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Contribution of conspecific soil microorganisms to tree seedling light responses: Insights from two tropical species with contrasting shade tolerance

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ABSTRACT

Light intensity drives whole-plant carbon gain, with consequences for biomass production and plant community dynamics in forest systems. Recent studies suggest that soil microbial communities may mediate the impacts of resource availability on plant performance, yet little is known about the net effect of conspecific soil microorganisms for tree seedling light responses. Here we examined the interactive effects of light availability and presence of conspecific soil microorganisms on tree seedling growth, morphology and nutrient content for two congeneric tropical tree species. The two *Bauhinia* tree species with contrasting shade tolerance were grown in sterilized or unsterilized soil medium, under either high (50%) or low (10%) light conditions in a greenhouse experiment. Plant light responses and soil feedback effects were determined after 12 weeks. Results showed that the light-demanding tree species was generally more responsive to both light and soil microbes compared with its shade-tolerant congener. Presence of soil microbes enhanced plant growth and biomass responses to increased light availability for the light-demanding species alone, driven by positive soil feedback effects in high light. Six plant traits (leaf mass fraction, stem mass fraction, specific stem length, leaf phosphorus concentration, leaf nitrogen: phosphorus ratio and root nitrogen: phosphorus ratio) showed significant interactive effects between light and soil treatment. Observed changes to leaf biomass allocation in response to light in the presence of conspecific soil microorganisms were consistent with optimality theory and adjustments to maximize resource acquisition under different light conditions. In addition, presence of soil microbes decreased the average plasticity of plant nutrient content and stoichiometry in response to light for the light-demanding *Bauhinia* species. Together these results highlight the importance of conspecific soil microbes for plant-light relations, with implications for plant-plant interactions and species coexistence.

1. Introduction

Light is a key resource for photosynthesis, driving plant growth and morphology as well as gene expression (Cheng et al., 2014; Givnish, 1988; Vialet-Chabrand et al., 2017). The impacts of light intensity on plants are of particular interest in the forest understorey, where plants encounter a highly heterogeneous and dynamic light environment (Chazdon et al., 1996). Indeed, tree seedling responses to light intensity may be critical for individual survival and population persistence, with consequences for both small-scale biotic interactions (plant-plant, plant-soil) and large-scale biomass production in managed forest systems (Binkley et al., 2013; Bloor and Grubb, 2003; Carvalho and Castillo, 2018).

Previous studies of tree seedlings in temperate and tropical forests have shown that tree seedlings adjust their morphological and physiological traits in response to light changes, so as to maximize carbon gain under low light/ shade conditions (Baltzer and Thomas, 2005; Niinemets, 1997; Popma and Bongers, 1991; Portsmuth and Niinemets, 2007; Valladares et al., 2000). For example, seedlings generally increase specific leaf area and biomass allocation to leaves under low light conditions, with benefits for light interception (Bloor and Grubb, 2004; Liu et al., 2016; Walters and Reich, 1999). Plants may also reallocate nutrients to leaves from other organs, and show increased investment in photosynthetic enzymes to maximize photosynthetic rates under low light conditions (Valladares and Niinemets, 2008). The magnitude of plant responses to variation in light intensity is often

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thought to differ between shade-tolerant and light-demanding species, since greater phenotypic plasticity to light changes is commonly observed in light-demanding species (Sánchez-Gómez et al., 2006; Valladares et al., 2000; Walters and Reich, 1999). In contrast to shade-tolerant tree species, light-demanding species typically have high growth rates, low leaf longevity and high light-saturated photosynthetic rates at the leaf level (Givnish, 1988). Fast growth rates are expected to promote the expression of phenotypic plasticity since greater morphological changes can be generated during a given time period compared to plants with slow growth rates (Van de Vijver et al., 1993). However, comparisons of phenotypic plasticity between shade-tolerant and light-demanding species may be confounded by phylogeny and genetic constraints, and comparisons within phyletic lines are required to identify clear ecological patterns (Kelly and Purvis, 1993; Van Kleunen and Fischer, 2005).

An increasing number of studies suggest that plant species' responses to abiotic factors may be mediated by soil microorganisms (Friesen et al., 2011; Fry et al., 2018; Yang et al., 2009). Indeed, recent experiments comparing plants grown in sterile and non-sterile soil mediums have highlighted the importance of soil microorganisms for plant traits and plant sensitivity to water stress (Kannenberg and Phillips, 2017; Xi et al., 2018). 'Live' soil contains a variety of microbial groups (e.g. Gram-positive and Gram-negative bacteria, mycorrhizal, saprophytic or pathogenic fungi) with different resource-use strategies and functional roles in soil, such as organic matter decomposition and mineralisation of phosphorus and nitrogen (Eldridge and Delgado-Baquerizo, 2018; Fanin et al., 2019). Soil pathogens and microbial mutualists can have direct effects on plant growth and functional traits by providing novel biochemical capabilities (e.g. nutritional and defence pathways), or by altering existing plant metabolic pathways (Berg et al., 2016; Friesen et al., 2011). Soil microbial communities may also have indirect effects on plant performance via changes in soil properties such as nutrient availability, which influence plant traits associated with resource acquisition and use (Bruelheide et al., 2018). Work in grassland and forest ecosystems has demonstrated coupling of microbial functional groups and plant traits, and suggests that fast-growing, resource-acquisitive plant species are often associated with bacteria-dominated soil microbial communities and fast nutrient turnover (de Vries et al., 2012; Grigulis et al., 2013), or abundance of particular bacterial groups (Pei et al., 2016).

The net effect of soil microbes on plants (i.e., soil feedback effects) can be negative, positive or neutral, depending on the microbial groups involved and the prevailing environmental conditions (Smith-Ramesh and Reynolds, 2017; van der Putten et al., 2013). For example, mutualistic mycorrhizal fungi which promote plant acquisition of soil nitrogen and phosphorus may enhance photosynthetic rates under nutrient-limited conditions (Friesen et al., 2011). Increased light availability is known to increase plant carbon allocation to mutualistic mycorrhizal fungi, thereby increasing positive effects of soil microbes on plant performance under high light conditions (Carvalho and Castillo, 2018; Johnson et al., 1997; Konvalinková and Jansa, 2016; Zheng et al., 2015). Increased light availability may also reduce the costs of plant defence against fungal pathogens, and increase plant resistance (Roberts and Paul, 2006). As yet, however, few studies have explicitly quantified the magnitude of soil feedback effects on tree seedlings under varying light conditions (McCarthy-Neumann and Kobe, 2010).

In the present experiment, we investigated the interactions between light intensity and presence of conspecific soil microorganisms for two *Bauhinia* tree species with contrasting shade-tolerance at the seedling stage. We examined the effects of high and low light intensity on plant growth, morphology and nutrient content for seedlings growing in sterilized or unsterilized soil medium under greenhouse conditions. Although changes in the nutrient content and stoichiometry of above- and belowground plant tissue have significant implications for biogeochemical cycling and ecosystem function (Elser et al., 2010), few studies of whole-plant responses to light have addressed plant N:P ratios. We focused on effects of conspecific soil microorganisms on host plant performance because conspecific cultures have been shown to have a greater influence on tropical tree seedlings than heterospecific cultures (McCarthy-Neumann and Kobe, 2010). Indeed, the *Bauhinia* genus is reported to have associations with mycorrhizal fungi and endophytic fungi with antibacterial properties (Bezerra et al., 2015; Brundrett, 2009; Vaidya et al., 2008). Three hypotheses were addressed: (1) presence of soil microbes has a more positive effect on plant growth and biomass under high-light compared to low-light conditions i.e., more positive soil feedback effects due to increased mutualistic mycorrhizal fungi; (2) light responses of seedling plant traits are modified in the presence of soil microbes; (3) the light-demanding tree species has a greater sensitivity to light compared to its shade-tolerant congener. We also assessed the linkages between seedling light responses and microbial community structure in the unsterilized medium.

2. Materials and methods

2.1. Plant material

Two congeneric savanna tree species with contrasting growth rates and shade-tolerance at the seedling stage were used as a model system; the shade-intolerant species *Bauhinia brachycarpa* and the shade-tolerant species *Bauhinia variegata.* Seedling shade tolerance was assigned based on expert knowledge at the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. These two woody legume species have a broad distribution across tropical and subtropical areas, providing fodder, fuel and medicinal uses (Filho, 2009). They are non-nodulating species and not considered to fix nitrogen (Sprent et al., 2017), but are known to have symbiotic associations with ectomycorrhizal fungi (Brundrett, 2009). Seeds were collected in 2016 and 2017 from the dry woodland ecosystem of the Yuanjiang Valley, China (23°28′N, 102°10′E) where the two species co-occur naturally. Seeds were surface-sterilized (1 min 75% ethanol, 3 min 2% NaClO, 1 min 75% ethanol, 3 min distilled water) and kept at 5 °C prior to use in a two-phase plant-soil feedback experiment.

2.2. Soil conditioning

In the first phase of the experiment, field soil was collected in the rhizosphere under adult individuals of *B. brachycarpa* and *B. variegata.* Field soil in the 0–15 cm soil layer for each tree species was collected from the Yuanjiang woodland ecosystem, homogenized and used for soil conditioning in 2016. Species-specific soil microbes were cultured by growing individual plants of *B. brachycarpa* and *B. variegata* in pots (14 cm diameter × 16 cm height) that were filled with a mix of field soil from under their respective adults, sterilized sand and peat (volume ratio of 1: 6: 3). This soil-conditioning approach capitalizes on the fact that adult plants have influenced the soil for a long period of time under natural conditions, and minimizes possible confounding effects of spatial variation in soil abiotic properties and soil microbial composition (Brinkman et al., 2010). Bulking soil together is an appropriate technique for soil conditioning since we were interested in the impact of the average conspecific soil community on our model species (Gundale et al., 2019).

Seeds were first germinated in plug trays filled with sterilized sands and left to grow for two weeks at the Xishuangbanna Tropical Botanical Garden (21°41′N, 101°25′E, 570 m a.s.l.). One tree seedling was then transplanted into each pot and left to grow under greenhouse conditions for nine months; seven pots were established for each species, leading to a total of 14 pots. Pots were randomly located in the greenhouse, watered regularly and re-arranged weekly to avoid possible positioning effects. After nine months, all above- and belowground plant biomass was removed from the pots. The conditioned soil was homogenised, and used for the main feedback experiment. Half of the soil was kept at 5 °C whereas the remainder was steam-sterilized for 3 h at 121 °C and then kept at 5 °C prior to the start of light response experiment.

2.3. Experimental design and light treatments

Light intensity and soil biota treatments were applied in a split-plot design (Quinn and Keough, 2002), with 12 blocks. Light treatments (high, low) were established on the whole blocks. Treatments of soil biota (‘live’, sterile) and species identity (*B. brachycarpa* and *B. variegata*) were set up within blocks. Our study was designed to investigate the effects of model species on themselves i.e. self-sterilized individual soil feedbacks (Brinkman et al., 2010; Kulmatiski and Kardol, 2008). In the absence of sterile, laboratory conditions, we cannot rule out possible contamination of our sterilized soil treatment by microorganisms in air/water during the experiment. However, it is reasonable to assume that the sterilized soil treatment did not have the cohort of conspecific microorganisms or mycorrhizae associated with each tree species (Xi et al., 2018). Each treatment combination was replicated six times, resulting in a total of 48 pots for the light response experiment.

In May 2017, surface-sterilized seeds were germinated in petri-dishes for 10 days, and seedlings were then transplanted into pots (10 cm diameter × 12 cm height) in the experimental greenhouse. Fourteen 10-day old seedlings were oven dried (70 °C, 72 h) for each species and weighed to determine average initial biomass of individual seedlings. All pots were filled with a mix of sterilized sand and either ‘live’ or ‘sterile’ conditioned soil using a sand: soil volume ratio of 9: 1. The volume of soil inoculum was low in order to minimize possible artefacts due to soil sterilization at the start of the experiment (Xi et al., 2018). Two weeks after transplanting, soil nutrients were supplied to each pot by adding 100 ml of strength 1 Hoagland solution (see details in Table S1). Light treatments were also initiated at this time. The low light treatment was achieved using a steel frame covered by a layer of polyethylene shade cloth (1.38 m × 0.76 m × 0.80 m). Shade houses were deployed over low light blocks throughout the experimental period, resulting in an average light transmission of 10% of full light (86.7 $\mathrm{w\,m^{-2}}$, measured by S- LIB- M003, HOBO, USA); the high light treatment had an average light transmission of 50% (400 $\mathrm{w\,m^{-2}}$, measured by S- LIB- M003, HOBO, USA). The low light treatment is similar to the light intensity of the understory of the Yuanjiang woodland ecosystem, whereas the high light treatment is similar to gap light conditions.

Pots were randomly located in each block and re-arranged weekly to minimize possible position effects. Plants were watered regularly to avoid water limitation and all pots received the same amount of soil water during the 12-week experimental period.

2.4. Plant measurements

All plants were destructively harvested for trait and biomass measurements after 12 weeks of growth (August 2017). Specific leaf area (SLA, leaf lamina area/ lamina dry mass, $\mathrm{m^2\,g^{-1}}$) and specific root length (SRL, the ratio of root length to dry mass of fine roots, $\mathrm{m\ g^{-1}}$) were determined following standard protocols (Pérez-Harguindeguy et al., 2013). Leaf area was determined for one fully-expanded fresh leaf per individual using ImageJ software after scanning with a flatbed scanner (Canon Lide 120, Canon, Japan). Leaves were then oven dried (70 °C, 72 h) and weighed to calculate SLA. Roots were washed to remove soil and organic debris. Root length was measured on a subsample of fresh roots per individual using SmartRoot software (Lobet et al., 2011) and a flatbed scanner (Canon Lide 120, Canon, Japan). Roots were then oven dried as above and weighed to determine SRL.

Oven-dried plant material was weighed to determine dry mass; leaf mass fraction (LMF), root mass fraction (RMF) and stem mass fraction (SMF) were calculated by dividing the leaf, root and stem biomass by the total plant biomass respectively. Specific stem length (SSL, the ratio of stem length to dry stem mass, $\mathrm{m\ g^{-1}}$) was determined using fresh stem length measurements and stem dry mass data. Dry leaf and root biomass were then finely-ground for measurements of N and P concentration in leaves and roots. N concentration was measured using an elemental analyser system (Flash EA1112HT, Thermo Finnigan, USA) (Matejovic, 1995). Plant P concentration was measured using a Spectrophotometer (Z-2300, Hitachi, Japan) after H_2SO_4-H_2O_2-HF digestion (Bowman, 1988).

Relative growth rate in mass (RGR, $\mathrm{g\ g^{-1}\ day^{-1}}$) was calculated for each harvested plant as the difference between their natural-logged mass at final harvest (lnM_{t1}) and the average value of natural-logged initial masses of individual seedlings ($ln\bar{M}_{t0}$), all divided by the interval of growth (t_1-t_0, days):

$$RGR = \frac{lnM_{t1} - ln\bar{M}_{t0}}{t_1 - t_0}$$

Net feedback effects of soil on plants (PSF) were calculated for each treatment combination using the equation (Brinkman et al., 2010):

$$PSF\ index = \frac{M_{L,i} - M_{S,i}}{M_{S,i}}$$

where $M_{L,i}$ and $M_{S,i}$ represents plant biomass in live and sterile soil treatments in the block i.

Net effects of high light intensity on plant biomass were assessed for live or sterile soil as:

$$Plant\ light\ response = \frac{M_{high} - \bar{M_{low}}}{\bar{M_{low}}}$$

where M_{high} and $\bar{M_{low}}$ represents plant biomass in high treatments and the average values of biomass in low light treatments respectively.

2.5. Soil microbial analysis

Soil samples were collected from all pots in ‘live’ soil treatments at final harvest, sieved at 2 mm and kept at −20 °C prior to measurement of species-specific soil microbiota. A subsample of sieved soil from each pot was oven-dried (105 °C, 24 h) to determine soil moisture content at final harvest. Soil microbial communities were determined using phospholipid fatty acid analysis (PLFA) (Bossio and Scow, 1998). Briefly, we extracted lipids from 8 g dry-weight equivalent of fresh soil using a one-phase mixture of chloroform, methanol and phosphate buffer (1: 2: 0.8, v/v/v). The lipids were fractioned into neutral, glycol- and polar lipids using solid-phase extraction columns by eluting with chloroform, acetone and methanol respectively. Phospholipids were dissolved in 200 μl hexane containing 19:0 as internal standards after a mild-alkaline methanolysis. The identification and quantitation of PLFAs were conducted using an Agilent 6890 Gas Chromatograph (Agilent Technologies, Palo Alto, CA) and the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE).

The abundance of individual fatty acids was expressed for each sample as μg PLFA $\mathrm{g^{-1}}$ dry soil. The fatty acid nomenclature used was described in Frostegård et al. (1993). The PLFA 18:2ω6,9c was used as an indicator of fungal biomass (Frostegård et al., 2011). PLFA specific to Gram$^+$ bacteria (a15:0, i15:0, i16:0, a17:0 and i17:0) and Gram– bacteria (16:1ω7c, cy17:0, 18:1ω7c and cy19:0) were used to represent the bacterial groups (Grayston et al., 2001). Fungi: bacteria ratio and Gram$^+$: Gram– bacteria ratios were calculated to estimate changes in microbial community structure.

2.6. Statistical analysis

Split-plot three-way ANOVA was conducted to analyse plant growth rates and trait data, with light treatments as the fixed whole-plot factor, soil treatments and species identity as the fixed sub-plot factors and block as the random factor. Soil microbial data in ‘live’ soil treatments and values of PSF index were analysed using split-plot two-way ANOVA, with light treatments as the fixed whole-plot factor, species identity as the fixed sub-plot factors and block as the random factor. Values for the plant light response index were analysed using two-way ANOVA, with soil treatments and species identity as the fixed factors. The amount of plasticity for each plant trait across light treatments was quantified using coefficients of variance (CV) following Bloor and Grubb (2004). Mean values for plasticity of morphological traits and nutrient concentration/ stoichiometry were analysed using two-way ANOVA, with soil treatments and species identity as the fixed factors. Differences between treatments were determined using Tukey’s honest significant difference *post hoc* tests. All analyses were conducted using R. Mixed-effect models were conducted using the nlme packages. Data were log-transformed to meet assumptions of variance homogeneity and residual normality where necessary.

3. Results

3.1. Plant growth rate responses to light availability and soil microbes

Light intensity had a positive effect on relative growth rates irrespective of soil and species treatment ($F_{1,10}$ = 225, $P <$ 0.001; +60% on average in high versus low light, Fig. 1). However, the magnitude of positive light effects varied depending on both the presence of soil microbes and the tree species (Light × Soil × Species interaction, $F_{1,30}$ = 4.73, $P =$ 0.038, Fig. 1). Positive effects of high light on seedling growth rates were higher in the presence of soil microbes for *B. brachycarpa* (+86% and +32% for “live” and sterile soil respectively), but there was no difference in the light response of *B. variegata* growth rates between soil treatments (+63% on average, Fig. 1). Overall plant biomass responses to light (i.e., light response index values) also varied depending on plant species and presence of soil microbes (significant Soil × Species interaction, $F_{1,20}$ = 16.26, $P <$ 0.001). Positive effects of high light were higher in the presence of soil microbes for *B. brachycarpa*, but there was no difference in the light response of *B. variegata* biomass between soil treatments (Fig. 2A).

In general, plant biomass performance was either similar or higher in pots with ‘live’ versus sterile soil depending on light treatment and species (significant Light × Species interaction on PSF values, $F_{1,10}$ = 6.02, $P =$ 0.034). In low light treatments there was no significant difference in soil feedback effects (PSF) between species, whilst in high light treatments, the values of PSF index for *B. brachycarpa* were more positive than for *B. variegata* (Fig. 2B).

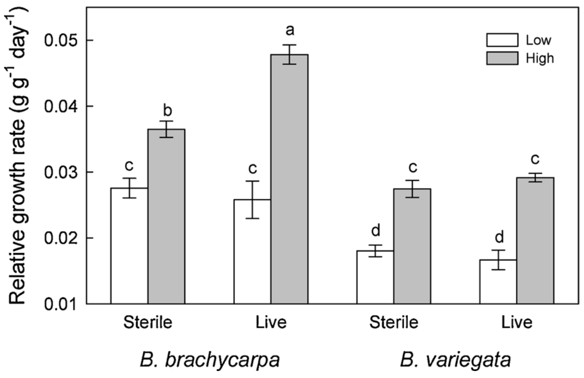

Fig. 1. Effects of light and soil treatments on seedling relative growth rates for *Bauhinia brachycarpa* and *B. variegata*. Means ± SE are shown (*n* = 6). Data with different letters are significantly different ($P <$ 0.05).

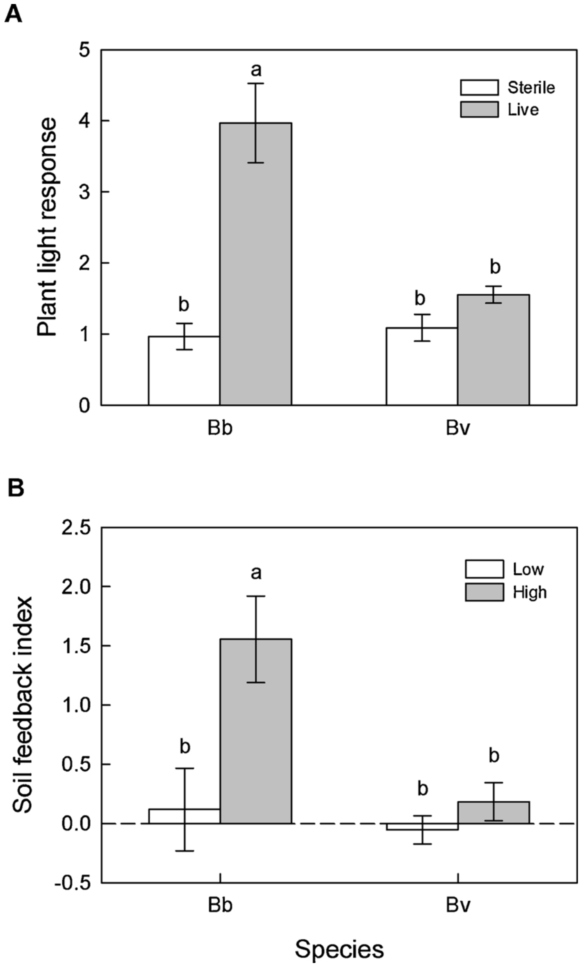

Fig. 2. Values for (A) plant light response index in live or sterile soil treatments, and (B) soil-plant feedback index under high and low light conditions. Indices are based on seedling biomass of *Bauhinia brachycarpa* and *B. variegata*, means ± SE are shown (*n* = 6). Data with different letters are significantly different ($P <$ 0.05).

3.2. Plant trait responses to light availability in the presence or absence of soil microbes

Plant morphological traits varied in their responses to experimental treatments (Table 1, Fig. 3). Values of LMF, SLA, SRL and SSL generally decreased under high light whereas RMF showed the opposite response to increased light availability (Fig. 3). The light-induced decreases in LMF and SSL depended on both the presence of soil microbes and the tree species involved (Table 1). Decreases in LMF were more pronounced in the presence of soil microbes, but the negative effects on LMF associated with high light and presence of soil microbes were greater for *B. brachycarpa* than *B. variegata* (-37% versus -22%, Fig. 3A). Light responses of SSL in *B. brachycarpa* were more pronounced in the presence of soil microbes (-68% versus -52% for “live” and sterile soil respectively), whereas *B. variegata* showed a smaller magnitude of light response for SSL in the presence of soil microbes (-49% versus -67% for “live” and sterile soil respectively, Fig. 3D). A similar response was observed for stem height; *B. brachycarpa* increased stem height in high light in the presence of soil microbes, whereas stem height increases in *B. variegata* were comparable in all soil conditions (Fig. S1). Light responses in SMF were also more pronounced in *B. brachycarpa* than in *B. variegata*; high light decreased SMF in *B. brachycarpa* in sterile soil but tended to increase SMF in the presence of soil microbes (Fig. 3C). Light responses in RMF, SLA and SRL did not interact with presence of soil microbes. However, light-induced decreases in SLA were greater for *B.*

Table 1

Interactive effects of light, soil treatments and species identity on plant morphological traits. F values derived from analysis of variance are shown: significant effects ($P < 0.05$) are shown bold type.

	LMF (g/g)		SLA (cm^2/g)		SMF(g/g)		SSL (cm/g)		RMF (g/g)		SRL (cm/g)	
Source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Light (L)	5.26	0.045	**63.91**	**< 0.001**	0.00	0.969	**62.43**	**< 0.001**	**20.09**	**0.001**	**112.33**	**< 0.001**
Soil (S)	**15.68**	**< 0.001**	**7.47**	**0.010**	1.64	0.210	1.45	0.239	**53.53**	**< 0.001**	0.24	0.627
Species (Sp)	1.72	0.200	**98.63**	**< 0.001**	2.69	0.112	**175.05**	**< 0.001**	**62.41**	**< 0.001**	0.11	0.748
L × S	**15.78**	**< 0.001**	1.83	0.186	**22.76**	**< 0.001**	0.05	0.830	3.64	0.066	0.01	0.944
L × Sp	0.00	0.951	**25.58**	**< 0.001**	0.29	0.594	0.04	0.851	1.60	0.216	0.58	0.452
S × Sp	**6.79**	**0.014**	**6.88**	**0.014**	0.21	0.648	1.12	0.298	**34.00**	**< 0.001**	1.15	0.292
L × S × Sp	**5.12**	**0.031**	0.67	0.418	**9.05**	**0.005**	**7.38**	**0.011**	3.45	0.073	0.42	0.521

$DF_{1,30}$ for all terms except light ($DF_{1,10}$); n = 6.

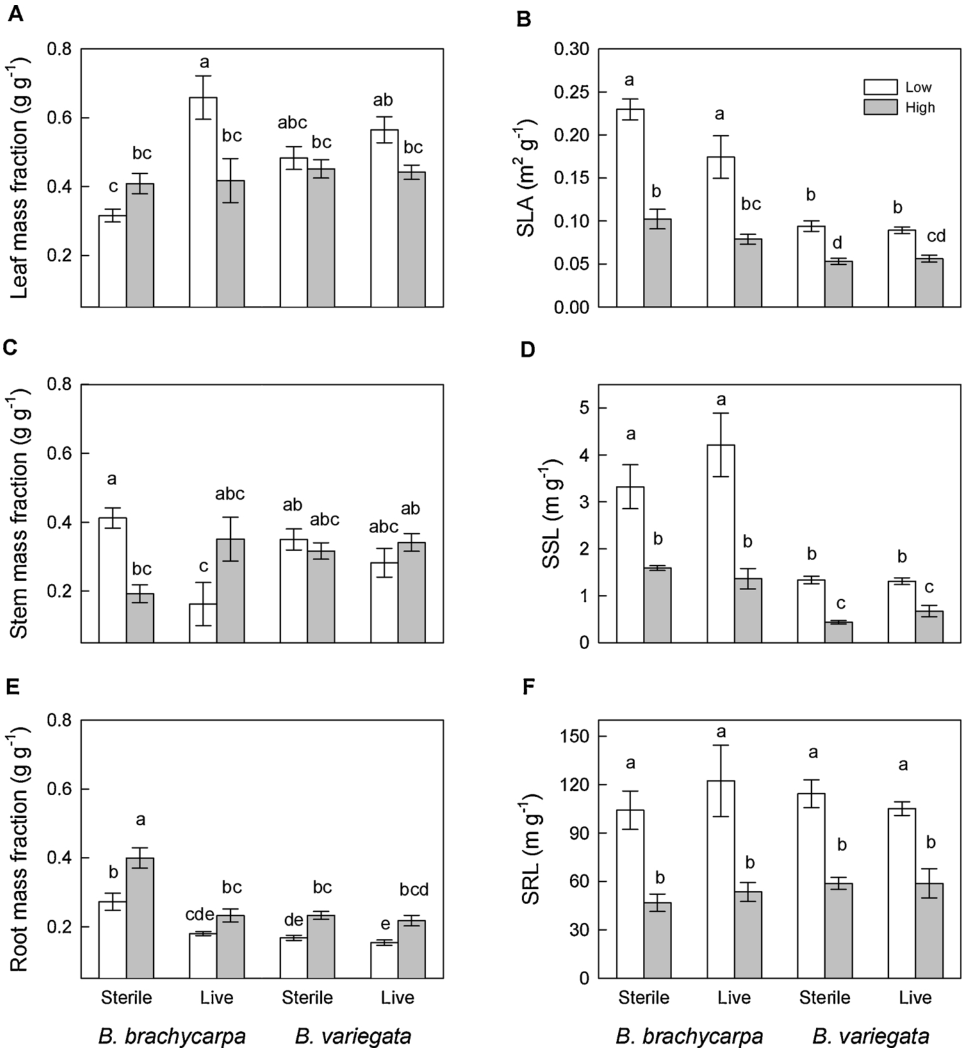

Fig. 3. Effects of light and soil treatments on (A) leaf mass fraction, (B) specific leaf area, (C) stem mass fraction, (D) specific stem length, (E) root mass fraction, and (F) specific root length for seedlings of *Bauhinia brachycarpa* and *B. variegata*. Means ± SE for plant morphological traits are shown ($n = 6$). Data with different letters are significantly different ($P < 0.05$).

brachycarpa than *B. variegata* (Fig. 3B).

High light intensity generally decreased N concentration in roots and leaves for *B. variegata* (average of -28% and -23% respectively, Table 2), but only decreased root N concentration in *B. brachycarpa* (Table 2, Fig. 4A and B). Light responses in root and leaf N did not interact with presence of soil microbes (Table 2). Total leaf and root N content generally increased with high light (Table S2, Fig. S1), but the light effects on leaf N content were only significant for *B. brachycarpa* (significant species x light interaction), driven by a strong light response in 'live' soil (Fig. S1).

In the absence of soil microbes, leaf P concentration decreased in high light treatments, whilst in the presence of soil microbes, there was no significant difference in leaf P concentration between light treatments (Table 2, Fig. 4C). Total leaf P content showed the reverse response pattern; leaf P content increased in high light treatments in the presence of soil microbes alone (Table S2, Fig. S1). Root P showed a negative response to high light irrespective of soil treatments (−21% on average, Fig. 4D). Root P generally increased in the presence of soil

Table 2
Interactive effects of light, soil treatments and species identity on plant nutrient concentration and stoichiometry. F values derived from analysis of variance are shown: significant effects ($P < 0.05$) are shown bold type.

	Leaf N (%)		Root N (%)		Leaf P (%)		Root P (%)		Leaf N: P		Root N: P	
Source	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Light (L)	**27.34**	**< 0.001**	**107.42**	**< 0.001**	4.42	0.062	**15.43**	**0.003**	**8.52**	**0.015**	0.01	0.930
Soil (S)	**19.41**	**< 0.001**	1.83	0.187	**103.58**	**< 0.001**	**201.25**	**< 0.001**	**133.99**	**< 0.001**	**153.70**	**< 0.001**
Species (Sp)	**14.45**	**< 0.001**	1.85	0.184	**5.86**	**0.022**	**27.34**	**< 0.001**	3.00	0.094	0.02	0.882
L × S	1.22	0.278	1.84	0.185	**8.78**	**0.006**	0.07	0.799	**36.83**	**< 0.001**	**6.87**	**0.014**
L × Sp	**6.57**	**0.016**	0.14	0.710	2.12	0.156	0.15	0.699	1.21	0.281	0.55	0.465
S × Sp	2.84	0.103	1.80	0.190	**12.88**	**0.001**	**43.24**	**< 0.001**	**17.66**	**< 0.001**	**18.04**	**< 0.001**
L × S × Sp	2.62	0.116	0.07	0.789	2.54	0.122	1.06	0.311	1.39	0.247	1.25	0.273

$DF_{1,30}$ for all terms except light ($DF_{1,10}$); n = 6.

microorganisms, but effects were greater *B. brachycarpa* than in *B. variegata* (Soil × Species interaction, Table 2, Fig. 4D). For total root P content, light responses depended on both the presence of soil microbes and the tree species involved (Table S2). Light-induced increases in total root P were more pronounced in the presence of soil microbes, but the positive effects on root P content associated with high light and presence of soil microbes were greater for *B. brachycarpa* than *B. variegata* (Fig. S1).

High light increased leaf N: P ratios in the sterile soil treatment but tended to decrease leaf N: P in the presence of soil microorganisms (Table 2, Fig. 4E). The same response pattern was also apparent for root N: P ratios (Fig. 4F). Leaf and root N:P were generally greater in sterile soil, but microbe-induced decreases in leaf and root N:P values were smaller in *B. variegata* compared to *B. brachycarpa* (Table 2, Fig. 4).

Overall, plant morphological traits, nutrient concentration and stoichiometry showed considerable variation in plasticity to light, with

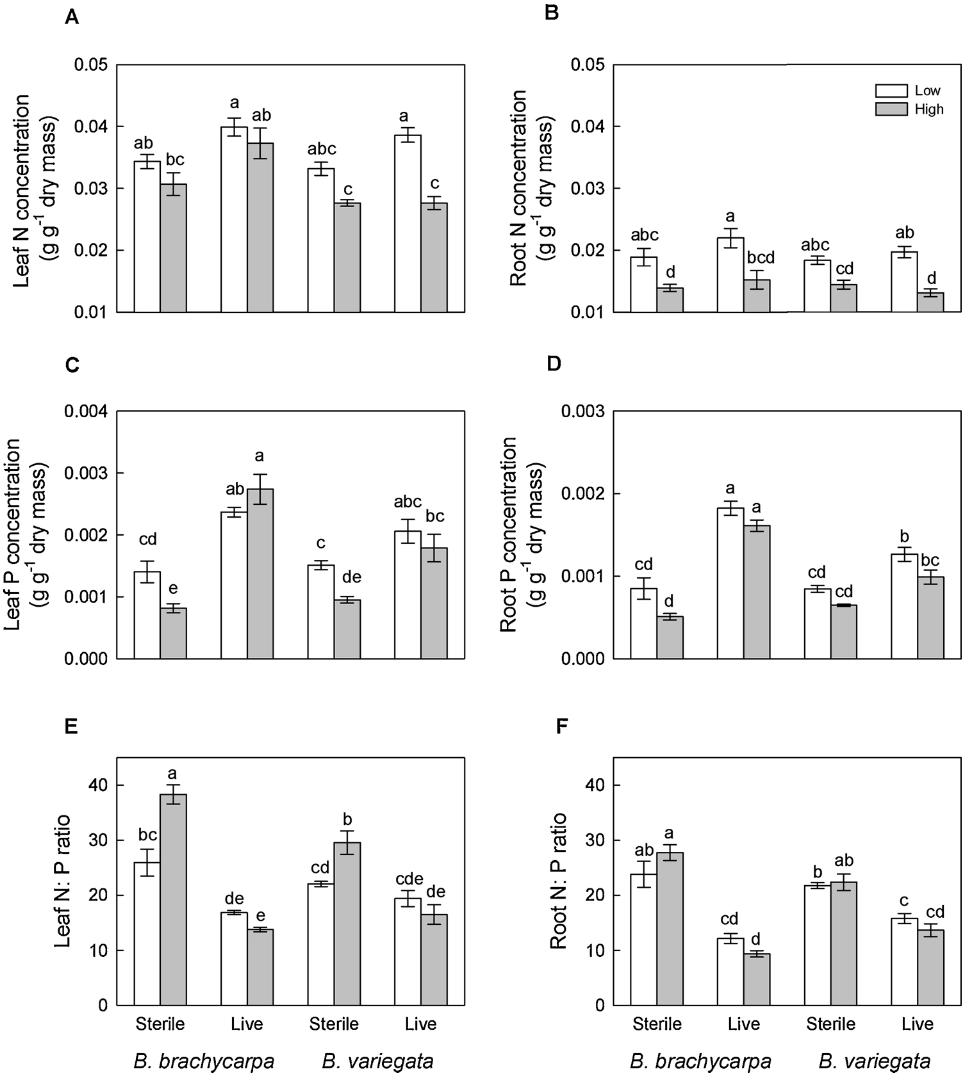

Fig. 4. Effects of light and soil treatments on (A) leaf N concentration, (B) root N concentration, (C) leaf P concentration, (D) root P concentration, (E) leaf N: P ratio, and (F) root N: P ratio for seedlings of *Bauhinia brachycarpa* and *B. variegata*. Means ± SE for plant biochemical traits are shown ($n = 6$). Data with different letters are significantly different ($P < 0.05$).

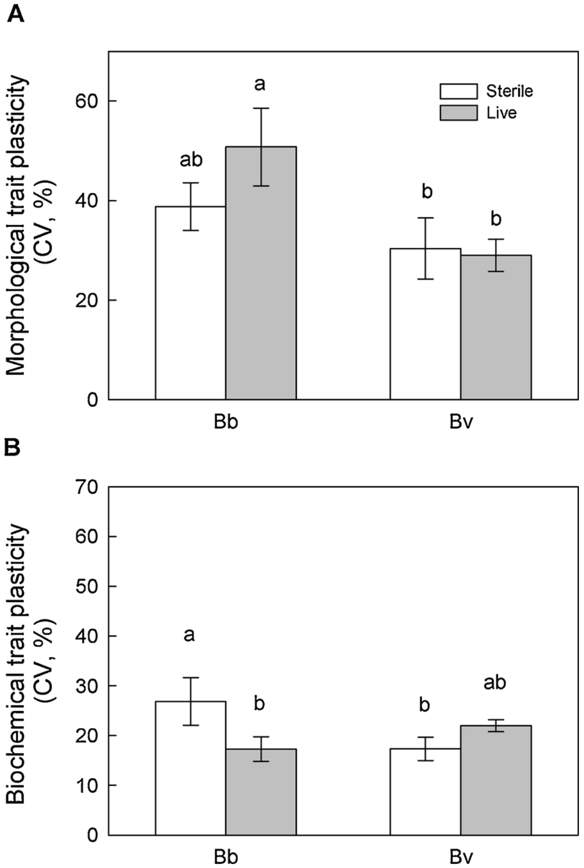

Fig. 5. Plasticity of seedling traits for *Bauhinia brachycarpa* and *B. variegata* grown under high and low light conditions. Mean coefficients of variation (CV, %) are shown for (A) morphological traits (based on leaf mass fraction, root mass fraction, stem mass fraction, SLA, SRL, SSL), and (B) biochemical traits (based on leaf N and P, root N and P, leaf N: P ratio, root N: P ratio). Means ± SE are shown; data with different letters are significantly different ($P < 0.05$).

mean CVs ranging from 11 to 69% in the different soil and species treatments. Average plasticity in plant morphological traits in response to light was greater for *B. brachycarpa* than in *B. variegata* and did not depend on soil treatment, although there was a tendency for increased morphological plasticity in the presence of soil microorganisms (Fig. 5A). In contrast, average plasticity in plant nutrient concentration and stoichiometry in response to light varied depending on both plant species and soil treatment; *B. brachycarpa* had lower plasticity in these ‘biochemical’ traits in live compared to sterile soil, whereas plasticity in *B. variegata* showed no difference in the two soil treatments (Fig. 5B).

3.3. Light effects on soil microbial community structure

High light increased total microbial biomass as well as the biomass of fungi and bacteria across tree species treatments, driven by strong responses in soil with *B. variegata* seedlings (Table 3, Fig. 6). In addition, high light availability increased the fungi: bacteria ratio but decreased the Gram$^+$: Gram– bacteria ratio across the two tree species (significant light effect, Table 3). Across light treatments, *B. variegata* was associated with greater total microbial, fungal and bacterial biomass than *B. brachycarpa*, and conditioned soil microbial communities with higher Gram$^+$: Gram– bacteria ratio (significant species effect, Table 3, Fig. 6). Microbial variables showed no light × species interactions (Table 3).

4. Discussion

The successful establishment and persistence of tree seedlings is highly dependent on the availability of light in forests (Pacala et al., 1996). This study was conducted to determine how tree seedling light responses are influenced by the presence or absence of soil microbes. In contrast to inoculation experiments that target specific microbial groups and minimize confounding effects of other soil microorganisms, our objective was to examine plant performance in the presence of the entire soil microbial community conditioned by conspecific plants in order to obtain valuable information on the net effects of a cohort of microorganisms. We also provide insights into plant-soil feedback under contrasting light conditions for tropical woody species, for which data is extremely scarce (Kulmatiski et al., 2008).

Our first hypothesis was that the presence of soil microbes would modify plant growth responses to light availability due to more positive soil feedback effects under high light conditions. This hypothesis was partially supported by our data; positive effects of high light on seedling growth rates were more pronounced (i.e., of greater magnitude) in the presence of soil microbes for *B. brachycarpa* alone. Positive soil feedbacks may be promoted by high nutrient availability, high abundance of mutualists and/or low vulnerability to pathogens (Smith-Ramesh and Reynolds, 2017). Assessment of mycorrhizal colonisation and isotopic tracer experiments exploring C transfers from the plant to soil microbes (Kaiser et al., 2015) would provide a clearer view of the relative importance of mycorrhizae for the positive soil feedback observed here.

In general, we found that *B. brachycarpa* conditioned microbial communities with a greater dominance of Gram– bacteria, a functional group associated with rapid soil nutrient release from litter and root exudates (Fanin et al., 2019). The decrease in relative abundance of Gram + bacteria observed here is consistent with the negative association between fast-growing tree species and Gram + bacteria reported for subtropical forests elsewhere (Pei et al., 2016). Lower nutrient limitation (due to fast nutrient turnover with Gram– bacteria) may therefore help explain the positive soil-plant feedbacks observed for *B. brachycarpa* in high light. Unlike seedlings of *B. brachycarpa,* the shade-tolerant and slower-growing *B. variegata* seedlings showed neutral soil-plant feedbacks in our high light treatment. Absence of positive soil feedbacks in *B. variegata* was not linked to lower microbial biomass or a change in fungi: bacteria ratios in soil in high light. These results agree with the suggestion that shade-tolerant species are less sensitive to microbially-mediated soil-plant feedbacks due to higher defence against soil pathogens and lower dependence on symbionts (Kobe and Vriesendorp, 2011; Reich et al., 1998).

Irrespective of plant species, we found that soil feedback effects were neutral under low light levels i.e. 10% full light. Reduced light

Table 3
Interactive effects of light treatments and species identity on soil microbial community composition. F and P values are given; significant effects ($P < 0.05$) are shown bold type.

		Total PLFA		Fungal PLFA		Bacterial PLFA		Fungi: bacteria ratio		G+: G− bacteria ratio	
Source	*Df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Light	1, 10	5.16	**0.046**	6.25	**0.031**	6.70	**0.027**	5.14	**0.047**	8.66	**0.015**
Species	1, 10	7.14	**0.023**	5.52	**0.041**	7.82	**0.019**	1.13	0.313	7.86	**0.019**
Light × Species	1, 10	2.35	0.156	2.46	0.148	1.98	0.190	0.02	0.879	0.04	0.845

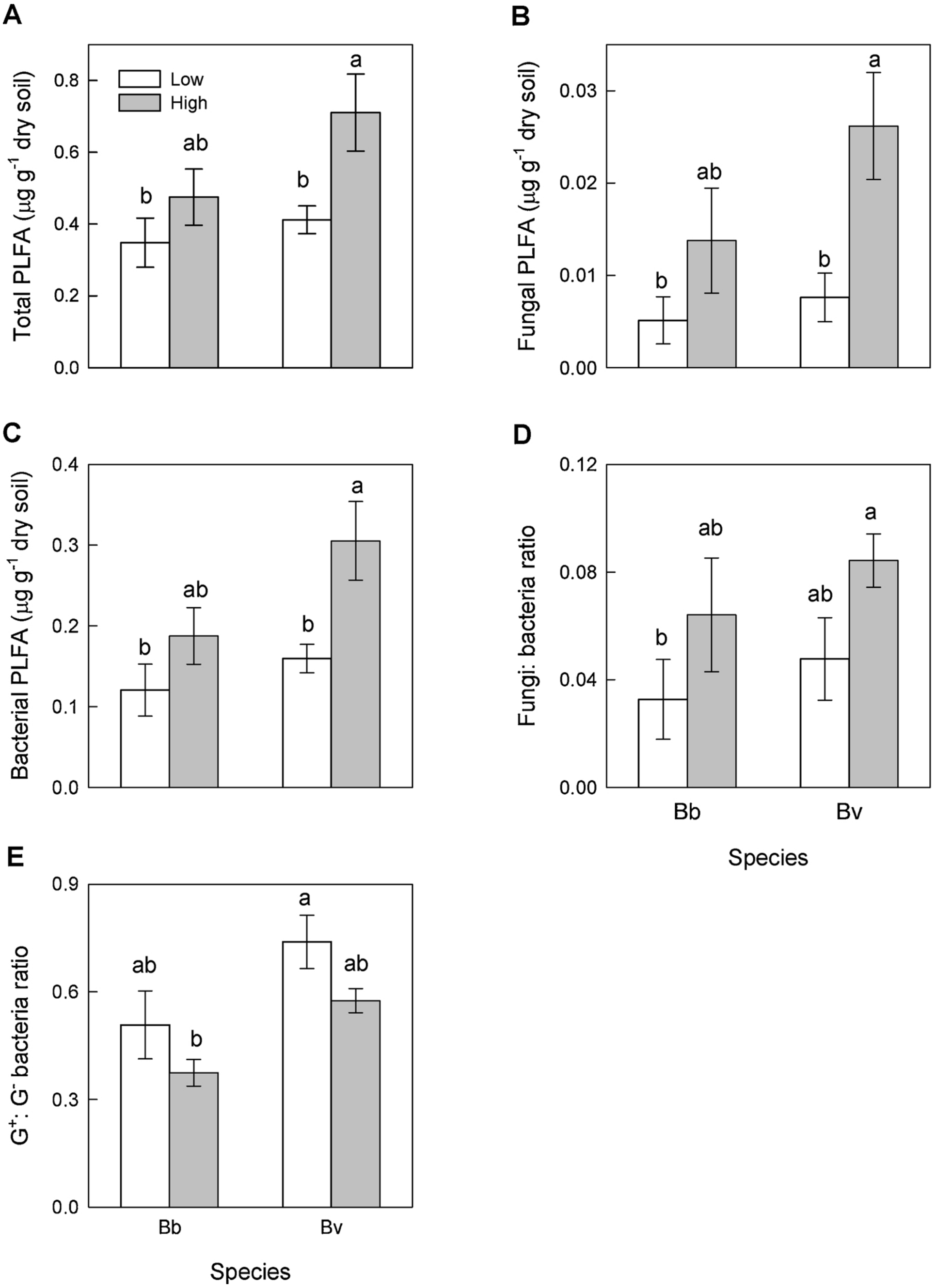

Fig. 6. Effects of light treatments on (A) total microbial PLFA, (B) fungal PLFA, (C) bacterial PLFA, (D) fungi: bacteria ratio, (E) Gram$^+$: Gram$^-$ bacteria ratio in soil conditioned by *Bauhinia brachycarpa* and *B. variegata*. Bb, *B. brachycarpa*; Bv, *B. variegata*. Means ± SE are shown (n = 6); data with different letters are significantly different ($P <$ 0.05).

intensity can decrease both overall carbon transfers to mycorrhizae (Bennett and Klironomos, 2019) and preferential carbon allocation towards beneficial mycorrhizal mutualists (Zheng et al., 2015), as well as promoting moist conditions which enhance pathogen growth. Indeed, work on herbaceous legumes suggests that the mycorrhizae-plant benefit relationship shows a threshold response along a light gradient, and that positive effects of mycorrhizae on plant growth disappear at 10% of the full light (Konvalinková and Jansa, 2016). The absence of strong soil feedbacks observed here under low light for both species is consistent with the idea that plant-soil interactions play a relatively more important role in structuring communities establishing in open canopy gaps and along forest edges rather than in forest understories (Smith and Reynolds, 2015). However, light-thresholds for the mycorrhizae-plant benefit relationship may vary among species (Ibáñez and McCarthy-Neumann, 2015), and it is possible that the 'positive to neutral' and 'always neutral' response of soil feedbacks found here in 50% and 10% light for our two species may change at extremely low light intensities.

Plants are generally considered to adjust their traits and patterns of biomass allocation to capture those resources in most limiting supply and maintain carbon gain (Bloom et al., 1985). In line with previous studies on tree seedling light responses (e.g. Bloor and Grubb, 2004; Liu et al., 2016; Poorter and Bongers, 1991; Reich et al., 1998; Walters and Reich, 1999), we found that all seedlings generally increased SLA, had more etiolated stems, decreased their biomass allocation to roots but increased SRL in response to reduced light availability. Given that microbes have the potential to influence most plant functional traits via both direct and indirect effects (Friesen et al., 2011), our second hypothesis was that presence of soil microbes would modify responses of plant traits to light availability. In fact, only half of the plant traits tested (i.e. leaf mass fraction, stem mass fraction, specific stem length, leaf P concentration, leaf N: P ratio and root N: P ratio) showed any interactions between the soil microbes and light intensity treatments, providing mixed support for our hypothesis. As with the interactions observed between soil microbes and light on seedling growth rates, the magnitude of interactive effects on aboveground allocation and SSL was greater for *B. brachycarpa* compared to *B. variegata*.

Mycorrhizal and rhizobial inoculation experiments have demonstrated that symbionts generate significant costs to plants by ‘consuming’ plant photosynthates, and can lead to decreases in plant growth and reproduction under low light conditions (Ballhorn et al., 2016; Konvalinková and Jansa, 2016). Numerous studies suggest that the physiological and morphological adaptations of plants to light may be boosted by the presence of mycorrhizal fungi in order to compensate for the increased carbon demands of the mycorrhizal plant (Konvalinková and Jansa, 2016). We found some evidence that conspecific soil microbes enhanced plant trait changes which could be of benefit under limiting light conditions; all seedlings allocated relatively more biomass to leaves in the presence of soil microbes compared with seedlings growing in sterilized soil. In a source-limited situation, the carbohydrate content in the plants may also decrease because the carbon sinks are more than sufficient to keep pace with assimilate production (Li et al., 2002; Paul and Foyer, 2001). Further work should measure seedling concentrations of non-structural carbohydrates, and examine the differences in the source–sink balance in the presence and absence of soil microbes along a light gradient.

In the present study, presence of conspecific soil microbes appeared to play an important role for P capture, as seedlings had consistently lower leaf and root P concentration (and total P content) in sterilized soil compared to ‘live’ soil. This likely reflects the absence of mycorrhizal fungi in sterilized soil; mycorrhizal roots have a greater capacity to take up phosphate from the soil solution due to fungal hyphae as well physiological and biochemical properties of the fungal/soil interface such as the presence of high-affinity phosphate transporters and the release of organic acids (Plassard and Dell, 2010). In the absence of soil microorganisms, soil nutrients appeared to be limiting for seedlings even under low light conditions. This agrees with previous work on tree seedlings where co-limitation by soil nutrients and water was shown to constrain plant responses to light intensity (Brown et al., 2014; Veenendaal et al., 1996). At the same time, we found that light-induced changes in leaf P and N:P ratio were buffered in the presence of soil microbes, suggesting a possible role of soil microbes in plant stoichiometric homeostasis (Peng and Wang, 2016). Presence of conspecific soil microorganisms may enhance the capacity of plants to maintain relatively constant elemental ratios within their tissues in the face of fluctuating resource availability, with significant implications for the functioning of food webs and the cycling of nutrients within ecosystems (Sterner and Elser, 2002).

Our final hypothesis was that the light-demanding *Bauhinia* species would have a greater sensitivity to light compared to its shade-tolerant congener. This was clearly the case in ‘live’ soil; the light-demanding *B. brachycarpa* showed a greater plant light response in terms of growth rates, biomass and plant morphological traits compared to *B. variegata*, in agreement with previous comparisons of shade-tolerant and light-demanding species (Valladares et al., 2000; Walters and Reich, 1999). Contrary to expectations, the two species did not show marked differences in light response in either biomass or average plasticity of morphological traits in sterilized soil. Moreover, *B. variegata* showed greater sensitivity to light in the average plasticity of ‘biochemical’ traits (nutrient concentration and stoichiometry) compared to *B. brachycarpa* for seedlings growing in sterilized soil. These findings suggest that ‘trait-based’ classifications of plants according to light-use strategies do not simply reflect inherent plant properties, but may instead reflect plant x environment interactions mediated by soil microorganisms. Our results also point to possible mechanisms linking soil feedback effects with plant coexistence (Comita et al., 2014). The expression of seedling responses to light availability combined with soil biota may promote a shifting competitive hierarchy between *B. brachycarpa* and *B. variegata* in natural ecosystems under changing light conditions; the competitive advantage of shade-intolerant *B. brachycarpa* seedlings in high light could be further enhanced by positive feedback effects of soil biota.

It is important to note that phenotypic plasticity and intraspecific trait variation reflect both environmental effects and genetic variation (Barbour et al., 2019). Although light × genotype interactions on tropical plant traits may be relatively limited (Nicotra et al., 1997), we cannot guarantee that the seedlings of our model species had a very diverse genetic background (due to the spatial proximity of collected seeds), and the importance of genetic diversity for the light plasticity of our study species remains to be investigated. Where genetic diversity promotes intraspecific variation in plant resource-use, soil feedbacks could also be impacted by the plant genotypes which have conditioned the soil (Bennett and Klironomos, 2019). Nevertheless, recent work suggests that effects of intraspecific plant variation on soil feedback effects are either absent or significantly smaller than effects of interspecific variation (Allen et al., 2018; Bukowski et al., 2018).

As far as we are aware, this is the first study to demonstrate the influence of a cohort of conspecific soil microbes on the light responses of plant morphological traits and nutrient concentration or stoichiometry. Overall, our results showed that light responses of aboveground plant traits appeared to be more sensitive to the presence of soil microbes than root traits. The light-demanding tree species was more responsive to both light and soil microbes compared with its shade-tolerant congener, with implications for biotic interactions and plant community dynamics. Positive effects of conspecific soil microbes on *B. brachycarpa* performance under high light conditions appeared to be mediated by light-induced changes in plant morphology and soil microbial community structure. Presence of conspecific soil microbes also appeared to buffer *B. brachycarpa* against changes in plant nutrient content and N: P stoichiometry, with potential consequences for ecosystem functioning. These findings confirm the importance of plant-soil interactions for plant responses to abiotic conditions, and suggest that plant growing medium may play a crucial role in the outcome of experiments examining plant responses to a fluctuating environment. Further studies are required to verify our findings for a broader range of plant species from diverse functional groups, and to explore the impact of both conspecific and heterospecific soil microbial groups on the expression of genes during plant light responses.

Author statement

We confirm that there are no known conflicts of interest associated with this publication and that there has been no significant financial support for this work that could have influenced its outcome. All authors have read and approved the manuscript being submitted. This article is the authors’ original work, has not received prior publication and is not under consideration for publication elsewhere.

Author contributions

NX conceived the ideas and designed methodology; NX and YW collected and analysed the data; NX, JMGB, CC and YW interpreted the results and wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2019.103826.

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