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No evidence that boron influences tree species distributions in lowland tropical forests of Panama

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Summary

- It was recently proposed that boron might be the most important nutrient structuring tree species distributions in tropical forests. Here we combine observational and experimental studies to test this hypothesis for lowland tropical forests of Panama.
- Plant-available boron is uniformly low in tropical forest soils of Panama and is not significantly associated with any of the > 500 species in a regional network of forest dynamics plots. Experimental manipulation of boron supply to seedlings of three tropical tree species revealed no evidence of boron deficiency or toxicity at concentrations likely to occur in tropical forest soils. Foliar boron did not correlate with soil boron along a local scale gradient of boron availability.
- Fifteen years of boron addition to a tropical forest increased plant-available boron by 70% but did not significantly change tree productivity or boron concentrations in live leaves, wood or leaf litter. The annual input of boron in rainfall accounts for a considerable proportion of the boron in annual litterfall and is similar to the pool of plant-available boron in the soil, and is therefore sufficient to preclude boron deficiency.
- We conclude that boron does not influence tree species distributions in Panama and presumably elsewhere in the lowland tropics.

Introduction

It was proposed recently that boron (B) might be the most important nutrient influencing tree species distributions in tropical forest on Barro Colorado Island (BCI), Panama (Steidinger, 2015). This was based on a niche-breadth analysis using data on extractable nutrients and tree community composition from the BCI large forest dynamics plot, in which B (along with potassium, K) was found previously to have the strongest effect on community structure of many measured soil nutrients (John *et al.*, 2007). The argument for the importance of B is based on the widely held assumption that B deficiency and toxicity occur over a relatively narrow range of soil B concentrations compared with other nutrients (Gupta *et al.*, 1985). However, there is little evidence in support of this assumption, which has been challenged on the basis of results for a number of crop plants and cultivars (Chapman *et al.*, 1997).

Boron is an essential plant micronutrient and is the most commonly deficient micronutrient for crops (Shorrocks, 1997). Boron is known to constrain tree growth in plantation forestry in Scandinavia, New Zealand and Southeast Asia once macronutrient limitation (principally nitrogen, N) has been corrected by the addition of fertilizer (Hunter *et al.*, 1990; Högberg *et al.*, 2006; Dell *et al.*, 2008). There are also reports suggesting that B can be the primary nutrient limiting growth in eucalyptus plantations in some parts of China (Dell *et al.*, 2008), while temporary B deficiency was reported for deciduous forests in Quebec, Canada, primarily for rapidly growing seedlings on sandy soils (Bernier & Brazeau, 1988). By contrast, B toxicity is common only in semi-arid environments, particularly in irrigated agriculture. For example, B toxicity in Australia is confined to regions with low annual rainfall (< 550 mm) in Western Australia, South Australia and Victoria, particularly on strongly alkaline (pH > 8) soils with sodic clay subsoils, which are poorly leached and have B concentrations > 12 mg B kg^{-1} (Nable *et al.*, 1997). Overall, there is little evidence of B deficiency or toxicity in natural forests in the tropics or elsewhere.

Here we examine the hypothesis that soil B influences species distributions in lowland tropical forests. We combine manipulative experiments on tropical tree seedlings with analysis of species responses to soil B availability across a regional-scale network of forest census plots and a local scale gradient in B availability on BCI. We also present a B budget for a well-studied site in Panama, present results of a long-term micronutrient addition experiment and suggest an explanation for the absence of B effects on tropical tree distributions in the region. We conclude that soil B does not influence the distribution of lowland tropical tree species in Panama.

Materials and Methods

Soil-extractable B determination

We quantified plant-available soil B in soils from a network of 79 lowland tropical forest census sites in Panama (Pyke *et al.*, 2001; Condit *et al.*, 2004, 2013; Engelbrecht *et al.*, 2007; Turner & Engelbrecht, 2011). The sites vary from inventory transects to plots of various sizes, including 40 × 40 m, 1 ha, 6 ha and 50 ha (BCI). The sites span a strong rainfall gradient, from *c.* 1800 to > 4000 mm yr^{-1} (Engelbrecht *et al.*, 2007). Soils include a variety of taxonomic orders (Oxisols, Ultisols, Alfisols and Inceptisols) and vary widely in chemical properties, including pH (3.3–7.0), organic carbon (2–10%) and readily exchangeable soil phosphorus (P) (< 0.1 to > 20 mg P kg^{-1}). For each site, a soil sample consisted of a composite of either five cores (inventory transects and 40 × 40 m plots), 13 cores (1 ha forest dynamics plots) or 25 cores (a 6 ha plot on the Caribbean coast and a 50 ha plot on BCI). Cores were taken from the surface soil (0–10 cm depth), because this zone integrates the nutrient cycle and contains the majority of the extractable nutrients and fine roots.

We extracted B from soils using two procedures: hot water extraction and Mehlich-III extraction. Hot water soluble B (Berger & Truog, 1939, 1944; Gupta, 1967) is the most widely used measure of plant-available soil B (Keren, 1996). We used hot 0.01 M $CaCl_2$ instead of deionized water (Jeffrey & McCallum, 1988), because this procedure yields similar concentrations to the hot water procedure but reduces interference from suspended clay in the extracts, which is important in the analysis of the high clay soils of Panama.

Briefly, air-dried soils sieved to < 2 mm were mixed with 0.01 M $CaCl_2$ in a 1 : 2 soil to solution ratio and placed in a shaking water bath at 85°C. After 20 min, samples were centrifuged (8000 ***g***, 10 min) and the supernatant was decanted. Boron was determined in the extracts by inductively coupled-plasma optical-emission spectrometry (ICP–OES) (Optima 7300DV; Perkin Elmer Inc., Shelton, CT, USA).

We also used Mehlich-III solution to determine extractable soil B (Mehlich, 1984). This procedure is a commonly used multi-element extraction to quantify plant-available nutrients, including B, and was used in the original analysis on BCI (John *et al.*, 2007). It includes water-soluble B, as well as additional B held on soil sorption sites. Briefly, air-dried soils were shaken for 5 min in Mehlich-III solution (0.2 N acetic acid, 0.25 M NH_4NO_3, 15 mM NH_4F, 13 mM HNO_3, and 1 mM EDTA) in a 1 : 10 soil to solution ratio. The samples were centrifuged (8000 ***g***, 10 min) and the supernatant was decanted. Boron was determined by ICP–OES as detailed in the previous paragraph. All extractions and preparation of standards for the two procedures were conducted in plastic to avoid B contamination from borosilicate glassware.

The most sensitive wavelength for B determination by ICP–OES at 249.772 nm suffers from a major interference by iron (Fe) (Sah & Brown, 1997; Taber, 2004; Turner *et al.*, 2016). This is a particular problem for Mehlich-III extracts of tropical forest soils, which contain considerable Fe concentrations, but does not affect 0.01 M $CaCl_2$ extracts, which contain insufficient Fe to interfere in B detection (Turner *et al.*, 2016). Here we used the most sensitive line (249.772 nm) to determine B in 0.01 M $CaCl_2$ extracts and the line without Fe interference (208.597 nm) to determine B in Mehlich-III extracts and total element digests.

Regional and local scale species distributions in relation to soil B

We incorporated plant-available soil B measurements as described in the previous section into a hierarchical Bayesian model to quantify regional-scale species distributions in response to soil B. The model was described in detail previously (Condit *et al.*, 2013) and included data on the distributions of > 500 tree species that occur in a network of 72 forest census sites in Panama, dry season soil moisture deficit, soil exchangeable calcium (Ca) and K, readily exchangeable P determined by extraction with anion-exchange resins (resin P), the micronutrients Fe and zinc (Zn), extractable inorganic N, and the potential toxin exchangeable aluminum (Al). We reran the original model except that we replaced inorganic N (the nutrient with the weakest influence on species distributions) with either hot-$CaCl_2$ extractable B or Mehlich-III extractable B. Significant associations between tree species distributions and soil nutrients are indicated by effect sizes – the first order parameter of the logistic model describing the distribution of a species in relation to a given nutrient. Positive effect sizes indicate that the species occurs predominantly at high concentrations of the nutrient, while negative effect sizes indicate that the species occurs predominantly at low concentrations of the nutrient.

At the local scale, Steidinger (2015) proposed that species distributions respond to soil B across a B availability gradient on BCI. To test this, we measured B in leaves of tree species that occur across the B gradient on BCI, and compared these concentrations with estimated B availability from kriged Mehlich-III extractable B concentrations (updated values corrected for Fe interference reported in Turner *et al.*, 2016). We sampled shade leaves from 10 individuals of each of six species: *Chrysophyllum argenteum* Jacq., *Guarea guidonia* (L.) Sleumer, *Hirtella triandra* Sw., *Protium panamense* (Rose) I.M. Johnst., *Tetragastris panamensis* (Engl.) Kuntze and *Trichilia tuberculata* (Triana & Planch.) C. DC. Trees were selected for sampling based on *K*-means clustering (Hartigan & Wong, 1979) to maximize variation in kriged estimates of soil base cation concentrations determined from Mehlich-III extractions (John *et al.*, 2007). This yielded a parallel variation in extractable B, given that B was strongly correlated with base cations on the 50 ha plot (Pearson $r > 0.7$; see Fig. 1 and table S4 in John *et al.*, 2007). We used the Poisson cluster method test to determine the association between tree species distribution and soil B (John *et al.*, 2007). Foliar B was determined by nitric acid digestion as described below, and compared with Mehlich-III extractable B concentrations using linear regression.

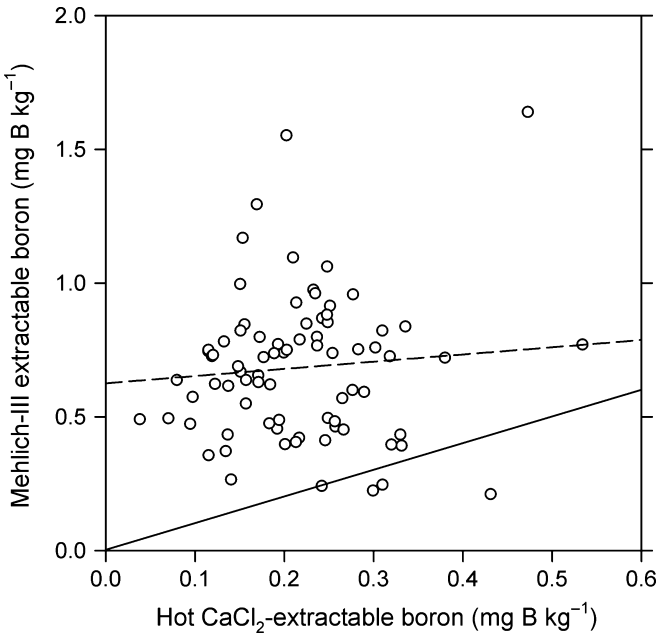

Fig. 1 Concentrations of soil boron (B) extracted by hot 0.01 M $CaCl_2$ and Mehlich-III solution in soils from 79 sites under lowland tropical forests in Panama. Each soil was a composite of multiple replicate cores of the surface soil (0–10 cm depth) at each site. The solid line is the 1 : 1 line. The dashed line is the regression between hot-$CaCl_2$ extractable B and Mehlich-III extractable B, defined by the equation [Mehlich B] = 0.269 × [$CaCl_2$-B] + 0.625; $R^2 = 0.008$, $P = 0.44$.

Growth responses of tropical tree seedlings to B

We grew seedlings of three tropical tree species underneath a glass rain shelter in a mixture of quartz sand and soil. The soil was obtained from a site under tropical forest on the Santa Rita Ridge, one of the most infertile sites in central Panama. We selected species that differed in their distributional affinity for B based on the results of the Poisson cluster model implemented by John *et al.* (2007). Initially, we grew *Hura crepitans* L. in full nutrient solution (Johnson *et al.*, 1957) with B concentrations of 0, 1, 4, 10, and 40 times the standard concentration (i.e. 0, 12.5, 50, 125 and 500 μM B) with nutrient solution added once per week. As the plants showed no sign of B toxicity (see Results section) we grew two additional species, *Ochroma pyramidale* (Cav. ex Lam.) Urb. and *Pentagonia macrophylla* Benth., with up to 100 and 150 times the standard B concentration (i.e. 1250 and 1875 μM B), with nutrient solution added twice per week. Small amounts of water loss by evaporation were replaced every 2 d using tap water that contained a trace concentration of B equivalent to that in rainfall (< 1 μM B). For the first experiment (*H. crepitans*) we used a 50 : 50 sand and soil mixture, while in the second experiment (*O. pyramidale* and *P. macrophylla*) we used a 95 : 5 sand and soil mixture to ensure an extremely low B concentration in the zero-B treatment. Thus, all plants received B from the substrate and water additions.

Seedlings were grown for different periods depending on growth rate until they attained similar biomass, with growth period ranging from 27 d for *O. pyramidale* to 70 d for *P. macrophylla*. The seedlings of each species were harvested for all treatments when the ratio of plant biomass to pot volume was *c.* 1 g : 1 l in the 12.5 μM B treatment. Leaves, stems and roots were separated, dried for 3 d at 60°C, weighed and ground. All species form arbuscular mycorrhizas and acquire their symbiotic fungi rapidly after germination. We have verified this in previous experiments, although we did not measure mycorrhizal colonization in this experiment.

Relative growth rate (RGR; mg g^{-1} d^{-1}) was calculated according to the following equation: $RGR = ((\mathrm{Log_e} W_f - \mathrm{Log_e} W_i)/(\Delta t))$, where W_f is the final dry mass, W_i is the initial dry mass and Δt is the duration of the experiment. Leaf mass fraction (LMF; leaf mass per unit whole plant mass; g g^{-1}), net assimilation rate (NAR; biomass increment per unit leaf area; g m^{-2} d^{-1}), leaf area ratio (LAR; leaf area per unit whole plant mass; cm^2 g^{-1}), specific leaf area (SLA; leaf area per unit leaf mass; cm^2 g^{-1}) and root mass fraction (RMF, root mass per unit whole plant mass; g g^{-1}) were calculated from harvest data. NAR was calculated according to the following equation: $NAR = ((W_f - W_i) \times (\mathrm{Log_e} A_f - \mathrm{Log_e} A_i))/((A_f - A_i) \times (\Delta t))$, where W_f and W_i are the final and initial dry mass (g), respectively, A_f and A_i are the final and initial leaf area (m^2), respectively, and Δt is the duration of the experiment (d).

Foliar B was determined by digestion in concentrated nitric acid under pressure in polytetrafluoroethylene vessels, with detection by ICP–OES spectrometry at 208.957 nm (although there was negligible Fe interference at the low concentrations present in the leaf tissue). Foliar B recoveries from the certified reference materials NIST 1515 (apple leaves) and NIST 1547 (peach leaves) were > 95% of the certified values.

Boron addition experiment in a lowland tropical forest

We quantified responses in trees and soil to 15 yr of experimental B addition at a site in lowland tropical forest on Gigante Peninsula, close to BCI. The long-term nutrient addition experiment is described in detail elsewhere (Yavitt *et al.*, 2009; Wright *et al.*, 2011; Turner *et al.*, 2015). A micronutrient treatment that includes B is applied to four 40 × 40 m (1600 m^2) plots, and four similar plots serve as untreated controls. Since 1998, the four micronutrient plots have received annual additions of dolomitic limestone (230 kg ha^{-1}) and a soluble trace element mix (25 kg ha^{-1}; Scott's Miracle Grow Company, Marysville, OH, USA) that contained 1.35% B, equivalent to 0.3375 kg B ha^{-1} or 33.75 mg B m^{-2}. This is a similar amount to the annual B return in litter fall at this site (see 'Boron budget'). To compare the control and micronutrient plots, we used a one-way ANOVA to compare differences in plant-available B determined by hot-$CaCl_2$ extraction for soils from 0 to 5 cm depth sampled in November 2012 (log-transformed values), and a one-way ANOVA to compare treatment effects on B in fine roots. We used a factorial two-way ANOVA with treatment (control vs micronutrient) and species as main effects to evaluate differences in B concentrations in wood and live leaves for three species (*Alseis blackiana*, *Heisteria concinna* and *T. panamensis*) sampled in 2013. We used a main effects ANOVA (no interaction) with

treatment and season as main effects to evaluate B concentrations in litter fall during the 2012 wet season and the 2013 dry season.

Boron budget

We quantified B stocks in trees and soil using data from the four control plots in the nutrient addition experiment on Gigante Peninsula. We measured B in wood and leaves of the three species that occur in all plots in the experiment (*A. blackiana*, *H. concinna*, *T. panamensis*). Samples were digested in concentrated nitric acid under pressure with detection by ICP spectrometry as described under 'Growth responses of tropical tree seedlings to B'. We used the average concentrations in wood and leaves of the three species to estimate total B stocks in the forest, using above-ground biomass estimates derived from allometric equations involving tree diameter and height (Chave *et al.*, 2015). This assumes that the three-species means are representative of the whole-plot means involving all species. We calculated total soil B in samples taken to 1 m depth in the soil profile (four cores per plot) by multiplying total B determined by nitric acid digestion and soil bulk density. In the same manner, we calculated stocks of plant-available B determined by hot-$CaCl_2$ extraction in the top 1 m of the profile and the surface 20 cm of the profile, given that the majority of the fine roots occur in the latter horizon (Yavitt & Wright, 2001). Finally, we estimated B inputs in rainfall by multiplying the annual rainfall on BCI (2600 mm; Windsor, 1990) by the global mean B concentration in marine rain of $10 \pm 2.3\ \mu g\ l^{-1}$ (Park & Schlesinger, 2002). The value for marine rain is higher than for continental rain (mean $4.89\ \mu g\ l^{-1}$), but is most likely to resemble the concentration in rainfall on BCI given its proximity to the Caribbean Sea (BCI is *c.* 25 km south of the coastline). The mean value for marine rain is greater than the median concentration ($6.6\ \mu g\ l^{-1}$). However, the mean concentration is similar to measurements of rainfall B on the northern Gulf Coast of Florida, USA, which were $8.3\ \mu g\ l^{-1}$ in summer and $11.9\ \mu g\ l^{-1}$ in winter (Martens & Harriss, 1976). Our estimate of B inputs to the forest does not include dry deposition, for which values are poorly constrained (Park & Schlesinger, 2002) but potentially important (Cividini *et al.*, 2010). We did not measure B leaching, but assume that it is similar to B inputs in rainfall, assuming that the B cycle is in equilibrium in the forest.

Results

Soil B concentrations in lowland tropical forests of Panama

Across a large number of soils in central Panama under lowland tropical forests but spanning a range of soil properties, plant-available B concentrations determined by extraction in hot $CaCl_2$ ranged between 0.04 and 0.53 mg B kg^{-1} (mean 0.22 mg B kg^{-1}). These values are in the range considered to represent B deficiency in agriculture and none were at levels at which B toxicity is typically expected to occur (> 5 mg B kg^{-1}) (Nable *et al.*, 1997). Mehlich-III extractable B concentrations were greater than hot $CaCl_2$ extractable B, ranging between 0.21 and 1.64 mg B kg^{-1} (mean 0.68 mg B kg^{-1}). The two measures of extractable B were not significantly correlated ($P = 0.44$; Fig. 1).

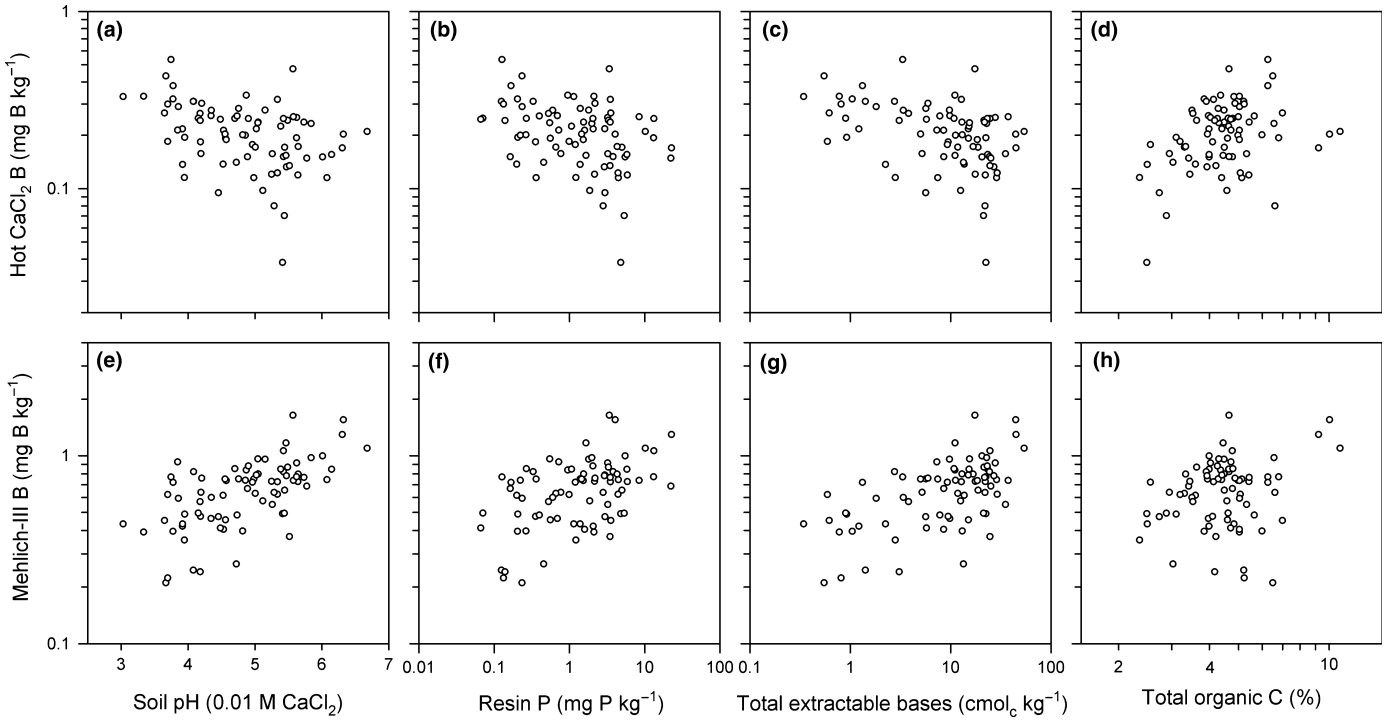

Fig. 2 Relationships between soil properties and plant-available soil boron (B) determined by extraction in hot 0.01 M $CaCl_2$ (a–d) and Mehlich-III solution (e–h). All measures except pH were log-transformed before analysis. P, phosphorus; C, carbon.

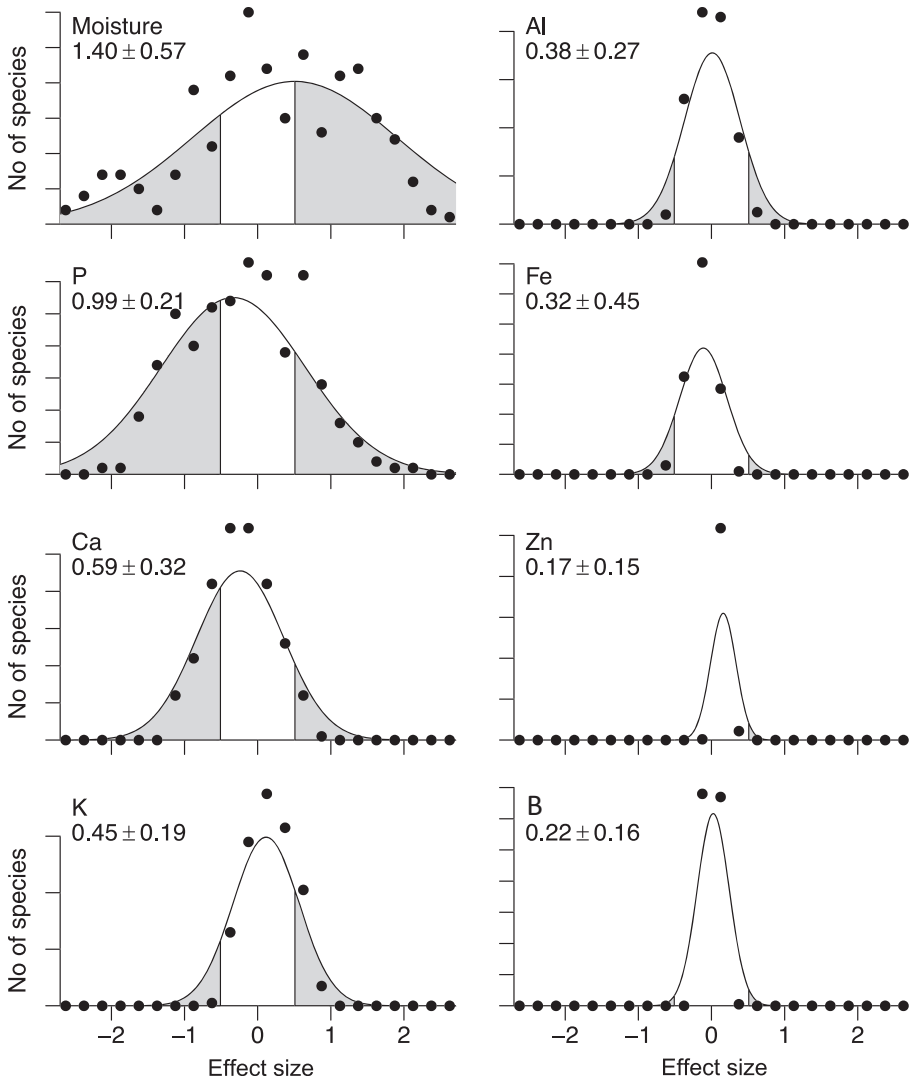

Fig. 3 Histograms of individual species responses to eight environmental factors, including plant-available boron (B) determined by extraction in hot 0.01 M $CaCl_2$. The horizontal axis is the effect size, which is defined as the first-order parameter of the logistic model and approximates the change in occurrence probability of a species across the range of each factor, relative to its mean occurrence. The shaded portions of each curve highlight species with strong responses (effect sizes greater than ± 0.5), which indicate species that occur predominantly at one end of the gradient. These first-order effects do not include modal responses. The curves are the fitted hyper-distributions of effect sizes with fitted standard deviation, while the points are the observed number of species within bins of 0.25, including only species with at least 10 occurrences. Moisture, dry-season moisture; Al, aluminum; P, resin phosphorus; Fe, iron; Ca, calcium; Zn, zinc; K, potassium.

Boron extracted in hot water was correlated negatively with soil pH in water and $CaCl_2$ (e.g. $r = -0.39$ for pH in $CaCl_2$, $P < 0.001$; Fig. 2a), resin P ($r = -0.36$, $P = 0.001$; Fig. 2b) and extractable base cations ($r = -0.42$, $P < 0.001$; Fig. 2c), and correlated positively with soil organic carbon ($r = 0.36$, $P = 0.001$; Fig. 2d). By contrast, Mehlich-III extractable B was correlated positively with pH (e.g. $r = 0.61$ for pH in $CaCl_2$, $P < 0.001$; Fig. 2e), resin P ($r = 0.48$, $P < 0.001$; Fig. 2f), base cations ($r = 0.58$, $P < 0.001$; Fig. 2g) and organic carbon ($r = 0.25$, $P = 0.028$; Fig. 2h). All measures except pH were log-transformed before analysis. Mehlich-III extractable Fe and K were not correlated significantly with hot water B, while Mehlich-III extractable Al and Fe were not correlated significantly with Mehlich-III extractable B (Supporting Information, Table S1).

Although the two measures of plant-available B were not significantly correlated, forward stepwise regression predicted Mehlich-III extractable B from hot-$CaCl_2$ extractable B and soil pH (measured in 0.01 M $CaCl_2$) according to the following model: $[\text{Mehlich B}] = 1.288 \pm 0.265 \times [\text{CaCl}_2\ \text{B}] + 0.272 \pm 0.030 \times [\text{pH}] - 0.912 \pm 0.177$ ($F_{2,76} = 42.7$, $R^2 = 0.53$, $P < 0.001$). Inclusion of organic carbon, Mehlich-III extractable Al or Fe, total exchangeable bases, or resin P did not improve the model ($P > 0.2$).

For BCI, Mehlich-III extractable B concentrations used by Steidinger (2015) were between 0.23 and 2.86 mg B kg^{-1} (John *et al.*, 2007). These measurements were made by ICP–OES using a B line that suffers from interference by Fe (Taber, 2004). Correcting this using an empirical equation derived from 230 soils under lowland tropical forest in Panama (i.e. comparing the difference in Mehlich-III extractable B measured at two lines with and without Fe interference, against Mehlich-III extractable Fe) allowed us to correct the reported B values for BCI (Turner *et al.*, 2016). These corrected Mehlich-III extractable B concentrations for the BCI 50 ha plot ranged between 0.08 and 2.09 mg B kg^{-1} (mean 0.58 ± 0.009 mg B kg^{-1}). The kriged values using the original and revised data were well correlated (Turner *et al.*, 2016), indicating that the previous statistical analyses do not require re-evaluation (John *et al.*, 2007; Steidinger, 2015). As in the cross-Isthmus plots, Mehlich-III extractable B on the BCI 50 ha plot was correlated

positively with pH, base cations (Ca, K, magnesium (Mg)) and micronutrients (manganese, Zn) (for both the original and the corrected data).

Regional and local scale species distributions in relation to soil B

At the regional scale, the inclusion of B had a negligible impact on modeled tree species distributions. Hot-$CaCl_2$ extractable B, widely used as a measure of soil B availability to plants, was not significantly associated with the distribution of any species (Fig. 3). Similarly, Mehlich-B made a near-negligible contribution to the model: there were fewer species with significant responses to Mehlich-B (eight of 271 species with > 10 individuals in plots) than to all nutrients apart from N (Fig. S1). Of the various soil nutrients included in the model, the majority of the species responded significantly to resin P, as in the original model (Condit *et al.*, 2013). Overall, species associated with low P tended to show weak negative responses to Mehlich-B, while species associated with high P tended to show weak positive responses to Mehlich-B. It therefore appears that the few significant Mehlich-B responses are simply byproducts of responses to resin P. Given that none of the species were associated with hot-$CaCl_2$ extractable B, and that this B pool does not correlate strongly with resin P, we conclude that B does not influence regional species distributions.

At the local scale, the distributions of three of the six species that we studied on the BCI 50 ha plot were significantly associated with high concentrations of soil B, while three species showed no significant association (Table S2). However, none of the six species showed a significant positive correlation between foliar B and Mehlich-III extractable soil B across the plot (Table S2), while one species, *G. guidonia*, showed a significant negative correlation. The range in Mehlich-III extractable B varied between 11- and 41-fold for the six species. Across these ranges of soil B, mean foliar B ranged from 0.06 ± 0.01 mg B g^{-1} for *C. argentea* to 0.23 ± 0.11 mg B g^{-1} for *H. triandra* (Table S2).

Growth responses of tropical tree seedlings to B availability

We found little evidence that B influences the growth rates of seedlings of three tropical trees species at anything other than extremely high B concentrations. There was no evidence of B deficiency at extremely low B availability, including in the 0 μM B treatment (i.e. with no B added to the nutrient solution). Indeed, there were no significant differences in any measure of plant performance between the 0 and 12.5 μM B treatments (Table 1), suggesting that even the small concentrations of B present in the sand–soil mixture (with Mehlich-III extractable soil B below the detection limit; 0.01 mg B kg^{-1}), the nutrient solution (as contaminants in the other nutrient preparations) and the small volume of tap water used to replace moisture loss by evaporation provided sufficient B for plant growth. By contrast, there was clear evidence of B toxicity at extremely high B (≥ 1250 μM B), as indicated by visual leaf damage and reduced plant size (Fig. 4), a marked increase in foliar B and a decline in RGR (Table 1).

Table 1 Responses of seedlings of three tropical tree species to variation in boron (B) availability

Boron treatment	Foliar B (μg B g^{-1})	Relative growth rate (RGR, mg g^{-1} d^{-1})	Leaf mass fraction (LMF, g g^{-1})	Net assimilation rate (NAR, g m^2 d^{-1})	Lear area ratio (LAR, cm^2 g^{-1})	Specific leaf area (SLA, cm^2 g^{-1})	Root mass fraction (RMF g g^{-1})
Hura crepitans							
0×	24.3 ± 3.2a	55.3 ± 3.7a	0.27 ± 0.03a	6.3 ± 0.5a	63.7 ± 2.6a	234 ± 25a	0.22 ± 0.02a
1×	17.8 ± 6.3b	54.5 ± 2.6a	0.27 ± 0.03a	6.3 ± 0.4a	62.5 ± 6.1a	234 ± 36a	0.23 ± 0.04a
4×	39.8 ± 8.7c	54.2 ± 2.9a	0.25 ± 0.01a	6.3 ± 0.4a	62.5 ± 3.5a	251 ± 15a	0.25 ± 0.03a
10×	35.8 ± 7.7c	51.9 ± 6.8a	0.28 ± 0.03a	6.0 ± 1.0a	62.0 ± 4.7a	222 ± 24a	0.23 ± 0.03a
40×	102.9 ± 20.5d	52.8 ± 4.2a	0.22 ± 0.02b	6.1 ± 0.5a	61.3 ± 5.0a	276 ± 27b	0.24 ± 0.04a
Ochroma pyramidale							
0×	20.9 ± 4.4a	153.3 ± 8.8a	0.54 ± 0.03a	7.4 ± 0.7a	217.4 ± 13.6a	403 ± 35a	0.33 ± 0.03a
1×	26.3 ± 2.9b	159.0 ± 8.6a	0.57 ± 0.03a	8.1 ± 0.9a	201.9 ± 24.3a	356 ± 52ab	0.29 ± 0.04a
10×	72.3 ± 11.9c	159.4 ± 6.3a	0.57 ± 0.02a	8.4 ± 0.5a	192.5 ± 9.6ab	337 ± 24b	0.30 ± 0.03a
40×	288.0 ± 43.2d	146.6 ± 6.2a	0.60 ± 0.01ab	7.0 ± 0.5ab	218.4 ± 8.6a	366 ± 20ab	0.25 ± 0.02a
100×	487.0 ± 82.3e	122.1 ± 11.4b	0.63 ± 0.03b	6.1 ± 0.7b	206.8 ± 11.6a	326 ± 18b	0.19 ± 0.04b
150×	530.1 ± 168.1f	87.4 ± 15.3c	0.63 ± 0.09b	4.8 ± 1.0c	187.0 ± 31.3b	295 ± 31c	0.18 ± 0.12b
Pentagonia macrophylla							
0×	11.3 ± 10.4a	66.9 ± 3.9a	0.58 ± 0.05a	5.2 ± 0.4a	127.0 ± 14.2a	219 ± 22a	0.31 ± 0.05a
1×	28.5 ± 5.5b	66.5 ± 2.6a	0.53 ± 0.10ab	5.4 ± 1.0a	124.7 ± 28.5a	235 ± 32a	0.38 ± 0.11a
10×	221.8 ± 31.8c	64.0 ± 4.6a	0.63 ± 0.04a	4.8 ± 0.7a	137.1 ± 23.4a	218 ± 27a	0.28 ± 0.04ab
40×	1511.4 ± 955.8d	62.7 ± 5.6a	0.66 ± 0.02a	4.9 ± 0.8a	129.2 ± 21.9a	196 ± 30a	0.22 ± 0.02b
100×	1674.8 ± 482.7d	43.2 ± 11.8b	0.48 ± 0.10b	5.1 ± 1.4a	71.8 ± 12.4b	152 ± 26b	0.29 ± 0.09ab

Values are mean ± SD of at least six replicate plants per treatment. Boron treatments refer to the B concentration (12.5 μM) in the standard nutrient solution (Johnson *et al.*, 1957). Differences among treatments were tested using generalized linear mixed-effects models for each species and response variable separately. All dependent variables were logarithmically transformed before analysis and results were considered statistically significant at $P < 0.05$. Differences among treatments for each species are denoted by characters after the mean values. We coded each seedling as random and B treatment as a fixed effect, and then used a Student's *t* test to determine differences among treatments.

Foliar B in the 12.5 μM B treatment was similar for the three species studied (17.8–28.5 μg B g^{-1}) , suggesting an approximate optimum foliar B concentration for tropical tree seedlings. Compared with these values, foliar B in the 0 μM B treatment was lower than the 12.5 μM B treatment for *O. pyramidale* and *P. macrophylla*, but higher for *H. crepitans*. However, foliar B increased dramatically as solution B increased further, particularly beyond the 125 μM B treatment, reaching 530 μg B g^{-1} in *O. pyramidale* and 1675 μg B g^{-1} in *P. macrophylla*.

For *H. crepitans*, the maximum B treatment (50 μM B) yielded the highest foliar B (103 μg B g^{-1}) and affected LMF and SLA, but did not significantly change RGR, NAR, LAR or RMF (Table 1). For *O. pyramidale* and *P. macrophylla* there were clear signs of leaf chlorosis at ≥ 50 μM B (Fig. 4). In addition to the marked increases in foliar B, there were corresponding reductions in RGR and SLA at ≥ 1250 μM B for *O. pyramidale* and *P. macrophylla* (Table 1). There were also significant changes in LAR, LMF and RMF at high B for both species, but NAR declined in *O. pyramidale* only at the highest B treatment (1875 μM B).

Soil and tree response to long-term B addition

Fifteen years of B addition as part of a micronutrient treatment increased plant-available B in soil significantly ($F_{1,6} = 21.5$, $P = 0.0035$; Fig. S2). In the upper 5 cm of soil, hot-$CaCl_2$ extractable B increased from 0.113 ± 0.021 mg B kg^{-1} in control plots to 0.192 ± 0.024 mg B kg^{-1} in the micronutrient treatment – a significant 70% increase. The greater plant-available B increased fine-root B significantly (control 0.017 ± 0.04 mg B g^{-1}, micronutrient 0.024 ± 0.004 mg B g^{-1}; $F_{1,6} = 6.4$, $P = 0.045$). However, there were no significant changes in B concentrations in wood, leaves or litter fall, although concentrations in the micronutrient treatment were slightly greater in each case. For wood B, neither the micronutrient main effect (control 0.036 ± 0.013 mg B g^{-1}, micronutrient 0.040 ± 0.010 mg B g^{-1}; $F_{1,15} = 0.267$, $P = 0.613$), the species main effect ($F_{2,15} = 2.58$, $P = 0.109$) nor the treatment × species interaction ($F_{2,15} = 0.423$, $P = 0.633$) was significant. For live leaves, neither the micronutrient main effect (control 0.060 ± 0.015 mg B g^{-1}, micronutrient 0.064 ± 0.015 mg B g^{-1}; $F_{1,16} = 0.588$, $P = 0.454$) nor the treatment × species interaction ($F_{2,16} = 1.03$, $P = 0.378$) was significant, but the species main effect was strongly significant ($F_{2,16} = 7.57$, $P = 0.0049$). For litter fall, neither the micronutrient main effect (control 0.064 ± 0.013 mg B g^{-1}, micronutrient 0.068 ± 0.021 mg B g^{-1}; $F_{1,11} = 0.176$, $P = 0.683$) nor the seasonal effect ($F_{1,11} = 0.396$, $P = 0.542$) was significant. There were no significant differences in the mass of litter fall or trunk growth rates in the micronutrient treatment ($P > 0.1$).

Boron budget

A simple B budget for Gigante Peninsula illustrates the likely significance of rainfall in supplying sufficient B for plant growth (Table 2). Assuming marine-influenced rainfall contains 10 μg B l^{-1}, the global marine rainfall concentration (Park & Schlesinger, 2002), then the annual rainfall on Gigante Peninsula (a long-term average of 2600 mm on nearby BCI) provides *c.* 26 mg B m^{-2} yr^{-1}. In comparison, the profile-weighted hot-$CaCl_2$ extractable B concentration in soil on Gigante is 0.062 mg B kg^{-1}, equivalent to 70.0 mg B m^{-2} in the top 1 m of soil. However, the majority of the fine roots in this forest occur in the upper 20 cm of the soil profile (Yavitt & Wright, 2001), where the plant-available B stock is 18.2 mg B m^{-2}. The input of B in rainfall is therefore 37% of the plant-available B pool to 1 m depth in the soil, but *c.* 50% greater than the plant-available B in the main rooting zone. Using the median B concentration in rainfall (6.6 μg B l^{-1}), the B input is of similar magnitude to

Fig. 4 Plant responses to increasing boron (B) concentration for two species, *Ochroma pyramidale* (upper panel) and *Pentagonia macrophylla* (lower panel) receiving an otherwise full nutrient solution (Johnson *et al.*, 1957). The standard solution B concentration was 12.5 μM. Boron concentrations in the nutrient solution increased from the left (no added B) to right (150 times the standard nutrient solution B for *O. pyramidale* or 100 times for *P. macrophylla*). The scale bar in the lower left is 10 cm long.

Table 2 Stocks of boron (B) in rainfall, plant biomass and soil in lowland temperate forest on Gigante Peninsula, part of the Barro Colorado Nature Monument, Panama

Pool	Boron concentration	Total mass	Boron stock[a] (mg B m^{-2})
Rainfall B[b]	10 μg B l^{-1}	2600 mm yr^{-1}	26.0 (17.1)
Total soil B[c]	18.8 ± 9.2 mg B kg^{-1} (profile weighted)	1129 kg m^{-2}	21 225
Soil soluble B[d]	0.062 ± 0.037 mg B kg^{-1} (to 1 m) 0.113 ± 0.025 mg B kg^{-1} (to 0.2 m)	1129 kg m^{-2} (to 1 m) 163 kg m^{-2} (to 0.2 m)	70.0 (to 1 m) 18.4 (to 0.2 m)
Foliar B[e]	*Alseis blackiana* = 0.070 ± 0.011 mg B g^{-1} *Heisteria concinna* = 0.051 ± 0.021 mg B g^{-1} *Tetragastris panamensis* = 0.059 ± 0.008 mg B g^{-1} Mean: 0.060 mg B g^{-1}	1350 g m^{-2}	81.0
Wood B[f]	*Alseis blackiana* = 0.044 ± 0.016 mg B g^{-1} *Heisteria concinna* = 0.034 ± 0.009 mg B g^{-1} *Tetragastris panamensis* = 0.033 ± 0.016 mg B g^{-1} Mean: 0.037 mg B g^{-1}	16 335 g m^{-2}	604.4
Fine root B[g]	0.017 ± 0.004 mg B g^{-1}	470 ± 111 g m^{-2}	8.0
Coarse root B[h]	0.037 mg B g^{-1}	2797 g m^{-2}	103.5
Leaf litter fall[i]	0.059 ± 0.013 mg B g^{-1} (dry season) 0.067 ± 0.014 mg B g^{-1} (wet season	630 g m^{-2} yr^{-1}	38.2
Plant B (total)			796.9

All masses and B stocks are calculated on an area basis (m^{-2}).
[a]Stocks are calculated in mg B m^{-2} of land surface (multiply by 10 to convert to g B ha^{-1}) by multiplying total mass/volume of a pool with its B concentration.
[b]The average marine rainfall B concentration (Park & Schlesinger, 2002). The B input assuming the median concentration in marine rainfall (6.6 μg B l^{-1}) is given in parentheses.
[c]Determined by nitric acid digestion and calculated for the profile to 1 m in four control plots.
[d]Determined by hot-$CaCl_2$ extraction and calculated for the profile to 1 m in four control plots.
[e]Leaf mass calculated from tree diameters based on an allometric equation (Chave *et al.*, 2008) using individuals ≥ 5 cm diameter at breast height (dbh) (the threshold used to develop the allometric relationship) for all species in the plots. Values are species means ± SD for shade leaves of four individual trees in the control plots.
[f]Mean values of wood cores to 10 cm into the trunk for trees in four control plots (n = 4 individuals for each species).
[g]Fine root mass is the mean ± SD of roots < 2 mm diameter to 1 m depth in the soil profile in the four control plots in the fertilization experiment. Fine root B was determined in roots from the 0–5 cm horizon in the four control plots.
[h]Coarse root mass was calculated as 20% of the above-ground biomass (Chave *et al.*, 2015) and corrected for fine root mass. The B concentration was assumed to be the same as wood.
[i]Values are from litter sampled during the dry and wet seasons from traps in each of the 32 plots in the N–P–K factorial nutrient addition experiment. Total litter fall on Gigante is *c.* 1100 g m^{-2}, with *c.* 60% falling as leaves (Wright *et al.*, 2011; Turner *et al.*, 2015). The B stock is calculated on the basis of two-thirds of the total leaf litter falling in the dry season.

the soil soluble B pool in the rooting zone. Total soil B, by contrast, is 21 225 mg B m^{-2} to 1 m depth in the profile, or 300 times greater than the soluble pool.

Foliar B varies markedly among the three species studied on Gigante, with concentrations from 0.051 mg B g^{-1} in *H. concinna* (Erythropalaceae) to 0.070 mg B g^{-1} in the medium-sized tree *A. blackiana* (Rubiaceae) (Dalling *et al.*, 2001), yielding an average concentration of 0.060 mg B g^{-1} for the three tree species. Multiplying this by leaf mass calculated from an allometric equation based on trunk diameter (Chave *et al.*, 2008) yielded 81.0 mg B m^{-2} in leaves – about three times the amount supplied annually in rainfall and of similar magnitude to the soluble B pool in the soil. By contrast, wood of the three species contained on average 0.037 mg B g^{-1}, yielding 604.4 mg B m^{-2} – more than seven times the B in leaves. Assuming that coarse roots (calculated as 20% of above-ground biomass, minus fine roots) contain a similar B concentration to wood, they contribute 103.5 mg B m^{-2}, while fine roots contain an additional 8.0 mg B m^{-2}. Wood therefore contains 76% of the total plant B (i.e. the sum of B in wood, roots and leaves; 796.9 mg B m^{-2}). Total plant B is 27 times smaller than total soil B.

Finally, total litter fall in plots that did not receive P (added P caused an increase in litter fall) across the 2006–2007 annual cycle was 1100 g m^{-2}, of which 630 g m^{-2} was leaf litter (Turner *et al.*, 2015). Leaf litter B varied seasonally, presumably reflecting reduced transpiration and B uptake during the dry season (Table 2). Two-thirds of the annual leaf litter fall occurs in the dry season, yielding a B turnover in annual leaf litter fall of 38.2 mg B m^{-2}. The amount of B in litter fall is therefore *c.* 1.5 times the amount in rainfall, 55% of the amount in the soil soluble pool to 1 m depth in the profile, and twice the amount in the soluble pool in the main rooting zone to 0.2 m depth.

Discussion

We found no evidence that B influences the distribution or performance of tree species in lowland tropical rain forests of Panama. This included evidence from measurements of plant-available B in soil and its influence on regional species distributions, growth experiments with tropical tree seedlings in which B treatments exceeded the range of concentrations found in lowland tropical forests, foliar B along a local scale soil B gradient on

BCI, and stand-level forest experiments that raised plant-available B by 70% with no effect on tissue B, litter production or tree growth.

Soil B concentrations in tropical forests

Measurements of plant-available B by two commonly used procedures in tropical forest soils spanning a range of taxonomic orders, pH and fertility status indicate uniformly low B throughout the region. Soluble soil B concentrations determined by extraction in hot 0.01 M $CaCl_2$, the most widely used measure of plant-available B (Keren, 1996), were < 0.6 mg B kg^{-1} and therefore typical for strongly weathered soils (Shorrocks, 1997). Although diagnosis of B status from soil tests is difficult (Nable *et al.*, 1997), these values are in the range considered to indicate potential B deficiency in agriculture (Gupta *et al.*, 1985; Shorrocks, 1997) and are far below levels at which B toxicity occurs in most crop plants (> 5–10 mg B kg^{-1}) (Nable *et al.*, 1997). None of the soils contained soluble B concentrations in the range considered adequate for crop production (i.e. 1–4 mg B kg^{-1}). However, soils with high clay concentrations, which characterize the majority of the soils in this study (Turner & Engelbrecht, 2011), can sorb B and maintain it in plant-available forms despite acidic conditions (Shorrocks, 1997). This might contribute to the maintenance of B availability in Panama despite low concentrations of plant-available B.

There is little comparable data on soluble B in tropical forests other than BCI, although Mehlich-III extractable B was undetectable in a lowland tropical forest at Yasuni National Park in Ecuador and a montane forest plot at La Planada, Colombia (John *et al.*, 2007). On BCI, Mehlich-III extractable B concentrations (after correction for iron interference) were up to 2 mg B kg^{-1} (Turner *et al.*, 2016), but plant-available B determined by Mehlich-III extraction is greater than by extraction in hot water (Fig. 1). Interpretation of potential toxicity based on Mehlich-III extractable B should therefore be adjusted accordingly, because B sorbed to soil surfaces, some of which is included in the Mehlich-III extraction, does not contribute to B toxicity (Goldberg, 1997). Our measurements of plant-available B in a wide range of soils suggest that B deficiency is possible, but it seems unlikely that B toxicity occurs in lowland tropical forests of Panama.

Regional and local scale species distributions in relation to soil B

At the regional scale, we found no evidence that tree species distributions are influenced by soil B, including both hot 0.01 M $CaCl_2$ and Mehlich-III extractable B. For hot-$CaCl_2$ extractable B, the most widely used measure of soil B availability, none of the species were associated significantly with soil B. A small number of species were related to Mehlich-B, but these relationships were weak and presumably an artifact of the positive correlation between Mehlich-B and resin P, because the latter explains the distribution of *c.* 60% of the tree species in the region (Condit *et al.*, 2013). This further suggests that the apparent influence of B on species distributions at the local scale on BCI (Steidinger, 2015) is an artifact of the strong correlations between B and other nutrients there, particularly base cations (John *et al.*, 2007). Two of the species in our growth experiment, *H. crepitans* and *P. macrophylla*, have significant positive associations with Mehlich-B on BCI, while *O. pyramidale* has a significant negative association with B (J. W. Dalling & R. John, unpublished results). However, based on their responses to experimental B treatments, it seems unlikely that the range of plant-available B concentrations is sufficiently wide to influence the distribution of these species on the plot.

At the local scale, we tested the hypothesis that soil B drives species distributions by determining foliar B in six species that span the gradient in Mehlich-III extractable B on BCI. We assumed that an effect of soil B availability on tree performance would be reflected in correlations between soil B and foliar B (Nable *et al.*, 1997; Shorrocks, 1997). Although foliar B was relatively high for some species, the absence of significant positive correlations between foliar B and soil B for any species, despite up to 41-fold variation in Mehlich-III extractable B, provides additional evidence that B does not drive local scale species distributions in this tropical forest.

It is noticeable that the results of the regional scale Bayesian approach differ markedly from the results of the local scale niche-breadth analysis on the 50 ha plot. The absence of significant species associations with B in the regional scale plot network probably reflects the much larger study area reducing the importance of dispersal limitation, and the much larger range of soil chemical properties reducing the importance of covariance between B and base cations. This means that although the niche-breadth analysis is a valid approach for hypothesis generation, causality must be inferred with caution. For this reason, John *et al.* (2007) gave relatively little emphasis to the large niche breadth for B, relative to other cations, on the 50 ha plot.

Growth responses of tropical tree seedlings to B availability

Strongly weathered soils typically contain low concentrations of plant-available B, yet we found no evidence of B deficiency from growth parameters for three species of tropical trees, despite including a treatment with a solution that did not contain B. Foliar B in the lowest B treatment was in the range considered suitable for crop plants (20–100 μg B g^{-1}) (Shorrocks, 1997; Marschner, 2006) and did not drop below the *c.* 10 μg B g^{-1} threshold below which B deficiency typically occurs (Shorrocks, 1997; Dell *et al.*, 2008). This indicates that tropical tree species in general can tolerate relatively low B availability.

It is possible that B deficiency might manifest in ways other than vegetative growth parameters determined here, by affecting any of the proposed roles for B in plant metabolism, including sugar transport and carbohydrate metabolism, cell wall synthesis and structure, lignification, RNA metabolism, respiration and membrane integrity (Marschner, 2006). Boron deficiency can also inhibit root elongation and influence seed viability, fruit production and P uptake. Although we could not assess seed or fruit

production, the lowest B treatment did not significantly affect foliar P (data not shown) or change the root to shoot ratio. Furthermore, B appears to be involved in the development of N-fixing nodules in at least some legumes (Bolanos *et al.*, 1994), although we did not include any of the many species of tropical Fabaceae in our study.

Boron toxicity was induced only at extremely high B concentrations, as indicated by chlorosis at leaf margins (Nable *et al.*, 1997; Marschner, 2006) that coincided with reductions in growth rates. Foliar B concentrations in all three species in high B treatments exceeded those considered to represent potentially toxic concentrations in crop plants ($> 100\ \mu g\ B\ g^{-1}$) (Nable *et al.*, 1997). Symptoms of toxicity occurred at $\geq 500\ \mu M$ B which, assuming 50% soil moisture, is equivalent to a hot-water extractable B concentration of $2.7\ mg\ B\ kg^{-1}$. This is > 10 times the mean water extractable B in the soils studied here and more than five times the maximum observed concentration (and therefore many more times less than the equivalent Mehlich-III extractable B; Fig. 1).

Although we studied only three of the many hundreds of species present in tropical forests in Panama, the absence of seedling responses to the lowest B treatment and a reduction in growth rates coinciding with the appearance of toxicity symptoms at $\geq 500\ \mu M$ B suggest that tropical trees are unlikely to suffer either deficiency or toxicity of B in nature. These results are similar to those reported for crop plants, in which optimum yields were maintained across a wide range of solution B concentrations from < 1 to > 100 μM B (Chapman *et al.*, 1997). Coupled with the low soluble B concentrations in soils, it seems unlikely that B deficiency and toxicity span a narrow range of B concentrations for tropical trees, nor that plants can accumulate B to toxic levels in lowland tropical forests.

Forest responses to experimental B addition

Fifteen years of B addition to lowland tropical forest on Gigante Peninsula increased plant-available B in soil and fine-root B, but did not significantly change B concentrations in leaves, wood or litter fall. Similarly, there were no significant changes in stem growth rates or litter fall, or in the soil microbial biomass (Turner & Wright, 2014). However, a previous study reported that litter decomposition was greater in the micronutrient treatment (Kaspari *et al.*, 2008). As the micronutrient treatment includes the base cations Ca and Mg, as well as a number of essential plant micronutrients, the role of B in the litter decomposition response is unknown. Although the micronutrient treatment contained a number of different nutrients in addition to B, we interpret the absence of significant changes in the trees in this treatment, despite a 70% increase in plant-available soil B, as additional evidence that B availability does not influence vegetation in the region.

Boron budget for a lowland tropical forest

The global B cycle is driven primarily by a large atmospheric flux derived from sea salt aerosols, with a smaller anthropogenic contribution from combustion of biomass and coal (Park & Schlesinger, 2002; Schlesinger & Vengosh, 2016). For a well-studied site in central Panama, close to BCI, we estimate that atmospheric B inputs are sufficient to sustain the B demand of the forest. A study of a temperate forest revealed a considerable amount of B in throughfall precipitation (Cividini *et al.*, 2010), suggesting that dry deposition represents an important additional B input to the forest. The annual input of B in rainfall, assuming the mean B concentration in marine rainfall, is equal to about one-third of the B in live leaves, about two-thirds of the B in annual litter fall, and exceeds the standing pool of soluble B in the soil rooting zone. Using the median B concentration in rainfall, the B input is of similar magnitude to the soil soluble B pool. We therefore assume that the small soluble B pool is replenished annually by rainfall and litter decomposition. The foliar B pool is dwarfed by B in wood, as is typical for forests worldwide (Lehto *et al.*, 2004), while wood B is many times smaller than the total B in the soil. Indeed, most of the B in the ecosystem is in the total soil B pool, presumably as tourmaline, one of the most common and highly resistant cyclosilicates (Keren, 1996). Together with the results of the micronutrient addition experiment, the B budget indicates that it is unlikely that trees suffer B deficiency at this site. This might not apply to continental locations such as the Amazon basin, where rainfall B inputs are likely to be smaller than in Panama (mean continental rainfall $B = 4.89\ \mu g\ l^{-1}$) (Park & Schlesinger, 2002). However, such regions are typically extremely poor in P and base cations (e.g. in Amazonia; Quesada *et al.*, 2010), which reduces the likelihood that B limitation will occur even with low B inputs in rainfall.

Boron is similar to P in terms of its distribution in the tropical forest soil studied here: soluble concentrations are $< 1\ mg\ kg^{-1}$, while total concentrations are primarily contained in recalcitrant forms that are not directly available to plants (compare Table 2 with values for total and soluble P on Gigante Peninsula) (Turner *et al.*, 2013, 2015). However, comparison of B in rainfall and leaf litter fall provides support for our assertion that B is unlikely to be of importance in structuring the vegetation in lowland tropical forests. While rainfall provides an amount of B of comparable magnitude to the annual B return in litter fall, rainfall P is an order of magnitude less than rainfall B (soluble reactive P in BCI rainfall is typically $\leq 1\ \mu g\ P\ l^{-1}$) and accounts for < 1% of the annual litter fall P ($319\ mg\ P\ m^{-2}$ in the 2006–2007 annual cycle) (Turner *et al.*, 2015). More importantly, live leaves contain *c.* 20 times more P than B, and the return of P in litter fall is approximately an order of magnitude greater than for B. In other words, despite similar availability in the soil, the annual plant requirement for P is considerably greater than for B.

Conclusions

Widespread low concentrations of plant-available B in lowland tropical forest soils and the absence of associations of tropical tree species to soil B at the regional scale provide strong evidence that B availability does not influence the distribution of tropical tree species in Panama. Instead, the significant responses of many tree species to moisture and readily

exchangeable soil P demonstrate that these two resources are the primary drivers of species distributions in tropical forests. The absence of a response of three species of seedlings to extremely low B and of mature forest to long term experimental B addition suggests that rainfall provides sufficient B for optimal growth. We conclude that B availability does not influence the distributions or productivity of tree species in Panama and probably elsewhere in the lowland tropics.

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Author contributions

B.L.T., P-C.Z., K.W. and J.W.D. designed the study. B.L.T., P-C.Z., K.W., R.C., S.J.W. and J.W.D. collected data. B.L.T., P-C.Z., K.W., R.C., S.J.W. and J.W.D. analyzed data. B.L.T. wrote the manuscript with input from all authors.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Histograms of individual species responses to eight environmental factors, including soil boron (B) determined by extraction in Mehlich-III solution.

Fig. S2 Change in plant-available boron determined by hot-$CaCl_2$ extraction in surface soil after 15 yr of micronutrient addition to lowland tropical forest on Gigante Peninsula, Panama.

Table S1 Pearson product-moment correlations between soil properties and plant-available boron concentrations

Table S2 The relationship between foliar boron (B) and estimated Mehlich-III extractable B in soil for six tree species growing on the Barro Colorado Island 50 ha forest dynamics plot in Panama

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