring generation experiment and soil

fungal community composition at the end of the parent generation experiment was analysed using a db-RDA. In this analysis, the growth and morphology of the plants were treated as the matrix of explanatory variables, and the Bray–Curtis dissimilarities among fungal OTUs were used as response variables. Significance of these relationships was examined using an ANOVA-like permutation test (999 permutations). The relationship between plant performance in the offspring generation experiment and soil chemical characteristics at the end of the parent generation experiment was analysed using a stepwise multiple linear regression with backward selection procedure. The relationship between plant performance in the offspring generation experiment and plant size (biomass per ramet) of their parent at the end of the parent generation experiment was analysed using a Spearman's rank correlation test.

3 | RESULTS

3.1 | Plant performance and soil (a)biotic properties at the end of the parent generation experiment

When treated with 5-azaC, *H. vulgaris* parents produced greater total biomass and more ramets in ambient light conditions than in shade conditions; but no shade effects were detected when they were treated with water (i.e. control; Figure S1A,B). Light condition, demethylation status of parent plants or the interaction did not influence plant size (biomass per ramet) of the parents (Figure S1C).

Conditioning by control versus 5-azaC-treated parental plants had no significant effect on soil fungal community composition (Figure 3a; permutation test, $p = 0.433$). However, exposing the parents to ambient light versus shade conditions led to clear differences in soil fungal community composition (Figure 3a; permutation test, $p = 0.001$). Moreover, the soil fungal community composition was marginally ($p = 0.069$) correlated to soil TP, but did not correlate with soil pH, TN or TK (Figure 3a; Table S4).

The relative abundance of two dominant fungal phyla (i.e. Ascomycota and Chytridiomycota; Figure S3A; Table S5A), two dominant fungal classes (i.e. Eurotiomycetes and Chytridiomycetes; Figure 3b; Table S5B) and five dominant fungal genera (i.e. *Acremonium*, *Penicillium*, *Neocucurbitaria*, *Capronia* and *Bipolaris*) were significantly greater in soil from parent exposed to ambient light than in soil from parent exposed to shade. These results were further confirmed by MetaStat analysis (Figure S3c–e) and LEfSe (Figure S4). Demethylation or its interaction with light condition did not influence the relative abundance of any dominant fungi at phylum (Figure S3A; Table S5A), class (Figure 3b; Table S5B) or genus (Figure S3B; Table S5C) level. However, the MetaStat analysis and the LEfSe showed that the relative abundance of certain taxa was different between soil from parents treated with water versus 5-azaC (Figures S3C–E and S4C).

We did not find any significant effect of parent plant treatment on pH, TN, TP or TK in the soil (Figure 3c; Table S6). Soil TP was marginally positively correlated with soil pH ($p = 0.054$) and soil TN

($p = 0.070$), and soil TN was also marginally positively correlated ($p = 0.074$) soil pH (Figure S5).

3.2 | Plant growth and morphology in the offspring generation experiment

Overall, total biomass of *H. vulgaris* offspring to light was significantly influenced by the interaction of light condition experienced by their parents and the soil legacy of the parental light condition (Figure 3f: a significant three-way interaction effect). Moreover, the planned contrast showed that demethylation status of the parent was also involved in influencing total biomass of *H. vulgaris* offspring in response to parental light conditions (Figure 3d,e).

When grown under ambient light condition and in soil from parents exposed to ambient light, offspring produced by control, ambient parents had greater total biomass than offspring produced by control, shaded parents (Figure 3d: left panel). This difference was smaller when the parents were treated with 5-azaC (Figure 3d: right panel), and disappeared when the offspring were grown under ambient light condition but in soil from parents exposed to shade (Figure 3e: leaf panel).

When grown under shade condition and in soil from parents exposed to shade, offspring produced by control, shaded parents had lower total biomass than offspring produced by control, ambient parents (Figure 3e: left panel). The growth difference was much greater when the parents were treated with 5-azaC (Figure 3e: right panel), and disappeared when the offspring were grown under shade condition but in soil from parents exposed to ambient light (Figure 3d: left panel).

A similar treatment effect was found for leaf biomass, stem biomass, root biomass, number of ramets, creeping stem length, leaf area and SIL of *H. vulgaris* offspring but not for SLA and internode length (Figure S6; Table S2). The growth and morphology measures in each of the treatment combinations are shown in the supplementary materials (Figure S7).

The log-scaled regressions showed significantly different relationships between stem biomass versus plant size, leaf area versus plant size and SLA versus plant size for the offspring grown in ambient and shaded light conditions (Figure S8).

3.3 | Relationships between offspring performance and parental plant size or soil (a) biotic properties

Total biomass, leaf biomass and stem biomass of *H. vulgaris* offspring were significantly related to soil fungal community composition (Table S7). Total biomass, leaf biomass, stem biomass, number of ramets and total stem length of *H. vulgaris* offspring were also significantly positively related to TP concentrations in the soil (Tables S7). Root biomass and number of ramets of *H. vulgaris* offspring were significantly negatively related to plant size of their parents (Table S7).

4 | DISCUSSION

In this study, we show that light conditions experienced by parents can significantly influence the responses of their offspring to light. This parental light effect was mediated by both the demethylation status of the parents and the soil that had been altered by the parents exposed to different light conditions. These results indicate that light-induced parental effects and soil legacy effects can interact to influence offspring performance, in our case, the clonal herb *H. vulgaris*.

4.1 | Hypothesis 1

The prediction that offspring produced by parents grown under ambient light conditions perform better than those produced by shaded parents when the offspring are grown under ambient light conditions was supported in soil from parents exposed to ambient light conditions. One explanation for this phenomenon is the provisioning effect, which states that offspring generated by parents grown in favourable conditions (e.g. ambient light conditions in our study) are of higher quality and with greater body size than those generated by parents grown in unfavourable conditions (e.g. shaded conditions in our study; Miao et al., 1991; Dong et al., 2018; Baker et al., 2019; Pigeon et al., 2019). However, in our study, the initial body size of the ramets (nodes each with a leaf, two internodes) used at the beginning of the offspring generation experiment was standardized (see Method section for details), and generally did not correlate with the performance of the offspring (Table S7). Many studies have also showed that changes in mineral composition, secondary metabolites and hormone content are involved in parental effects (González et al., 2017; Herman & Sultan, 2011; Latzel et al., 2020; Miao et al., 1991). For the current study, we cannot exclude the possibility that the observed parental effect of parental light condition may result from the contrasting chemical composition between parents exposed to ambient light versus shade conditions and further studies are needed to examine this.

By contrast, the prediction that offspring produced by shaded parents perform better than those produced by parents grown under ambient light conditions when the offspring are grown under shade conditions was not supported. Therefore, parental shading effects may not be adaptive for the shaded offspring of *H. vulgaris*. The absence of adaptive parental shading effects is likely because the parental shading effects severely inhibited the growth and development of clonal offspring generated by shaded parents, which may have potentially induced maladaptive changes in morphological traits (i.e. smaller and thinner leaves, shorter and finer internodes of clonal offspring produced by shaded versus ambient parents; Figure S6L–M, R–S). The allometric relationships further confirmed that differences in offspring plant sizes induced by parental shading effects may lead to divergent growth responses of the offspring to light (Figure S8). However, this result does not necessarily mean that the potential of parental environment conditions to steer the

performance of clonal offspring under stressful environments is overestimated. Future explorations are needed on how many generations these maladaptive phenotypic changes persist and whether the strength and direction of these changes shifts among different offspring generations.

4.2 | Hypothesis 2

Our results also show that the application of 5-azaC, a DNA demethylation agent, reduced the parental light effect for offspring grown under ambient light conditions and in soil from parents exposed to ambient light, but enhanced the parental light effect on offspring grown under shade conditions and in soil from parents exposed to shade. Therefore, our second hypothesis was not fully supported. In general, the effect of parental environment condition on offspring is thought to be established through methylation, that is, addition of methyl groups (e.g. Akkerman et al., 2016; Boyko & Kovalchuk, 2011; Herman & Sultan, 2016; Puy et al., 2021). Therefore, the application of the chemical demethylation agent should lead to the weakening or removal of parental effects. However, Baker et al. (2018) reported that parental shading effects on offspring can also be established via demethylation, that is, removal of methyl groups. Both the two processes are likely involved in influencing the performance of offspring in response to light conditions experienced by their parents under the contrasting light and soil conditions. It is important to note, though, that demethylation may result in the expression of alleles normally silenced by DNA methylation, uncovering previously hidden phenotypic variations in plants (Feng et al., 2010; Ji et al., 2018; Münzbergová et al., 2019). Further molecular work on analysing of DNA methylation status is needed to disentangle how exactly the parental effects are transmitted.

4.3 | Hypothesis 3

Interestingly, our study shows that the parental light effect on offspring differed between soils from parents exposed to shade and ambient light conditions. In our study, the fungal genera *Acremonium*, *Penicillium*, *Neocucurbitaria*, *Capronia* and *Bipolaris* were more abundant in soil from parents exposed to ambient light conditions, and soil fungal composition was significantly associated with the growth of *H. vulgaris* offspring. The growth of *H. vulgaris* offspring was also correlated to soil TP despite that the total soil phosphorus levels were not influenced by parental light conditions. Therefore, the different parental light effects in the two soils are likely due to contrasting abiotic and biotic properties of the soil. Moreover, the difference in parental light effects in the two soils varied depending on light conditions experienced by the offspring. This result indicates that current light availability is important in driving the influences of soil legacies on parental effects. It is important to note that changes in the soil microbial community are dynamic and that the

soil composition may have changed further during the period of the offspring generation experiment (Hannula et al., 2021). In our study, it is likely that shading offspring may have differently steered the soils, as well as altering legacy effects on the offspring responses to parental light conditions.

5 | CONCLUSIONS

We conclude that the light conditions experienced by parents can strongly influence the responses of their offspring to light via both parental effects and soil legacy effects. The parental effects were attributed to changes in DNA methylation status and the soil legacy effects were associated with changes in soil fungi and soil phosphorous levels. Our results highlight the importance of parental effects, soil legacy effects and current light availability in driving clonal plant population growth, and hence these factors should not be examined independently. Further molecular work should determine how the parental effects are transmitted to clonal offspring of *H. vulgaris*, how this works for other species, and how soil legacies influence these transmission processes.

AUTHORS' CONTRIBUTIONS

W.X. and F.H.-Y. conceived the idea and designed the methodology; W.X. and L.H. conducted the experiment and collected the data; W.X. and L.H. analysed the data with guidance of T.M.B.; W.X., L.H., F.H.-Y. and T.M.B. wrote the first version of the manuscript. All authors discussed the results, contributed substantially to the draft and gave final approval for publication.

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CONFLICT OF INTEREST

There are no conflict of interests to declare.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository <https://doi.org/10.5061/dryad.prr4xgxmg> (Xue et al., 2022).

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SUPPORTING INFORMATION

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