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Interference by Iron in the Determination of Boron by ICP-OES in Mehlich-III Extracts and Total Element Digests of Tropical Forest Soils

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ABSTRACT

Boron detection in soil extracts by inductively-coupled plasma optical-emission spectrometry (ICP-OES) can be influenced by iron interference, particularly in strongly weathered tropical forest soils. Boron concentrations in Mehlich-III extracts of 230 soils under lowland tropical forest in Panama were markedly overestimated at the most sensitive ICP-OES wavelength (249.772 nm) compared to a less sensitive but interference-free wavelength (208.957 nm) due to iron interference. Hot-water extracts contained insufficient iron to interfere in boron detection, but boron quantification in total element digests was affected strongly by iron interference at 249.772 nm. The relationship between boron overestimation and iron was used to correct a database of 300 Mehlich-III extractable boron measurements made at 249.772 nm for soils from a large forest dynamics plot. We recommend that boron measurements in tropical forest soils by ICP-OES should use the less sensitive 208.957 nm wavelength to avoid interference by extracted iron.

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KEYWORDS

Boron; CaCl₂-extraction; ICP-OES; Mehlich-III extraction; total element digestion; tropical forests

Introduction

Boron (B) is an essential micronutrient for plants, being involved in a number of physiological processes (Marschner 2006). Boron is one of the most common micronutrient deficiencies, particularly for plants growing on strongly weathered soils, while toxicity can also occur in some circumstances, notably in semiarid soils (Nable, Bañuelos, and Paull 1997; Shorrocks 1997). Although studied mainly in agriculture, it has been suggested that B is ecologically important in tropical forests, where its availability is correlated with the distribution of tree species at local scale (John et al. 2007; Steidinger 2015).

Boron availability has been assessed conventionally by extraction in hot water or 0.01 M calcium chloride (CaCl₂) (Berger and Troug 1939; Berger and Truog 1944; Jeffrey and McCallum 1988). However, B availability has recently been assessed by Mehlich-III extraction (Redd et al. 2008; Shuman et al. 1992), a procedure applied widely as a multi-element extraction for plant-available nutrients, including phosphorus, base cations (calcium, potassium, magnesium, sodium), and micronutrients (e.g., copper, iron, manganese, zinc) (Mehlich 1984; Sims 1989). Although it is primarily used in temperate agriculture, the procedure has also been employed in ecological studies, including the evaluation of nutrient availability in tropical forests (Andersen, Turner, and Dalling 2010; John et al. 2007; Turner et al. 2013).

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Although there are a number of procedures for the detection of B in soil extracts (Keren 1996), it is now most commonly determined by inductively-coupled plasma optical-emission spectrometry (ICP-OES, Thermo Fisher Scientific, Waltham, MA), which allows multiple elements to be determined simultaneously on the same extract (Redd et al. 2008; Shuman et al. 1992; Taber 2004). In most cases, each element can be determined at a number of wavelengths, which vary in sensitivity. There are four wavelengths available for determination of B by ICP-OES in environmental samples, but the two most sensitive wavelengths suffer from iron (Fe) interference (Sah and Brown 1997). In particular, the most sensitive wavelength of 249.772 nm is subject to interference from the Fe signal at 249.782 nm (Sah and Brown 1997; Taber 2004). This interference is assumed to be negligible for the analysis of water or 0.01 M CaCl₂ extracts, which contain little Fe (Jeffrey and McCallum 1988). However, it is an important limitation for analysis of Mehlich-III extracts, which can contain considerable concentrations of Fe due to the acidic nature of the extraction solution (pH 3). This is likely to be a particular problem for strongly weathered soils, typical of much of the lowland tropics, because they contain high concentrations of acid-soluble Fe oxides. It therefore seems likely that an alternative wavelength should be used to quantify B in Mehlich-III extracts of tropical forest soils. The problem is also likely to apply to other soil analyses in which B is determined by ICP-OES in solutions containing sufficient concentrations of Fe. For example, acid digestions for total elements are typically analyzed by ICP-OES, raising the possibility that total soil B might be routinely overestimated if detection occurs at 249.772 nm.

We assessed the potential interference by Fe in the quantification of B in Mehlich-III extracts, hot-water extracts, and total element digestions of lowland tropical forest soils. First, we determined B by ICP-OES at two wavelengths in Mehlich-III extracts of 230 soils under lowland tropical forest in Panama, and compared the overestimation with the concentrations of Fe in the extracts. Using an equation derived from this analysis, we corrected data for 300 soils used to develop a B availability map for a large forest dynamics plot on Barro Colorado Island (BCI), Panama, where B has been proposed to be of particular importance in structuring tree species distributions. Using the corrected data, we present revised B availability data for the plot for use in future studies. Finally, we quantified hot-water extractable B and total B in soils from long-term nutrient experiments in lowland tropical forest on Gigante Peninsula, close to BCI. By determining B by ICP-OES at wavelengths with and without Fe interference, we estimated the potential overestimation of total B in strongly weathered tropical forest soils.

Methods

Sampling locations

We quantified Mehlich-III extractable B in soils from a network of 80 lowland tropical forest census sites in Panama (Condit et al. 2004, 2013; Engelbrecht et al. 2007; Pyke et al. 2001; Turner and Engelbrecht 2011) and 150 locations associated with long-term (25 year) litter fall measurements on BCI and San Lorenzo National Park, Panama (Harms et al. 2000; Wright and Calderón 2006; Wright et al. 1999, 2008). The forest census sites spanned a strong rainfall gradient (approximately 1800–3200 mm y⁻¹) and a wide range in soil 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). Each soil sample was a composite of either five soil cores (for transects and 40 × 40 m plots), 13 cores (for 1 ha forest dynamics plots), or 25 cores (for larger 6–50 ha plots). Soils from sites associated with long-term litter fall measurements were sampled using a single soil core (6.25 cm diameter × 10 cm depth) taken directly adjacent to each of the litter traps. All soil samples were taken from the surface soil (0–10 cm depth), because this zone integrates the nutrient cycle and contains the majority of the fine roots. Samples were air-dried (10 days at approximately 22 °C), crushed to break up large aggregates, and sieved <2 mm to remove roots and stones.

Mehlich-III extraction and ICP-OES spectrometry

Boron was extracted from soils in Mehlich-III solution (Mehlich 1984). Briefly, air-dried soils (sieved <2 mm) were shaken for 5 min in Mehlich-III solution (0.2 N acetic acid, 0.25 M ammonium nitrate (NH₄NO₃), 15 mM ammonium fluoride (NH₄ F), 13 mM nitric acid (HNO₃), and 1 mM ethylenediaminetetraacetate (EDTA) in a 1:10 soil to solution ratio. The samples were centrifuged (8000 × g, 10 min) and the supernatant decanted. Boron and Fe were determined in the extracts by ICP-OES (Optima 7300DV, Perkin Elmer Inc., Shelton, CT, USA). All measurements were performed at two wavelengths: 249.772 and 208.957 nm. The former wavelength is most sensitive (greater signal intensity at a given concentration) but is subject to interference from Fe, while the latter wavelength is less sensitive but does not suffer from Fe interference (Taber 2004). All extractions and preparation of standards were conducted in plastic to avoid B contamination from borosilicate glassware.

Correction of boron concentrations on the Barro Colorado Island 50 ha forest dynamics plot

Boron was determined previously on the 50 ha forest dynamics plot on BCI by Mehlich-III extraction and ICP-OES using the 249.772 nm wavelength (John et al. 2007). Using these data, John et al. (2007) found that B availability was significantly associated with the spatial distribution of a large number of tree species, while Steidinger (2015) used niche breadth analysis to further suggest that B might be the most important nutrient structuring tree species distributions in this tropical forest. Using the relationship between Mehlich-III extractable Fe and the difference between the two measures of Mehlich-III extractable B for the 230 soils, we calculated corrected values for B in the 300 analyses of BCI soils from the original John et al. (2007) dataset. The corrected values were calculated using the following equation:

[Corrected Mehlich-B] = [Mehlich-B measured at 249.772 nm] – [Mehlich-Fe] × 0.00256, where Mehlich-B is in mg B kg⁻¹, Mehlich-Fe is in mg Fe kg⁻¹, and 0.00256 is the slope of the regression model between Fe and the difference between B determined at 249.772 and 208.957 nm (see below). Using these corrected data, we used kriging as described previously (John et al. 2007) to re-estimate B concentrations at the 20 × 20 m quadrat scale across the 50 ha forest dynamics plot.

Total soil boron

We measured total B in soils from all 15 plots in a long-term litter manipulation experiment in lowland tropical forest on Gigante Peninsula, part of the Barro Colorado Natural Monument, Panama. The experiment is described in detail elsewhere (Sayer et al. 2012; Vincent, Turner, and Tanner 2010). Soils are Oxisols developed on Miocene basalt. Briefly, we sampled soil to 1 m in five increments (0–5, 5–10, 10–20, 20–50, and 50–100 cm) at four locations in each of the 15 plots in the experiment. Soils were air-dried and sieved as described above, and then ground to a fine powder in a ball mill. Samples (100 mg) were digested in 2 mL of concentrated nitric acid for 6 h under pressure at 180 °C in PTFE vessels (PDS-6 Pressure Digestion System, Loftfields Analytical Solutions, Neu-Eichenberg, Germany). The digests were diluted to 50 mL with deionized water and B and Fe were determined by ICP-OES as described above.

Hot 0.01 M CaCl₂ extraction of plant-available boron

We quantified plant-available B by extraction in hot 0.01 M CaCl₂ (Jeffrey and McCallum 1988) in surface soils (0–5 cm) from 36 plots in a long-term nutrient addition experiment on Gigante Peninsula, Panama. This procedure is equivalent to hot-water extractable B (Berger and Troug 1939; Berger and Truog 1944), but the inclusion of CaCl₂ eliminates problems from silicate clays. The fertilization experiment is described in detail elsewhere (Turner et al. 2013; Wright et al. 2011;

Yavitt et al. 2011). Soils were air-dried and sieved (<2 mm) prior to extraction. Soils were mixed with 0.01 M CaCl₂ 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 decanted. Boron and Fe were determined in the extracts by ICP-OES as described above (Optima 7300DV, Perkin Elmer Inc., Shelton, CT, USA).

Results

Comparison of Mehlich-III extractable boron determined at two wavelengths

Mehlich-III extractable B concentrations in soils from the 80 forest census plots, plus an additional 150 sites on BCI and San Lorenzo National Park (i.e., a total of 230 sites), were determined at both 249.772 nm and a slightly less sensitive line at 208.957 nm that is not influenced by Fe. The two measures of B were strongly correlated (Pearson Product-Moment Correlation, $r = 0.845$, $p < 0.0001$) (Figure 1a). However, concentrations determined at 249.772 nm were much greater, indicating a considerable overestimation of extractable B. The relationship was described by the equation: [Mehlich-B at 208.957 nm] = $0.866 \pm 0.036 \times$ [Mehlich-B at 249.772 nm] - 0.260 ± 0.033 ($n = 230$, $R^2 = 0.72$, $p < 0.001$).

The difference in B concentrations measured at the two wavelengths was correlated strongly with the Mehlich-III extractable Fe concentration in the extracts (Figure 1b), confirming the influence of Fe on B concentrations at 249.772 nm. We calculated regression models with a calculated intercept ($Y = 0.00221x + 0.0617$; $R^2 = 0.95$, $p < 0.001$) and with the intercept passing through the origin ($Y = 0.00256x$; $R^2 = 0.98$, $p < 0.001$). As extracts containing zero Fe should yield zero B interference, we used the latter relationship to re-calculate Mehlich-III extractable B concentrations in the BCI dataset (see below).

Correction of boron concentrations on Barro Colorado Island

Mehlich-III extractable B concentrations of kriged data reported by John et al. (2007) and used subsequently by Steidinger (2015) were reported to be between 0.23 and 2.86 mg B kg⁻¹ (mean 0.96 ± 0.013 mg B kg⁻¹).

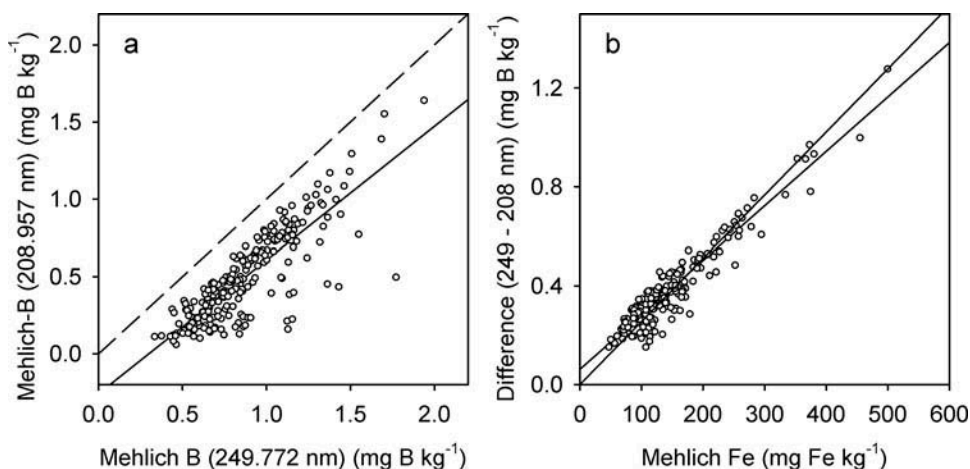


Figure 1. (a) Mehlich-III extractable boron (B) in soils from 230 sites under lowland tropical rainforest in Panama determined by inductively-coupled plasma optical-emission spectrometry at two wavelengths (208.957 and 249.772 nm). The 1:1 line is shown as a dashed line, demonstrating the markedly greater values measured at 249.772 nm. The relationship is described by the equation: $Y = 0.866 \pm 0.036 x - 0.260 \pm 0.033$ ($n = 230$, $R^2 = 0.72$, $p < 0.001$). (b) The difference between Mehlich-III extractable B determined at the two wavelengths plotted against Mehlich-III extractable iron (Fe), showing the effect of Fe interference in B determination at 249.772 nm. The two lines are the regression models with a calculated intercept ($Y = 0.00221 \pm 0.00005 x + 0.0617 \pm 0.0008$; $R^2 = 0.90$, $p < 0.001$) and with the intercept passing through the origin ($Y = 0.00256 x$; $R^2 = 0.98$, $p < 0.001$).

These measurements were made by ICP-OES at 249.772 nm, and were therefore affected by Fe interference. Using the empirical relationship between Mehlich-Fe and the overestimation of Mehlich-III extractable B at 249.772 nm described above from our analysis of 230 soils under lowland tropical forests in Panama (Figure 1b), we corrected the original values for the BCI 50 ha plot by subtracting the apparent B caused by Fe interference. Kriging these corrected values yielded Mehlich-III extractable B for the BCI 50 ha plot 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 (Figure 2) and the maps of B availability across the plot were similar (Figure 3). The original and corrected data are in an online supplementary file.

Total soil boron

Total B determined by nitric acid digestion and ICP-OES detection at 208.957 nm in soils to 1 m depth in a litter manipulation experiment on Gigante Peninsula varied between the detection limit (4.5 mg B kg⁻¹) and 27.8 mg B kg⁻¹ (mean 9.4 mg B kg⁻¹). In contrast, total B determined in the digests at 249.772 nm was between 198.6 and 325.5 mg B kg⁻¹ (mean 288.0 mg B kg⁻¹), approximately 30-fold greater than values determined at 208.957 nm (Figure 4a). The two measures were not correlated significantly.

The difference in total B concentrations measured at the two wavelengths (184–320 mg B kg⁻¹) was correlated strongly with the total Fe concentration in the digests (Figure 4b), further confirming the influence of Fe on B concentrations at 249.772 nm. As for Mehlich-III extractable B, we calculated regression models with a calculated intercept ($Y = 0.00234 \pm 0.00001 x + 2.34 \pm 1.123$; $n = 75$, $R^2 = 0.88$, $p < 0.001$) and with the intercept passing through the origin ($Y = 0.00256 x$; $n = 75$, $R^2 = 1.00$, $p < 0.001$). For both relationships, the regression models are virtually identical to those derived for Mehlich-III extractable B. For regression through the origin, the slope for total B is identical to the slope for Mehlich B (0.00256).

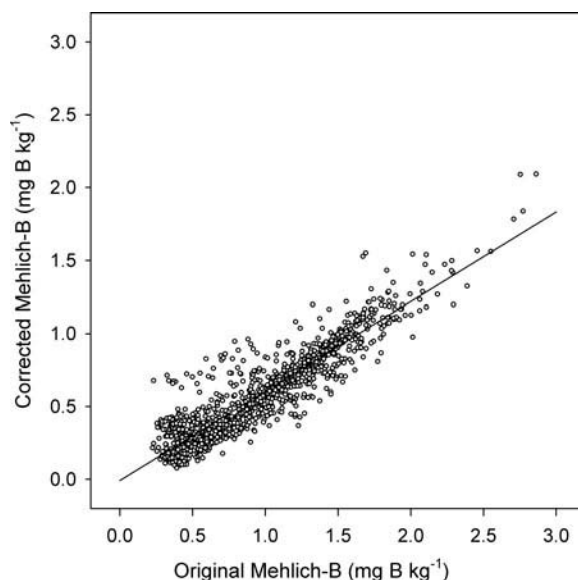


Figure 2. Relationship between the original Mehlich-III extractable boron (B) concentrations determined by inductively-coupled plasma optical-emission spectrometry at 249.772 nm for the Barro Colorado Island large forest dynamics plot and the corrected concentrations accounting for iron interference. The relationship is described by the equation: $Y = 0.613 \pm 0.008 x - 0.008 \pm 0.008$; $n = 1250$, $R^2 = 0.84$, $p < 0.0001$. The values are from Kriged estimates at the 20 × 20 quadrat scale.

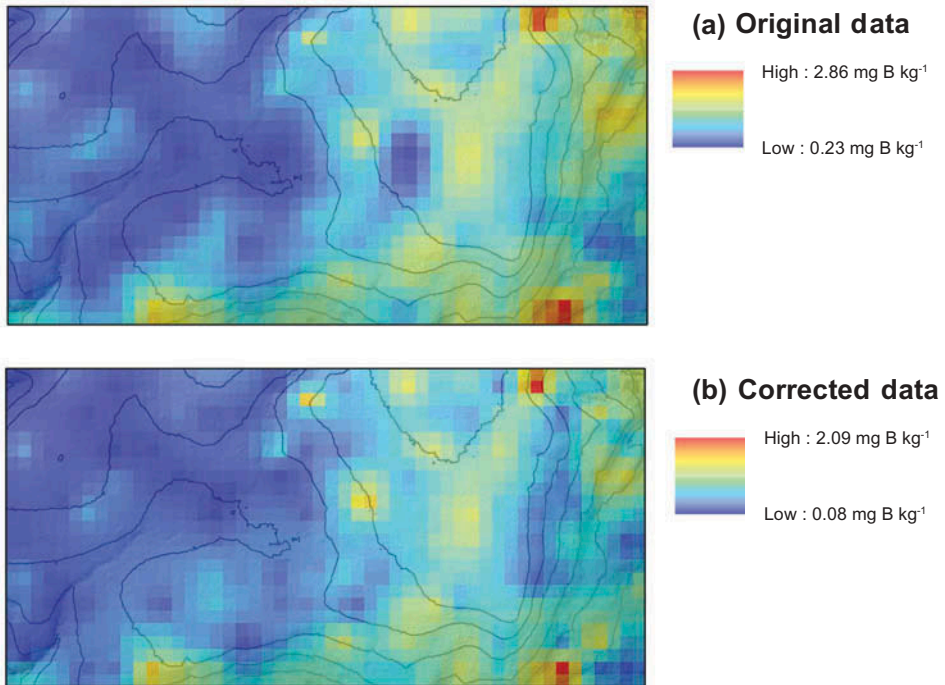


Figure 3. Maps of boron (B) availability as determined by Mehlich-III extraction plotted using (a) the original data determined by inductively-coupled plasma optical-emission spectrometry at 249.772 nm (i.e., including interference by iron) and (b) the revised data corrected for iron interference. The maps also show topographic contours at 5 m intervals.

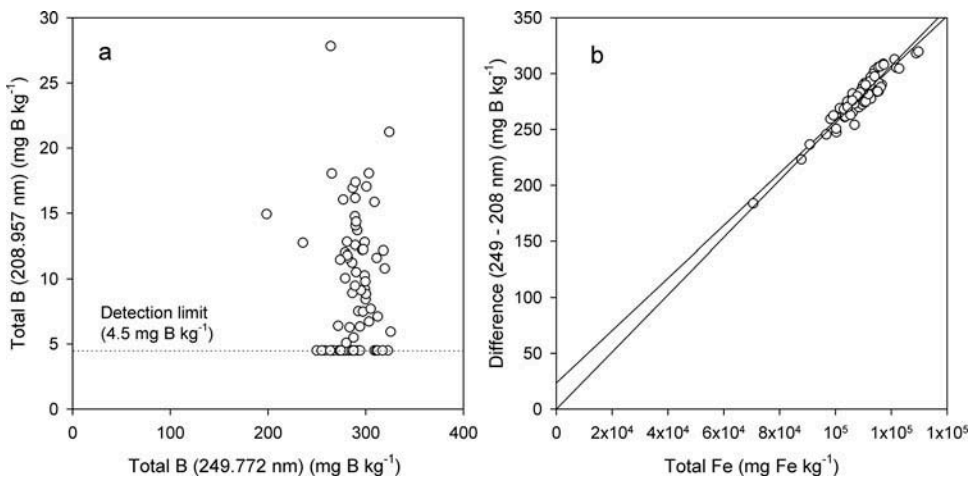


Figure 4. (a) Total boron (B) in soils in five depth increments to 1 m in 15 plots in a litter manipulation experiment under lowland tropical rainforest in Panama, determined by inductively-coupled plasma optical-emission spectrometry at two wavelengths (208.957 and 249.772 nm). The dashed line shows the detection limit for measurement at 208.957 nm (4.5 mg B kg⁻¹). The plot demonstrates the markedly greater B values measured at 249.772 nm. (b) The difference between total B determined at the two wavelengths plotted against total iron (Fe), showing the effect of Fe interference in B determination at 249.772 nm. The two lines are the regression models with a calculated intercept ($Y = 0.00234 \pm 0.00001 x + 2.34 \pm 1.123$; $n = 75$, $R^2 = 0.88$, $p < 0.001$) and with the intercept passing through the origin ($Y = 0.00256 x$; $n = 75$, $R^2 = 1.00$, $p < 0.001$). As zero Fe should yield zero interference, the latter relationship is considered to be the most accurate.

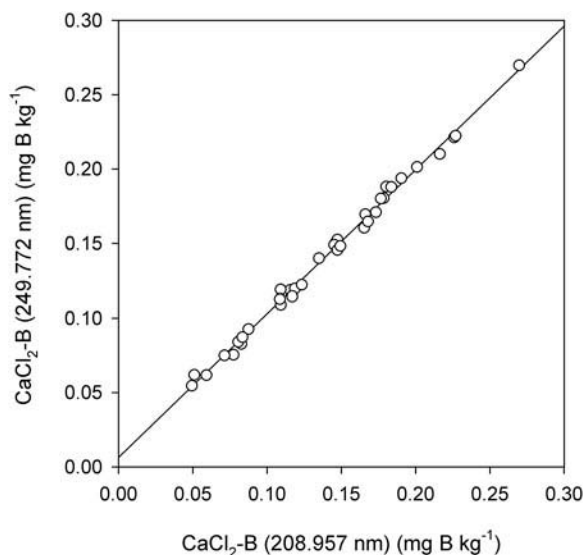


Figure 5. Plant-available boron (B) in soils from 36 plots in a nutrient addition experiment in lowland tropical rainforest in Panama, extracted in hot 0.01 M CaCl₂ and determined by inductively-coupled plasma optical-emission spectrometry at two wavelengths that are susceptible to iron interference (249.773, Y-axis) and that do not suffer from iron interference (208.957 nm, X-axis). The relationship was described by the equation: [CaCl₂-B at 249.772 nm] = 0.966 ± 0.012 × [CaCl₂-B at 208.957 nm] + 0.006 ± 0.002; *n* = 36, *R*² = 0.995, *p* < 0.001).

Hot 0.01 M CaCl₂ extraction of plant-available boron

Measurements of plant-available B in hot 0.01 M CaCl₂ extracts with detection by ICP-OES spectrometry at two B wavelengths were similar and strongly correlated (Figure 5). There was therefore no evidence of Fe interference. The relationship was described by the equation: [CaCl₂-B at 249.772 nm] = 0.966 ± 0.012 × [CaCl₂-B at 208.957 nm] + 0.006 ± 0.002; *n* = 36, *R*² = 0.995, *p* < 0.001). Iron concentrations in the extract were <0.5 mg Fe kg⁻¹, too low to cause interference in B measurement at 249.772 nm. For example, 0.5 mg Fe kg⁻¹ would produce a B signal of 0.001 mg B kg⁻¹, below the detection limit for B by this procedure.

Discussion

Determination of B in Mehlich-III extracts using the most sensitive wavelength (249.772 nm) markedly overestimated extractable B due to interference by Fe in the extracts. The same overestimation occurred for total B in acid digests, and appears to be a particular problem for strongly weathered tropical forest soils that contain high concentrations of extractable and total Fe. The second most sensitive B wavelength (249.677 nm) also suffers from Fe interference, although this can be corrected using a side line indexing procedure (Li-qiang and Zhu 1986). However, Fe interference did not affect B determination in hot CaCl₂ extracts, because Fe concentrations were too low to cause a detectable change in the B signal by ICP-OES.

Several previous studies have used the 249.772 nm wavelength for soil B in Mehlich-III extracts, or did not report the ICP wavelength used, suggesting that interpretation might have been influenced by overestimation of Mehlich-III extractable B. For example, Redd et al. (2008) concluded that Mehlich-III was unsuitable as an index of plant-available B because it yielded the weakest relationship to crop yield of any of the extractants tested. However, the ICP wavelength was not reported, raising the possibility that the results were influenced by overestimation of Mehlich-III extractable B due to Fe interference. In contrast, Taber (2004) quantified Mehlich-III extractable B using a wavelength that did

not suffer from Fe interference, and found that Mehlich-B correlated strongly with tissue B concentrations in three crop plants.

For both Mehlich-III extracts and total element digests, the regression models for Fe against the difference in B measured at the two ICP wavelengths were virtually identical, indicating that subtracting a B concentration equivalent to $0.00256 \times \text{Fe}$ can be used to correct previous analyses of soil B determined at 249.772 nm in any soil extracts or digests. This of course requires that Fe was also measured in the sample, but routine multi-element detection in Mehlich-III extracts and soil digests suggests that Fe concentrations will be available in most cases. As an example, we applied this correction to a data set of Mehlich-III extractable B in a long-term forest dynamics plot in Panama (John et al. 2007). The correlation between the original and corrected B concentrations, and the similarity between the original and revised maps, indicate that the original analyses (John et al. 2007; Steidinger 2015) are likely to remain unaffected by correction of the B data. However, B concentrations in the corrected data set are much lower than the original values, indicating that the possibility of B toxicity in this forest, postulated by Steidinger (2015), is unlikely.

Conclusions

Determination of B availability using Mehlich-III extraction and total B by acid digestion in tropical forest soils should be performed by ICP-OES detection at 208.957 nm to avoid interference from Fe. Previous measurements of B that used the most sensitive B wavelength of 249.772 nm can be corrected for Fe interference using a universal equation. Plant-available B extracted in water or CaCl_2 can be determined at the most sensitive wavelength (249.772 nm), because the extracts do not contain sufficient Fe to cause a detectable increase in the B concentration.

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