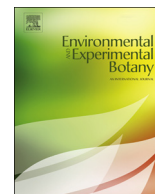




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# Phenotypic plasticity, not ecotype differentiation, explains the broad ecological niche of a tree species in African dry woodlands

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## ABSTRACT

In the African tropical dry woodlands, very few woody species are able to thrive both on *Macrotermes* termite mounds and in the surrounding matrix. *Dalbergia boehmii* is one such broad-niched species. In a previous study, populations on and off mounds were found to have different functional trait values. Here, in two reciprocal transplant experiments, both in situ and ex situ (pot experiment) we test whether the plants from mounds and from the matrix have evolved genetically divergent traits and are locally adapted (edaphic ecotypes), or whether trait differences arise from phenotypic plasticity. Growth, survival and morphological functional leaf traits were monitored. Seedling survival did not differ between plants of different origins and habitats. No constitutive difference or no heritable features were found between the plants of the two origins. However, functional traits varied between habitats in the in situ experiment, with higher specific leaf area (SLA) and lower leaf dry matter content values on termite mounds, indicating plastic response. The magnitude of the plastic response was larger in situ compared to the pot experiment, suggesting that other ecological factors, in addition to soil, drive trait plasticity. We conclude that the broad niche of the species is due to its ability to express adaptive phenotypic plasticity, not to locally adapted genotypes.

## 1. Introduction

Species showing a broad ecological amplitude have the ability to maintain populations across a broad range of ecological conditions. Such species can occupy a broad niche either by means of phenotypic plasticity or by evolving specialised populations adapted to a relatively narrow range of conditions (Van Valen, 1966; 1974). The evolution towards either plasticity or local adaptation depends on the scale of the spatial heterogeneity of key ecological factors compared to the scale of gene flow (Via and Lande, 1985). In habitats where the scale of variation in ecological factors is more restricted than the scale of gene flow, plasticity is more likely to evolve than adaptation. The intraspecific variation, either plastic or genetic, increases a species' ability to colonise new environmental conditions (Joshi et al., 2001; Byars et al., 2007). Adaptive genetic differentiation of plants at short distances has been documented in response to different soil factors (Knight et al., 2006; Sambatti and Rice, 2006; Hufford et al., 2007; Baythavong, 2011; Vidaller et al., 2018).

Soil properties influence spatial distributions and diversity of tropical tree communities with narrow and broad niche species. The specialisation of species to particular environmental conditions as one of the mechanisms potentially supporting high tree species richness in tropical forests has received much attention (Ashton, 1969; Chesson, 2000; Baltzer et al., 2005; Fine et al., 2005; Pringle et al., 2016; Cuma Mushagalusa et al., 2018; Werden et al., 2018). However, to date, no study has addressed the mechanisms underlying the broad ecological niche of some tree species of tropical forest. Yet, plants experience generations of selection by local environmental conditions due to their limiting gene dispersal through seeds and pollen (Sork, 2016; Grant et al., 2019; Hodgins and Yeaman., 2019). This selection may result in adaptive genetic differentiation (van Kleunen, 2007v; Nicolaus and Edelaar, 2018). Through ecotype formation, populations genetically differentiate at short distance according to variations in soil properties (Sakaguchi et al., 2018). Thereby, the phenotypic differences observed among populations are the manifestation of genetic variations and the different populations are locally adapted to their respective habitats

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(Rúa et al., 2016). Reciprocal transplant experiments are the tool of choice to test for local adaptation in plant populations (Kawecki and Ebert, 2004).

A very striking pattern of short distance edaphic variation also exists in the dry tropical woodlands and savannahs of Sub-Saharan Africa, maintained by the mound-building activity of termites. A growing number of studies in different regions of Africa have yielded convergent results, showing vegetation differences between termite mounds and the surrounding landscape, with however, local peculiarities in the amplitude of the difference (Joseph et al., 2012; Kirchmair et al., 2012; Van der Plas et al., 2013; Erpenbach et al., 2013; Davies et al., 2014; Seymour et al., 2016). The most influential factors seem to be the higher clay contents, base cation contents and stores of available water in termite mound soil (Konaté et al., 1999; Dangerfield et al., 1998; Sileshi et al., 2010).

In Upper Katanga, the density and size of termite mounds reach unrivalled values (ca. 3–5 mounds /ha, up to 20 m diam. 8 m height). There, the woody flora comprises two groups of narrow-niched species, i.e. a group of termitophilous species and a group of termitofuge species (Cuma Mushagalusa et al., 2020). Only 5% of species occupy a broad niche, being present in both habitats. In one of such species, *Dalbergia boehmii* (Fabaceae), differences in functional traits were found between termite mounds and the matrix. The individuals on mounds have larger values of specific leaf area (SLA), lower leaf dry matter content, lower leaf thickness, larger foliar concentrations of nutrients (Cuma Mushagalusa et al., 2020). This combination of traits suggests that individuals on and off mounds occupy different positions in the leaf economics spectrum. Indeed, individuals on mounds display a syndrome of faster resource capture and use, consistent with the higher nutrient status and larger stores of available water on termite mounds, in comparison to individuals in the surrounding matrix (Van der Plas et al., 2013; Joseph et al., 2014). Interestingly, the intraspecific trait differences in this species occurred in the same direction as the differences between the two groups of narrow-niched species. The question therefore arises as to whether the intraspecific variation of broad-niched species is due to phenotypic plasticity or genetic variation, or both. If genetic variation is implicated, is it due to local adaptation?

Here, we test whether the intraspecific trait variation of *Dalbergia boehmii*, a broad-niched tree species occurring both on and off termite mounds, is due to phenotypic plasticity in response to the habitat, or to genetic divergence related to the formation of distinct ecotypes. We also test if the plants on and off mounds show local adaptation to their habitat. To our knowledge, this study represents the first reciprocal transplant experiment testing for the existence of edaphic ecotypes in tropical Africa.

Specifically, we address the following questions:

- 1) Are the trait differences between the two groups of plants observed in their natural habitats maintained when they are cultivated on the same soil (either of the termite mound or of the matrix)?
- 2) Do plants of both origins express phenotypic plasticity in functional traits in response to the soil? If so, is the pattern of trait response when plants are cultivated ex situ similar to the differences between plants of both origins in a reciprocal transplant experiment in situ?
- 3) Are the plants on and off termite mound locally adapted?

To answer these questions, we use two reciprocal transplant experiments, i.e. one ex situ using pots filled with soil from mounds and from the matrix, and one in situ.

## 2. Material and methods

### 2.1. Studied species

*Dalbergia boehmii* (Fabaceae, subfam. Faboideae) is a small tree of tropical dry woodlands in the Sudanian and the Zambebian region. It is

widespread in tree savannahs and dry woodlands of Upper Katanga (Meerts and Hasson, 2016). In a previous paper (Cuma Mushagalusa et al., 2020), we showed that it is one of the very few woody species observed as frequently on and off termite mounds (44 and 56 % of occurrences, respectively) in Upper Katanga.

The climate of this region is Sudanian, corresponding to Cwa in Köppen's classification (Peel et al., 2007). Upper Katanga represents the northern part of the Zambebian centre of endemism (Malaisse, 1996; White, 1983). The vegetation consists of dry tropophilous woodlands, locally known as miombo woodlands, from the name of the most characteristic trees, i.e. *Brachystegia* (Fabaceae Caesalpinioideae) (Duvigneaud, 1958; Malaisse, 1978a; Schmitz, 1971; Werger and Coetzee, 1978). The miombo of Upper Katanga is of the wet type, corresponding to regions with annual rainfall > 1000 mm. Wet miombo is characterised by larger canopy height (ca. 20 m) and by a higher frequency of evergreen species (Malaisse, 1997).

### 2.2. Ex situ experiment

In 2016, during the dry season, fruits (one-seeded pods) of *Dalbergia boehmii* were collected on and off mounds in four sites in the region of Lubumbashi, i.e. Baya (11°52'50,56"S, 27°27'55,85"E 1143 m a.s.l.), Mikembo (11°28'36,43"S, 27°39'58,00"E, 1181 m a.s.l.), Kiswishi (11°32'15,68"S, 27°28'06,40"E, 1319 m a.s.l.) and Luiswishi (11°29'50,13"S, 27°36'31,64"E 1152 m a.s.l.). The pods were collected from eight trees in each type of habitat (i.e. matrix and mound) at each site. The seeds were extracted from pods and pooled by type of habitat. On each tree, 80 pods were collected and in each pod, seeds were visually sorted to exclude empty or damaged ones.

The soil for the ex situ experiment was collected at the site Mikembo (See Bauman et al., 2016; Muledi et al., 2017 and Cuma Mushagalusa et al., 2018 for a description of this site and soil analyses). Soil was collected from 17 randomly-selected termite mounds and 17 adjacent 25-m square plots in the surrounding matrix. This corresponds to a subsample of the 48 termite mounds and 102 plots sampled in our previous work (Cuma Mushagalusa et al., 2018; depth: 0–15 cm). The soil collected was pooled and homogenised according to the origin (matrix and mound soils mixed and homogenised separately).

Polyethylene bags (4 L; 35 cm height, 12 cm diam.) were filled either with soil from termite mounds (hereafter T soil) or with soil from the matrix (hereafter M soil). Twelve seeds were sown in each bag in September 2016. For each sampling site, the seeds from each habitat (mounds and matrix) were grown on both T soil and M soil. There were 20 replicates for each combination of site and habitat, for a total of 320 bags in the experiment. The bags were randomised twice a month in numbering 320 positions corresponding to 320 bags using a pseudo-random number generator (Monte-Carlo method) to generate a list of integers 1–320.

The bags were kept under shade cloth at the experimental garden of the Faculty of Agronomic Sciences of the University of Lubumbashi (DR Congo) (11°27'S, 27°28'N; 1200 m a.s.l.; monthly mean temperature: 16–33 °C, annual rainfall 1200 mm, rain season November–April) and the experimental garden was protected against grazing animals by a fence. Watering was provided until the beginning of the rain season. The seedlings were thinned to 1/bag in November.

### 2.3. In situ experiment

In July 2014, pods were collected from eight trees on termite mounds and eight trees in the matrix in two sites (Mikembo and Kiswishi). The seeds were extracted from pods, pooled in two groups (termite mounds and matrix) irrespective of site and sown in polyethylene bags 20 cm high and 8 cm wide, filled with soil from either termite mound or the surrounding matrix (0–15 cm depth) collected from Mikembo, as specified above. The bags were kept in the greenhouse of the Faculty of Agronomic Sciences of the University of

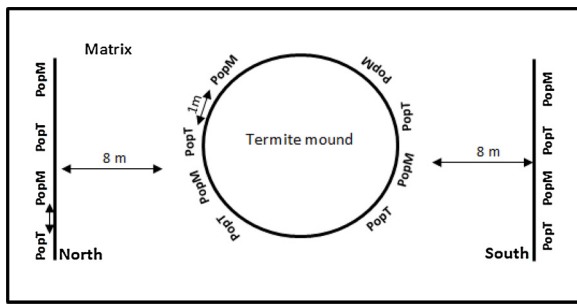


Fig. 1. Design of the in situ seedling transplantation experiment. Four seedlings (two originating from the termite mounds, two from the matrix), were planted on two diametrically opposite slopes of a termite mound; four seedlings planted in the matrix, 8 m away from mound, on two diametrically opposite locations. Figure adapted from Cuma Mushagalusa et al. (2018).

#### Lubumbashi.

In November 2014, at the beginning of the rain season, a total of 240 seedlings were transplanted in situ at Mikembo sanctuary, on 15 termite mounds and in the surrounding matrix within the 10-ha permanent plot, as follows. Each termite mound and both adjoining matrix plots were considered as a block. Two seedlings of both origins (M and T) were planted in two rows (distance between seedlings within a row: 1 m) on the diametrically opposite slopes of the mound and on two corresponding points in the matrix at ca. 8 m away from the mound in N and S direction (Fig. 1). Each termite mound and both adjoining matrix plots were considered as a block to control for soil heterogeneity across the study site (see Muledi et al., 2017).

## 2.4. Data collection

### 2.4.1. Growth and survival

In the ex situ experiment, the percentage of germination was monitored weekly for a duration of six weeks. Nine months after sowing, seedling height was measured to the nearest millimeter (from soil to apical bud) and the number of leaves was recorded.

In the experiment in situ, the height and the number of leaves of each seedling were recorded on the day of transplantation (Supporting information Appendix S2). Initial height as well as initial leaf number were submitted to a crossed ANOVA with soil and origin as main factors in order to test for differences in the initial size and leaf number. Neither of these analyses showed significant differences (Supporting information Appendix S3) so that initial size and number of leaves were not further considered in the main data analyses. Growth was expressed in two ways, as the increment of height and the increment of leaf number between the transplantation and the end of the experiment in December 2017 (i.e. plants aged 36 months). The survival rate of the seedlings was also calculated.

### 2.4.2. Leaf traits

Leaves were collected from all the seedlings in the bags in April 2017 i.e. nine months after sowing and in situ after 36 months from surviving seedlings. Five undamaged leaves were collected from each seedling. The leaves were weighted to the nearest 0.1 mg (fresh mass FM) and photographed. The leaves were oven-dried at 50 °C for 48 h and weighted (dry mass DM). Leaf area (LA) was determined from leaf pictures using ImageJ. Photographs were analysed by including a scale bar (graph paper) and taking image on a level plane. Specific leaf area (SLA) was calculated as the ratio of the fresh LA on the DM. Leaf dry matter content (LDMC) was calculated as DM/FM. Leaf thickness was estimated as FM/LA (Wilson et al., 1999), assuming fresh leaf has the same density as water.

## 2.5. Data analysis

Height, leaf number, height increment, leaf number increment, and functional traits were transformed using a box-cox transformation prior to analyses (Box and Cox, 1964; see Supporting information Appendix S1 for Lambda values estimated).

For the in situ experiment, we first used linear mixed models and generalised linear mixed models (results are presented in the Supplementary material S7) to model seedling growth and leaf traits, and survival, respectively. We used linear mixed models of the response  $y$  (height increment, leaf number increment, or leaf trait) against the fixed effects (factors) ‘habitat’ and ‘origin’, and included a random effect for the block they belonged to. Each termite mound and its two adjoining matrix plots were considered as a block. Within each block, the average value of the two seedlings from the same origin planted in the same line (Fig. 1) was used to avoid pseudo-replication. The model can be summarised as:

$$Y_{ij} \sim N(\mu_{ij}, \sigma_{residuals}^2)$$

$$\mu_{ij} = \beta_0 + \beta_{hab} * habitat_i + \beta_{orig} * origin_i + \beta_{hab,orig} * habitat_i * origin_i + \epsilon_{block j}$$

$$\epsilon_{block j} \sim N(0, \sigma_{bloc}^2)$$

where the  $i$  observations of  $y$  in block  $j$  are function of an intercept ( $\beta_0$ ), the two factors (each with two levels) and a bloc-level error of mean 0 and variance  $\sigma_{bloc}^2$ . The residuals of the model, after considering the among-bloc variance, are  $\sigma_{residuals}^2$ . We modelled the individual probability of survival with a generalised linear mixed model using a binomial law and a log-link function to linearise the parameters. This allowed testing whether the survival of each origin was higher when transplanted in its own habitat vs. the habitat of the other origin. The corresponding equation was:

$$Y_{ij} \sim \text{Bernoulli}(p_{ij})$$

$$\eta_{ij} = \log(p_{ij} / 1 - p_{ij}) = \beta_0 + \beta_{hab} * habitat_i + \beta_{orig} * origin_i + \beta_{hab,orig} * habitat_i * origin_i + \epsilon_{block j}$$

$$\epsilon_{block j} \sim N(0, \sigma_{bloc}^2)$$

Because the proportion of variance explained by the block error term was null in all linear mixed models (see extensive linear mixed model table in Supporting information Appendix S7), we simplified the models to only include the fixed effects, reducing them to three-way ANOVAs for seedling growth (height and leaf number increments) and leaf traits while yielding the same results. In the ANOVAs, a significant origin  $\times$  habitat interaction indicated that the effect of the habitat (on or off termite mound) on the response (e.g. height increment) depended on the origin of the plant (on vs. off termite mounds).

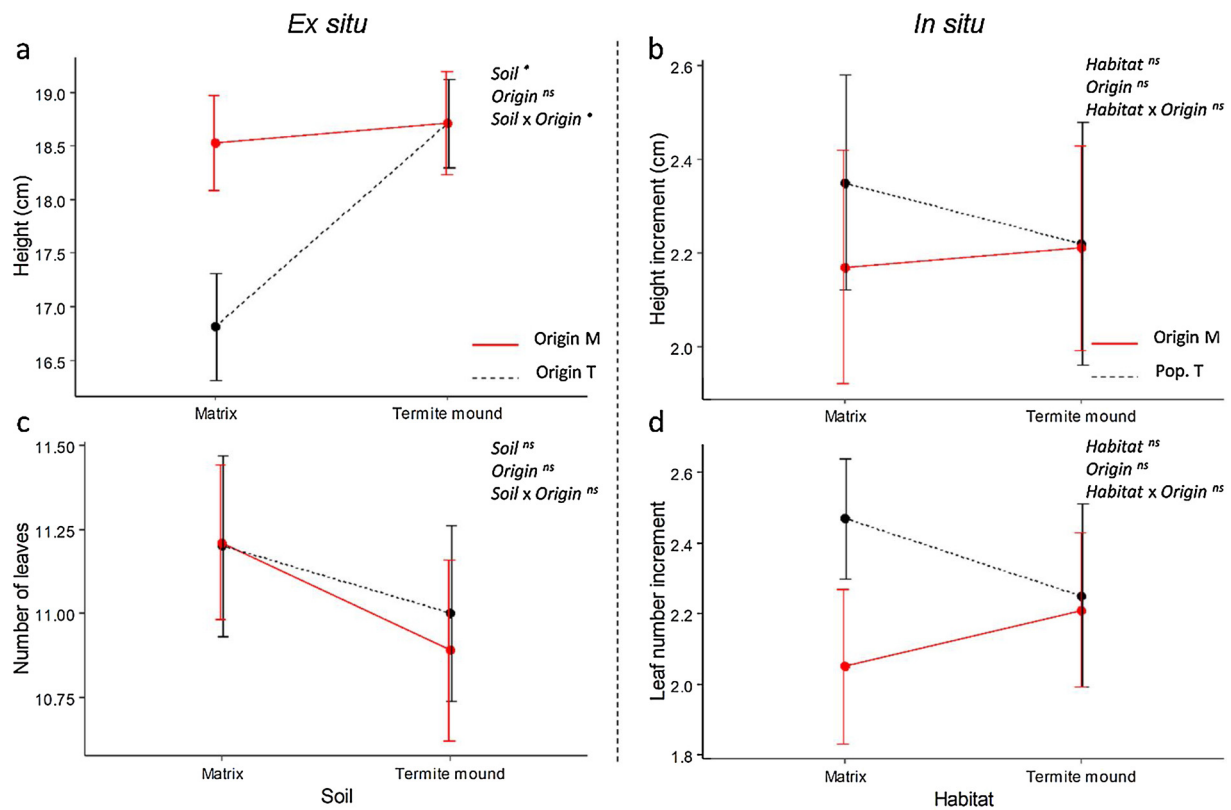
The results for growth and survival were interpreted following both criteria of Kawecki and Ebert (2004), i.e. comparing the same origin between the matrix and the termite mounds (“at home” vs. “away”) and comparing the two origins within each habitat (“local” vs. “foreign”).

For the analyses of height, leaf number and leaf traits based on the ex situ experiment, we used a two-way crossed ANOVA with the factors soil ( $j$  levels, with  $j = 2$ ) and origin ( $k$  levels, with  $k = 2$ ), where the origins were nested in the  $l$  sites, to account for the fact that the seeds came from different sites. For each  $i$  observation:

$$Y_{ijkl} = \beta_{soil} * soil_{ij} + \beta_{orig} * origin_{il(k)} + \beta_{soil,orig} * soil_{ij} * origin_{il(k)} + \epsilon_{ijkl}$$

where  $\beta$  are the model coefficients and  $\epsilon$  is the error term. Here also, a significant origin  $\times$  soil interaction indicated that the effect of the soil (termite mound or matrix soil) on the response depended on the origin of the plants (on vs. off termite mounds). Post-hoc tests (Tukey) were performed for significant interactions, using the *emmeans* package.

All analyses were performed using the R statistical environment,



**Fig. 2.** Mean height (a, b) and number of leaves (c, d) of *Dalbergia boehmii* from two origins (termite mounds and matrix, respectively), transplanted on mounds and in the matrix habitat in situ and on termite and matrix soil in an ex situ experiment with 15 and 20 replicates respectively. Error bars are standard errors. The plain red and dashed black lines are the matrix origin and termite mound origin, respectively. The significance levels of the soil or habitat (ex situ and in situ, respectively), origin, and interaction effects of the three-way ANOVA are represented in the upper-right corner of each figure: ns: non significant, \*:  $P \leq 0.05$ , \*\*:  $P \leq 0.01$ , \*\*\*:  $P \leq 0.001$ .

version: R v.3.4.0; (R Core Team, 2018; R code for analyses in Supporting information Appendix S8).

### 3. Results

#### 3.1. Ex situ experiment

##### 3.1.1. Growth

Seedling height was significantly larger on T soil compared to M soil (18.7 cm vs 17.7 cm;  $F = 5.24$ ,  $P < 0.05$ ), but leaf number did not differ significantly. The soil  $\times$  origin interaction was nearly significant for height ( $F = 3.57$ ,  $P = 0.059$ ) (Fig. 2a). The post-hoc tests for this interaction indicated that the T origin plants performed better on T soil than on M soil (18.7 vs. 16.8 cm) ( $t = -2.955$ ,  $P = 0.0176$ ). For leaf number, the interaction was not significant (Fig. 2c).

##### 3.1.2. Functional traits

SLA ranged from 134 cm<sup>2</sup>/g (plants of origin M on M soil) to 138 cm<sup>2</sup>/g (plants of origin T on T soil) (Fig. 3a). LDMC ranged from 35.0 % (plants of origin T on T soil) to 36.2 % (plants of origin M on M soil) (Fig. 3c). Leaf area ranged from 8.4 cm<sup>2</sup> (plants of origin T on M soil) to 9.0 cm<sup>2</sup> (plants of origin M on matrix soil) (Fig. 3e), and leaf thickness ranged from 0.207 mm (Pop T on M soil) to 0.201 mm (plants of origin M on T soil) (Fig. 3g). Neither the origin factor nor the origin  $\times$  soil interaction were significant for the studied traits. However, the SLA differed significantly between plants growing on the two soils ( $F = 6.487$ ,  $P < 0.05$ ) and so did LDMC ( $F = 14.815$ ,  $P < 0.001$ ). Seedlings had larger SLA and lower LDMC when cultivated in T soil compared to M soil (SLA: 137.6 vs 134.6 cm<sup>2</sup>. g<sup>-1</sup>; LDMC: 35.0 vs 36.1 %).

#### 3.2. In situ reciprocal transplant experiment

##### 3.2.1. Growth

Height increment ranged from 2.17 cm (plants of origin M in the matrix) to 2.35 cm (plants of origin T in the matrix) (Fig. 2b) and leaf number increment ranged from 2.05 (plants of origin M in the matrix) to 2.47 leaves (plants of origin T in the matrix) (Fig. 2d) over the growth period of 36 months. Neither height nor leaf number increments differed significantly among habitats, origins, and the interaction habitat  $\times$  origin was not significant.

##### 3.2.2. Leaf traits

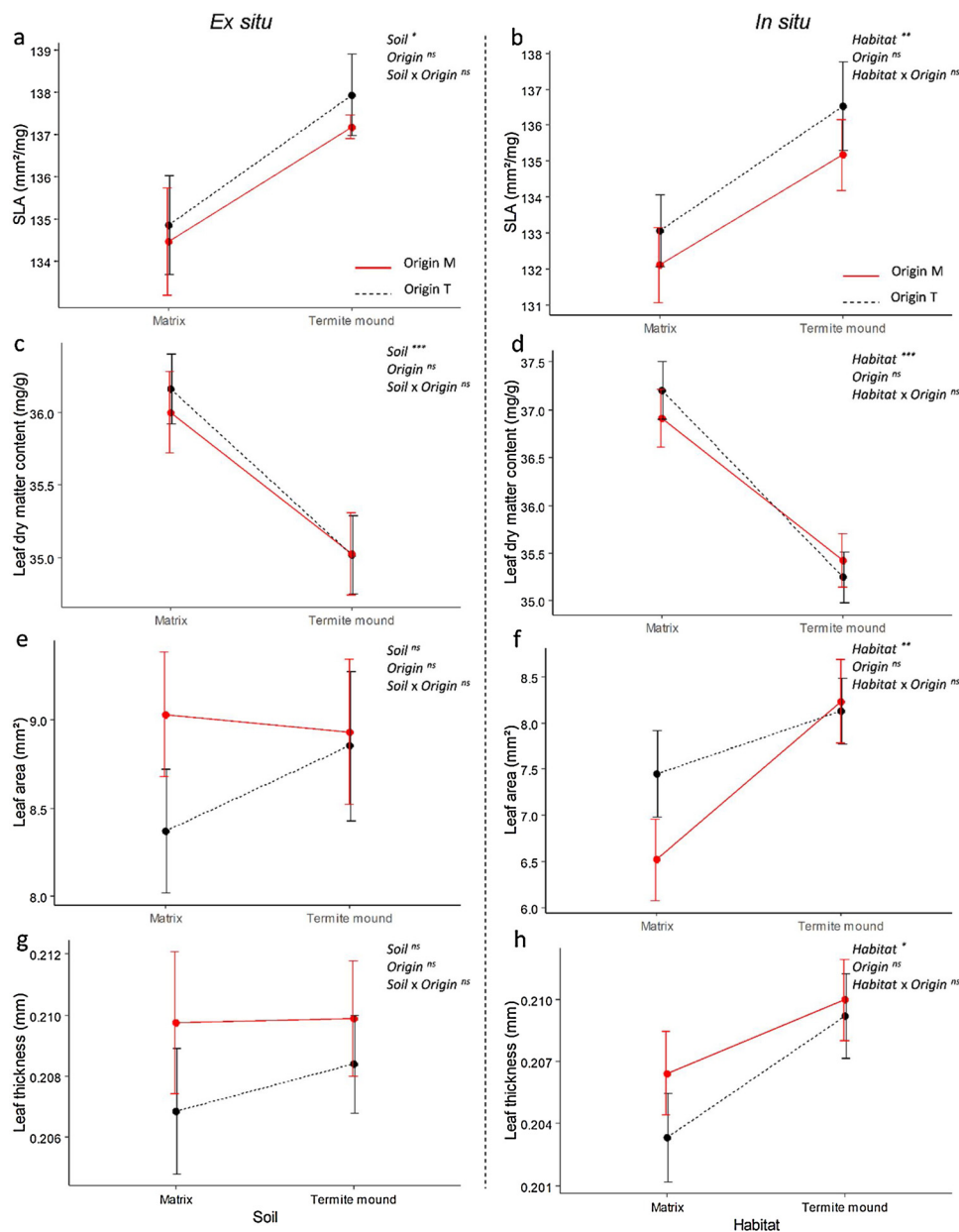
SLA ranged from 132 mm<sup>2</sup>/mg (plants of origin M in the matrix) to 137 mm<sup>2</sup>/mg (plants of origin T on termite mounds) (Fig. 3b), and was ca. 2.4 % higher on termite mounds compared to the matrix (habitat effect;  $F = 9.902$ ,  $P < 0.01$ ).

LDMC varied between 35.3 % (plants of origin T on termite mounds) to 37.2 % (plants of origin T in the matrix) (Fig. 3d), and was ca. 4.6 % higher in the matrix compared to the termite mounds (habitat effect;  $F = 34.681$ ,  $P < 0.001$ ).

LA ranged from 6.5 cm<sup>2</sup> (plants of origin M in the matrix) to 8.2 cm<sup>2</sup> (plants of origin M on termite mounds) (Fig. 3f) and also presented an habitat effect, with trait values ca. 14.6 % higher on termite mounds compared to the matrix (habitat effect;  $F = 7.494$ ,  $P < 0.01$ ).

Leaf thickness, finally, ranged from 0.203 mm (plants of origin M on termite mounds) to 0.210 mm (plants of origin T in the matrix) (Fig. 3h). This trait, too, varied significantly with the habitat ( $F = 5.117$ ,  $P < 0.05$ ), being 2.3 % higher on termite mounds compared to the matrix. Neither origin nor origin  $\times$  habitat interaction explained a significant proportion of the variation of any of the traits.





**Fig. 3.** Means values of leaf functional traits in two origins of *Dalbergia boehmii* (from termite mounds and the matrix, respectively), transplanted on mounds and in the matrix habitat in situ and on termite and matrix soil in an ex situ experiment with 15 and 20 replicates respectively. Error bars are standard errors. SLA (a, b), leaf dry matter content (c, d), leaf area (e, f) and leaf thickness (g, h). The plain red and dashed black lines are the matrix (M) and termite mound (T) origins, respectively. The significance levels of the soil or habitat (ex situ and in situ, respectively), origin, and interaction effects of the three-way ANOVA are represented in the upper-right corner of each figure: ns: non significant, ·:  $P \leq 0.1$ , \*:  $P \leq 0.05$ , \*\*:  $P \leq 0.01$ , \*\*\*:  $P \leq 0.001$ .

Extensive ANOVA tables are presented in Supporting information Appendices S4 and S5 respectively for ex and in situ experiment.

### 3.2.3. Survival

Survival rate varied between 58.3 % (Pop T and Pop M in the matrix) and 61.6 % (origin T on termite mound). Survival was not significantly explained by the origin ( $P = 0.894$ ) nor by the habitat ( $P = 0.691$ ) or the interaction between the two factors ( $P = 0.893$ ) (Fig. 4). For more details, see table of logistic regression analysis presented in Supporting information Appendix S6.

## 4. Discussion

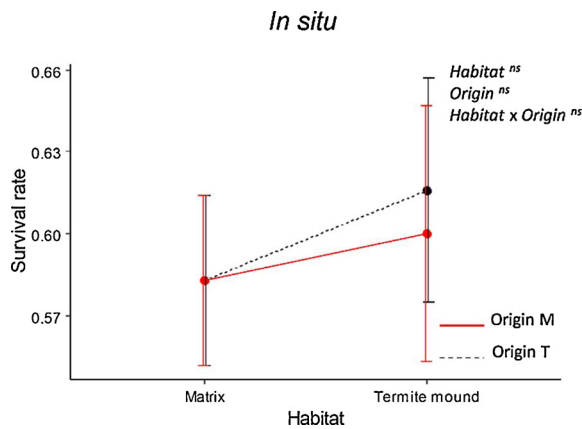
*Dalbergia boehmii* is a tree with a broad ecological niche in the dry miombo woodlands of Upper Katanga. In a previous study, we found intraspecific variation in leaf functional traits between plants on termite mounds and in the surrounding woodland matrix (Cuma Mushagalusa et al., 2020). Here, we tested whether that variation was due to phenotypic plasticity in response to the steep habitat heterogeneity or had a genetic basis, and in case of the latter, if local adaptation has evolved

between the two origins.

### 4.1. No evidence for genetic divergence in foliar traits

Foliar traits did not present statistically significant differences when the seedlings originated on and off termite mound were cultivated in the same substrate, be it in the experimental garden or in the in situ reciprocal transplant. Our data provide no evidence for a genetic divergence in foliar traits at the seedling state in *D. boehmii*. It could be argued that our trait values on seedlings are not representative of adult trees, since foliar traits can change with plant age (Damián et al., 2017; Fortunel et al., 2020).

The lack of genetic divergence of traits is not surprising, however, considering the fine spatial scale of habitat variation in the dry woodlands of Upper Katanga. The density of termite mounds ranges from 3 to 5/ha (Mujinya et al., 2013) and the soil surface covered by mounds is ca. 8% (Malaisse, 1978b). No data on the breeding system of *D. boehmii* is available. Autoincompatibility has been reported in other species of the genus *Dalbergia* (Bawa and Webb, 1984; Seavey and Bawa, 1986). The flowers are visited by insects (pers. obs.). The fruits are flat thin



**Fig. 4.** Survival of two two origins of *Dalbergia boehmii* (from termite mounds (T, stippled line) and from the matrix (M; continuous line)), reciprocally transplanted on mounds and in the matrix with 15 replicates. Error bars are standard errors. The plain red and dashed black lines are the matrix (M) and termite mound (T) origins, respectively.

Pods easily dispersed by wind. The average population density is in the range three to five individual/ha. Therefore, gene flow is probably significant between the mounds and the matrix, thus likely counteracting any disruptive selective pressure on local populations.

#### 4.2. No evidence for local adaptation

In spite of widely divergent environmental conditions between termite mound and the matrix, our results did not reveal any evidence of local adaptation. None of the two criteria of Kawecki and Ebert (2004) was met. In both experiments (i.e. ex situ and in situ), neither plant regardless his origin performed significantly better “at home” than “away”. The second criterion of local adaptation (i.e., the “foreign vs. local” criterion) was not met either, since the local population did not survive or grow systematically better than the foreign population. This is particularly illustrated by the termite mound origin compared to the matrix origin when grown on termite mounds (in situ) or on termite mound soil (ex situ). The reciprocal holds true for plants grown in situ in the matrix or ex situ in matrix soil.

In a previous paper using three couples of congeneric species (one termitophilous, one termitofuge), with the same experimental protocol as in the present study, we did find strong evidence for local adaptation of both the termitophilous and the termitofuge species, in the three genera investigated. In that experiment, local adaptation was expressed mostly in terms of survival, not growth. Thus, our failure to detect local adaptation here is unlikely to be due to experimental limitations.

There was however some evidence for better growth in the matrix origin plant compared to the termite mound origin ones when grown in the matrix (18.5 cm vs 16.8 cm). This slight growth difference, without a difference in survival, may not translate into a better reproductive success of the local population and would require additional evidence.

These findings are consistent with our inability to detect heritable features or constitutive differences in leaf traits.

#### 4.3. Phenotypic plasticity

Foliar traits showed a significant response to growth conditions in both the in situ and ex situ experiment. The pattern of response was consistent in both experiments. SLA was higher and leaf dry matter content was lower on termite mounds soil compared to the matrix soil.

Thus, the plastic response in the broad-niched *D. boehmii* has the same direction as the divergence in traits between the narrow-niched termitophilous and termitofuge congeneric species previously compared (Cuma Mushagalusa et al., 2018). The direction of plasticity in *D.*

*boehmii* is consistent with previous work reporting increased SLA, and decreased leaf dry matter content in response to increased soil fertility (Jager et al., 2015; Siefert and Ritchie, 2016; Geekiyanage et al., 2018).

The direction of trait response to the two habitats is remarkably similar for the three data sets, i.e. the adult trees sampled in situ, the seedlings grown ex situ and the seedlings grown in situ.

The magnitude of the response increased in the order seedlings ex situ < seedlings in situ < adult trees (SLA: 2.1 %–2.4 % - 11.3 %; LDM: 2.9 % - 4.6 %–10.5 %).

Soil fertility and granulometry are certainly not the sole ecological differences between mounds and matrix. Firstly, it is well known that termite mounds have larger stores of available water (Konaté et al., 1999; Turner, 2006) and this was verified in Upper Katanga (Cuma Mushagalusa et al., 2018). In the seedlings cultivated in bags, this difference in water economy cannot be reproduced. Secondly, termite mounds have low light intensity at ground level, and a buffered microclimate compared to the matrix (Joseph et al., 2016). Thus, soil chemical properties explain only a part of the plastic response observed in situ, which may explain the larger plastic response in situ compared to the experiment in bags.

Intraspecific variation in functional traits has received increasing attention in the last decade (Siefert et al., 2015). Our results show that edaphic heterogeneity has significant impact on local intraspecific trait variation in *D. boehmii*, as found in other broad-niched species (Lenssen et al., 2004; Fajardo and Piper, 2011; Pakeman, 2013; Harzé et al., 2016; De Smedt et al., 2018).

Leaf economic traits (SLA, LDMC) often display large intraspecific trait variation (Siefert et al., 2015). In contrast to leaf economic traits, traits related to leaf size (area and thickness), did not show differences in the experiment ex situ but only in the in situ experiment with the amplitude of the intraspecific variation of 146% and 2,3% respectively. These traits which are typically considered independent of the leaf economics spectrum (Siefert et al., 2015) have been linked with adaptation to broad climatic gradients (Craine et al., 2012). The variation of these traits at this fine scale is probably driven by the fact that termite mounds differ from the surrounding matrix for many ecological factors, including, water availability (Turner, 2006; Cuma Mushagalusa et al., 2018) and temperature (Joseph et al., 2016; Ashton et al., 2019) and may act as microclimate.

#### 4.4. Adaptive plasticity?

The question arises as to whether the observed plastic response is adaptive. In other words, does *D. boehmii*'s capacity to occupy a broad niche rely on its ability to express that plastic response? Answering this question would require comparing *D. boehmii* with a narrow-niched congeneric species (either termitophilous or termitofuge). However, indirect evidence suggests that the observed plastic response is actually adaptive. First, termite mounds have higher levels of available resources (nutrients and water) compared to the matrix. Such conditions are expected to favour a syndrome of faster resource capture and use. It is indeed what we found in the adult plants of *D. boehmii* on termite mounds and in the cultivated seedlings. Higher SLA, lower LDMC, indicate that the traits are shifted along the leaf economics spectrum in the direction expected in more resource-rich conditions (Wright et al., 2004; McGill et al., 2006).

The plastic response within *D. boehmii* is remarkably similar to the constitutive difference in the traits of termitophilous and termitofuge species that we found in a previous study (Cuma Mushagalusa et al., 2020). The few previous work examining plant traits on termite mounds reached similar conclusions (High values of SLA on termite mound and High LDMC in the matrix) (Van der Plas et al., 2013; Joseph et al., 2014).

## 5. Conclusion

*Dalbergia boehmii*, one of the few broad-niched species capable of colonising both termite mounds and the matrix in dry tropical woodlands, does not appear to have evolved edaphic ecotypes. The significant intraspecific variation in functional trait appears to be mostly if not solely accounted for by phenotypic plasticity. The pattern of plasticity strongly suggests that the response is adaptive. Further work should verify if the same conclusion applies to other broad-niched species in the dry tropical woodlands of the Zambezi region.

## Author contributions

FC conducted the field experiment, analyzed the data and wrote the paper; PM wrote the research project, designed the experiment and contributed to writing the manuscript; DB contributed to the data analysis and commented on a draft; MS and BM supervised the research in DR Congo, YM and MK participated to the field experiment and data collection.

## Declaration of Competing Interest

None.

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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.2020.104186>.

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