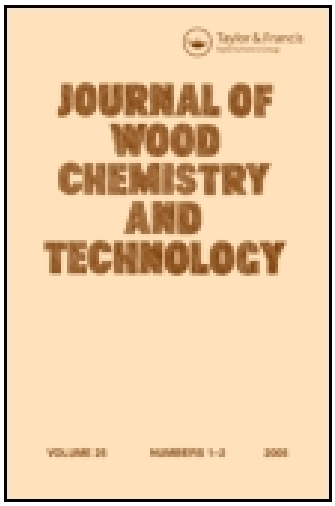

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An In Situ Method to Measure and Map Bark pH

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AN *IN SITU* METHOD TO MEASURE AND MAP BARK PH

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Bark pH is an essential parameter which partly governs the chemistry of the bark as well as its suitability as a microhabitat to a wide range of epiphytic organisms. Bark pH is known to vary with tree species, epiphytic cover, stemflow channelization, and anthropogenic influences. To date, reliable methods to quantify the spatial and temporal dimensions of bark pH have remained elusive. The aim of the present study was to develop and validate an *in situ* method to measure the surface pH of bark with high spatial resolution. Agar-agar panels, prepared with a pH indicator, were used to quantify and map the spatial variation of bark pH for cacao trees in Indonesia. Fine-scale changes of bark pH were clearly detectable and quantifiable with our bark pH mapping method. Bark pH was found to vary as a function of bark microrelief and the presence of epiphytes. The use of pH reference panels validated the bark pH measurements obtained from our method. The bark pH measurement method developed, described, and validated in this article is inexpensive and straightforward. It has the potential for wide adoption by scientists across disciplines who are interested in bark pH and its effect on life in the cortisphere. Unlike conventional methods to measure bark pH in deionized water or KCl extracts, our method is able to identify fine-scale spatial changes in bark pH that are relevant for the colonization of bark by organisms.

KEYWORDS. Cortisphere, epiphyte, bark microrelief, bark chemistry, stemflow, cacao

INTRODUCTION

Bark of woody plants, also known as the cortisphere,[1] is a complex surface which provides habitat to a variety of epiphytes and microbes.[2–6] A suite of internal and external factors, such as species-specific bark chemistry and atmospheric deposition, create varying microhabitats for organisms inhabiting tree bark.[5,7–8] Parameters which affect the spatial heterogeneity of corticolous organisms populating bark include availability of nutrients, moisture conditions, stemflow, and pH.[3,9–15] The importance of bark pH was recognized by Barkman,[16] who postulated that the influence of bark pH to the number of species on the bark surface depended on its buffering capacity. Based on stemflow pH measurements, bark surface pH does seem to vary for individual trees over time and with respect to their position in the forest edge or interior.[17] Hauck et al.[18] found that even a small increase in bark pH can lead to substantial dieback of lichens;

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Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/lwct.

specifically, *Lecanora conizaeoides*, a common lichen species in Europe. Thus, it is critical to have the capability to accurately and precisely measure and map bark pH directly on tree surfaces.

To date, reliable methods to quantify the spatial and temporal dimensions of bark pH have remained elusive. Consequently, neither the internal nor external factors driving bark pH patterns are adequately understood. Farmer et al.[10] reviewed some different methods to measure bark pH. Most methods involved grinding bark pieces into a powder and subsequently soaking them in deionized water or potassium chloride (KCl) solutions and measuring the pH of the solution. The only *in situ* method presented was the use of micro pH-electrodes. The latter is a very challenging method as it is nearly impossible to establish close contact to rough bark surfaces and only point measurements are retrieved. Thus, there is an urgent need for a method that is capable of quantifying variable bark pH levels with fine-scale spatial resolution over a long time span to better explain bark-organism interactions.[8] With such a method, bark pH could be causally linked to other bark properties (e.g., water content, nutrient status, microrelief), stemflow channelization on the tree bole, and the distribution of epiphytic lichens, bryophytes, and microbes. High-resolution maps of tree bark pH would, therefore, likely elucidate the complex interactions among bark pH, stemflow, life in the cortisphere, and corticular pathology.

The main objective of this study was to develop, test, and validate an *in situ* field method for direct and rapid measurement of the pH of the tree bark surface with high spatial resolution. As such, we present our method to measure bark pH for cacao trees (*Theobroma cacao* L.) in Indonesia. Because of the straightforward nature of our method to acquire precise and accurate measurements of bark pH, researchers interested in the hydrochemistry and biology of the cortisphere should be able to easily employ our technique to link bark pH, a key control for bark-inhabiting organisms,[19] with a suite of processes operating in the cortisphere.

STUDY AREA

The study site was a cacao plantation in central Sulawesi, Indonesia (1° 30′ S, 120° 02′ E) at an elevation of 792 m above sea level near the village of Toro and the Lore Lindu National Park. The climate of the area is characterized by a monsoonal precipitation dynamic, impacted by both the Intertropical Convergence Zone and El Niño Southern Oscillation, with total mean annual rainfall of approximately 2,500 mm at 641 m above sea level.[20] Mean annual air temperature is 24.5°C. Relative humidity averages 88.5%. The local area has experienced high rates of land cover change as native lowland and submontane rainforest has been converted to cacao agroforestry. Mean dbh (diameter at breast height, 1.37 m) of test trees in the cacao plantation was 9.6 cm with tree height ranging from 3.40 m to 4.55 m.[20] The cacao trees ranged from 4–10 years in age with a stand density of 1,425 trees ha^{-1}.[20] Epiphytic moss and corticolous lichen species were present in some locations of the bole of the cacao trees sampled, which provided us with the opportunity to demonstrate the ability and efficacy of our method to quantify pH of bark surfaces with and without epiphyte coverage. Our sampling efforts in the cacao plantation in central Sulawesi (Indonesia) were part of the STORMA (Stability of Rainforest Margins) project.

MATERIALS AND METHODS

Overview

The determination of bark pH essentially involves three steps (Figure 1). First, agar-agar panels are blended with a pH indicator to have unbiased contact with the bark surface. Next, the agar-agar panels are affixed to tree trunks to record bark pH. Different colors of the pH indicator in the agar-agar panels represent differences in bark pH. Lastly, high-resolution spatial images of bark pH are obtained by scanning and analyzing differences in the color of the agar-agar panels via an image processing and analysis program. It is important to note that our bark pH measurement and mapping method

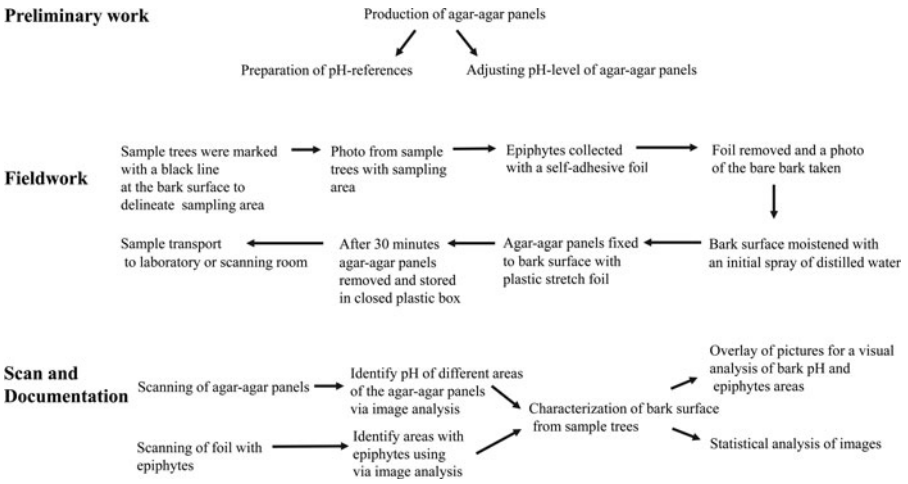

FIGURE 1. Step-by-step methodology for the determination of bark pH.

provides an approximation of bark pH, rather than an absolute pH, since the net balance between H^+ and OH^- ultimately determines pH. Our methodology for the field measurement of bark pH is quick and efficient, totaling 2.5 hours (two hours for the preparation of the agar-agar panels and 30 minutes for *in situ* measurement on an individual tree). Following digital image analysis, our method renders the spatial heterogeneity of bark pH with a suitable accuracy and precision to be useful for ecological research.

For comparison of our method with more conventional methods to measure bark pH, we cross-checked our results with those obtained from a bark wetting experiment. Bark samples (each piece approximately 2 × 2 cm) were harvested from cocoa trees. Plain bark samples, as well as those with mosses, lichens, and with mosses removed, were taken in four replicates each. Bark samples were soaked in deionized water or KCl extracts for 120 minutes with the bark side immersed in the solution to avoid any influence of the xylem water on the extract solution. Bark pH was then measured with a standard pH probe (WTW LF 330) after 5, 30, 60, and 120 minutes. In addition to the comparison of our method with more conventional techniques, we calibrated and validated our bark pH measurement and mapping method via a reference panel of known pH as explained in the Bark Scanning and Image Analysis subsection of Materials and Methods.

Preparation of the Agar-Agar Medium

Prior work has employed agar-agar panels to determine pH changes in response to root exudation.[21,22] For our study, we modified the agar-agar panels to enable the measurement of tree bark pH. Agar-agar panels (6.4 cm × 16.3 cm) with a blended pH indicator were produced. In a solution with 300 ml of distilled water, 3.75 g of agar-agar was suspended via stirring and boiled; while boiling, 3 mL of the pH indicator was added to prepare a solution with a 500 ml volume. Three separate batches with pH 2, 7, and 9 (adding 1 molar H_2SO_4 or NaOH) were prepared. To produce the pH indicator, 1 g bromocresol purple was added to 80 ml of distilled water. For the basic solution, 1 molar NaOH (the pH was kept < 9) was added via constant stirring until the bromocresol purple was completely dissolved. The pH indicator was then fixed at 6 with the addition of 1 molar H_2SO_4. The agar-agar panels (with initial pH sets of 2, 7, and 9) were manufactured from the hot agar-agar medium, in a plastic form (6.4 cm × 16.3 cm), and cooled to room temperature. We prepared panels with different pH starting points for testing on the bark surface.

As a reference and validation for the pH analysis of the agar-agar panels, smaller pieces of agar-agar with a pH of 2, 4, 7, and 9 (to fix buffer solutions) were produced. The agar-agar at pH 2 shows a lemon yellow color and

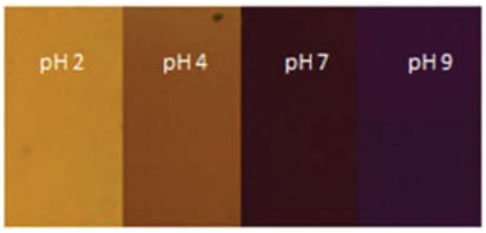

FIGURE 2. pH reference panels for the determination and validation of the pH measurements by the agar-agar panels.

changes to red-brown at pH 4, dark red at pH 7, and to a dark blue at pH 9 (Figure 2).

Determination of Bark pH

The boles of the selected cacao trees had a patchwork of epiphytic-covered and epiphytic-free areas of the bark surface. To quantify bark pH, the following sampling protocol was implemented: (1) using a template, the sample trees were marked at a height of 1 m with a black line at the bark surface with an area of 6.4 cm × 16.3 cm (the same area as the agar-agar panels); (2) any mosses and lichens occupying the area of the bark surface to be sampled were carefully transferred and fixed as originally situated on the sample tree on a sheet of self-adhesive plastic foil (with the area of 6.4 cm × 16.3 cm), which was brushed onto the bark surface; (3) the bark surface was moistened with an initial spray of distilled water (pH 7) to counterbalance any antecedent bark moisture; and (4) the agar-agar panels were fixed at the bark surface with stretch foil. The agar-agar panel was pressed into bark furrows and irregularities. After 30 minutes had elapsed, the agar-agar panels were removed and photographed. To ascertain whether the initial pH of the agar-agar panels influenced actual measurements of bark pH, agar-agar panels with an initial pH 9 (four panels) and 2 (eight panels), respectively, were prepared. This wide pH range allowed us to test the pH range on tree bark. To prevent a "mirror effect" from the surface of the agar-agar panels and ensure sample robustness for the image scanning and processing procedures, the agar-agar panels were transported to the laboratory in closed, airtight plastic boxes (Figure 1).

Bark Scanning and Image Analysis

The agar-agar panels and the self-adhesive plastic foil with mosses and lichens were scanned with a BenQ color scanner (Model S2W 4300U). Photoshop Cs was then used to orthorectify the scanned bark images to correct for distortion and to permit image overlays and subsequent image analyses. It is important to note that all samples taken from the bark surface were carefully kept in their original position as they were transferred from the tree bole to the foil to ensure accurate analysis of bark-covered areas in the image analyses.

Image overlays permitted the identification of locations where mosses and lichens were present and differences in pH levels within those locations. Areas of the bark with a differing pH without epiphytes were also detectable through the image analysis. Because variations in bark morphology could exert a detectable effect on bark pH, we developed a nominal classification for bark microrelief. The bark microrelief classification scheme had values ranging from 0 to 3, whereby a value of 0 represented a plain smooth bark surface without distortions, 1 a rough bark surface without distortions, and 2 a rough bark surface with distortions. Given the 30-minute duration while the agar-agar panels were affixed to the sample trees, it is likely that few ions diffused into the agar-agar panels to interfere with the image analysis.

To produce data for statistical comparison among locations of the bark surface with differing epiphytic coverage, pH level, or bark morphology, the sampled area of the bark surface (6.4 cm × 16.3 cm) was divided into 50 cells by overlaying a digital grid in Arc View. Each individual cell, or subpicture, had an edge length of 1.28 cm to 1.63 cm. Each cell was referenced by a unique label to enable the location of individual features of interest. Determination of the percentage of area within each cell covered by epiphytes and with different pH values permitted the statistical comparison among cells with different features and proportions of epiphytic coverage. For each subpicture, the grey value of the cell was split, the overall number

of pixels (total area of the subpicture) counted, and then the grey value of the colors between 0 and 256 were analyzed. Reference grey values of epiphytes and pH levels allow an identification of areas with moss or lichen (values for green color) coverage and for different pH levels (referencing agar-agar panels). For the calculation of area proportions, the counted pixels of each pH level were related to the total pixel and multiplied by 100. All image analyses were done with the computer programs Photoshop CS, Arc View, Microsoft Office, and Image J.

For determination and validation of the pH measurements of the agar-agar panels, the pH reference panels (with respective pH levels of 2, 4, 7, and 9; Figure 2) were scanned and typical signal peaks were detected with typical grey values and typical color range (Table 1) for each pH level defined to permit accurate interpretation of the agar-agar panels from the sample trees. These calibration procedures demonstrated that the best results were found for color ribbon 2 with clear peaks for the lower pH levels of 2 and 4. The peaks for pH 7 and pH 9 overlap (shown in Figure 3, grey values under 30). As such, a clear differentiation between pH values of 7 and 9 was not possible, although experimental observations indicate that pH was seldom > 9; accordingly, all color pixels over the range of pH 7 are defined as pH 7 and higher. The pH levels between the reference panels were fixed by the color range between the peaks.

TABLE 1. Color value for the different pH steps

pH step	9	7	7-4	4	7-2	2
Color range	6–23	4–13	14–54	55–79	80–155	156–168

To assess the accuracy of area estimation of the bark surface covered by lichens and moss from the image analysis routine, scanned subpictures with epiphytes were printed out. The weight of the whole picture was then taken, the lichen and mosses cut out, and the weight of the excised parts were input in relation to the total picture. Weighting differences between the total area and the area colonized by epiphytes were used to calculate the percent of area covered by mosses and lichen in the subpicture. The results indicate that the image analysis accurately measured the actual bark surface area

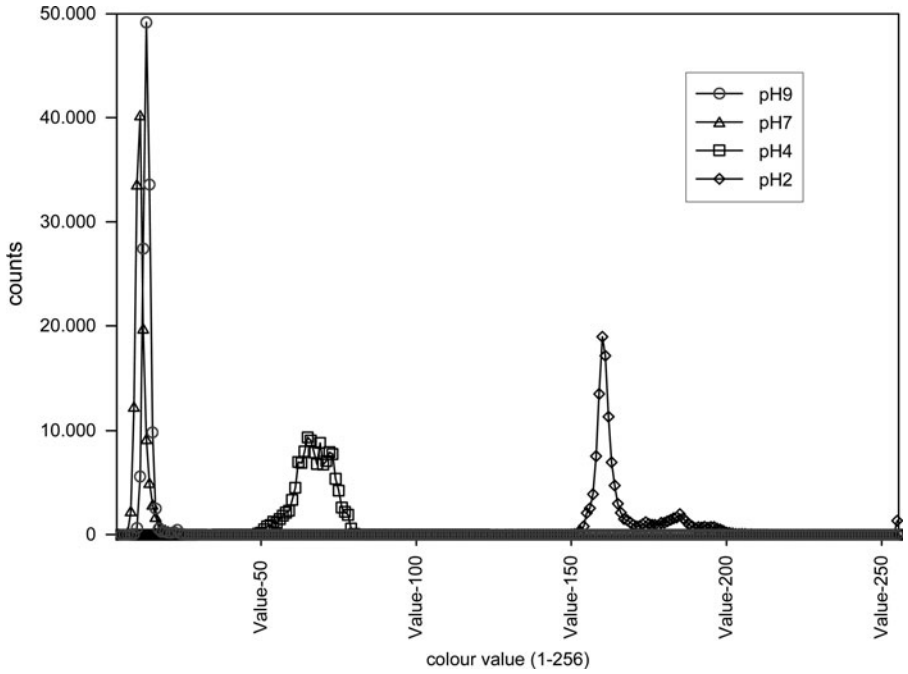

FIGURE 3. Histogram of the typical pH peaks from the reference panels at color belt 2 in the color range 0–256.

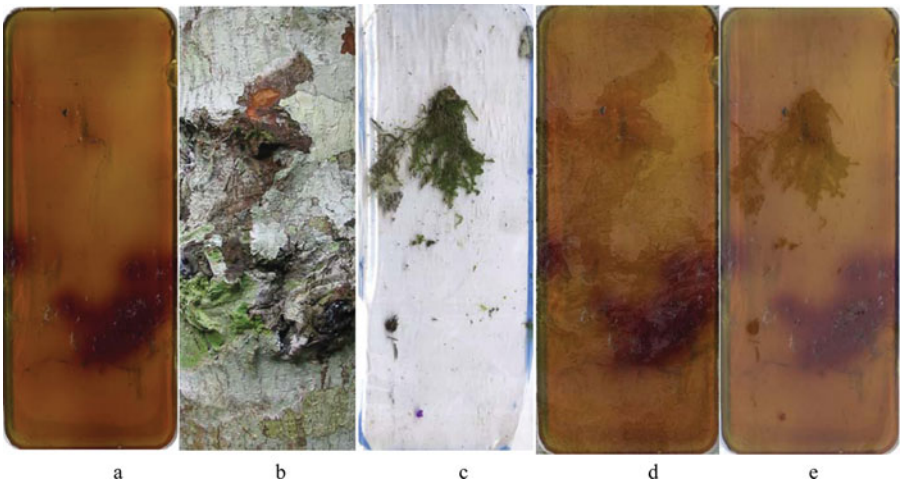

FIGURE 4. (a) An agar-agar panel of cacao tree T21; (b) the bark surface of cacao tree T21; (c) moss-covered area of cacao tree T21; (d) image overlay of the bark and agar-agar panel (a and b) depicting areas of variable pH; and (e) image overlay of the moss vegetation and the agar-agar panel (a and c) illustrating areas of different pH.

covered by epiphytes with a R^2 of 0.980 for color belt 2 and a R^2 of 0.989 for color belt 3.

RESULTS

Quantification and Mapping of Bark pH

Fine-scale changes of bark pH were clearly detectable and quantifiable with our bark pH mapping method (Figures 4a-c). Most pH panels fixed at pH 2 exhibited microscale spatial heterogeneity of bark surface pH. The fine-scale spatial variation of bark pH was evident for cacao tree T21 (Figure 4a) with an agar-agar panel fixed at pH 2. Most of the bark surface of T21 had a pH which ranged between 2 and 4 (Figure 4a). In the lower parts of the panel of T21, the reddish color indicated a sizeable area of bark with a pH between 4 and 7, as opposed to the lower right center, where the bark surface had a pH > 7 as indicated by the purplish-red color (Figure 4a). The largest area of the bark, with pH > 7, corresponds spatially with the irregularity of the distorted bark surface where a node has formed over an area once occupied by a branch (Figures 4a–b). The area where moss was present on the bark surface (Figure 4c) also corresponds to an area with a pH different than most of the bark surface (Figures 4a, 4c). In fact, an overlay of the pictures of the pH panel and the bark surface substantiates and confirms the spatial synchronization of the node with substantially higher bark pH (Figure 4d, Table 2 [cells F2-H4]), as well as the impact of the moss on a slightly elevated bark pH (Figures 4d and 4e), as indicated by the color change. The actual area of elevated bark pH, however, was not noticeably different between the moss-covered areas and bare bark surfaces (Table 2 [cells C2–E4]). The slightly blurred impression of Figures 4d and 4e is a result of the image overlaying process. Agar-agar panels with a fixed pH of 9 showed few differences in the spatial variability of bark pH, indicating a homogenous pH field.

Because digital images were acquired of the trunk bark surface of all sample trees, it was possible to analyze every pixel and assign each a color value representative of bark pH at that particular location per the image processing routine employed. This analysis revealed that the fine-scale changes in bark pH were attributable to the presence or absence of epiphytes (mosses and lichens) and variations in bark microrelief from surface distortions and irregularities. For the cacao trees sampled with an agar-agar panel starting with pH 2, the majority of the bark surface of most trees was found to have a pH > 4, with some trees (such as T4, T13, and T14) having the largest

TABLE 2. Associations among bark microrelief, moss-covered areas, and bark pH for tree T21

Position[a]	pH >7 (% area)	pH 7–4 (% area)	pH 4 (% area)	pH 4–2 (% area)	pH2 (% area)	Moss (% area)	Nominal bark microrelief value
A1	0	12	50	38	1	0.10	0
A2	0	3	53	44	0	0.06	0
A3	0	5	82	13	0	0.05	0
A4	0	2	70	28	0	0.14	0
A5	0	11	18	71	0	6.34	0
B1	0	20	77	2	0	0.01	0
B2	0	0	99	1	0	0.21	0
B3	0	0	91	8	0	3.27	0
B4	0	0	25	75	0	0.11	0
B5	0	5	10	84	0	5.13	0
C1	0	41	58	1	0	3.87	2
C2	1	3	96	1	0	17.60	2
C3	0	10	84	6	0	92.14	0
C4	0	0	5	95	0	4.98	0
C5	0	10	3	87	0	0.81	0
D1	0	22	78	0	0	34.25	1
D2	0	0	100	0	0	45.77	1
D3	0	3	52	45	0	89.95	2
D4	0	0	3	97	0	35.66	0
D5	0	10	47	43	0	2.57	0
E1	0	33	67	0	0	14.55	0
E2	0	2	98	0	0	6.63	0
E3	0	0	56	44	0	20.51	0
E4	0	0	14	86	0	6.53	0
E5	0	10	84	5	0	1.27	0
F1	43	54	2	1	0	0.32	1
F2	0	65	35	0	0	4.00	2
F3	0	0	100	0	0	1.93	1
F4	17	27	56	0	0	0.19	1
F5	38	32	29	0	0	2.85	2
G1	0	30	68	2	0	0.01	2
G2	20	38	41	1	0	0.24	2
G3	36	32	31	0	0	0.02	2
G4	77	20	3	0	0	0.84	1
G5	90	8	1	1	0	2.65	1
H1	0	25	74	1	0	6.21	2
H2	3	68	28	1	0	4.17	2
H3	89	10	1	0	0	0.35	1
H4	92	8	0	0	0	0.19	1
H5	25	45	29	1	0	4.87	1
I1	0	36	64	0	0	0.21	0
I2	0	32	68	0	0	0.09	0
I3	15	66	18	0	0	0.02	0
I4	7	59	34	0	0	0.01	0
I5	0	33	66	1	0	0.01	0
J1	0	13	72	15	0	0.23	0
J2	0	11	87	2	0	2.27	0
J3	3	73	24	0	0	0.01	0
J4	2	71	26	0	0	0.03	0
J5	0	50	43	6	1	0.02	0

[a]Position refers to the location in the grid of the bark image. The grid has five columns and 10 rows, each constituting a subpicture within the image. Each cell within the grid is 1.28 cm in length and 1.63 cm in width. Please note that the summation of some pH area percentages (for a given subpicture) may not exactly equal 100 due to rounding.

TABLE 3. Overview for the different trees with bark pH, moss growing areas, and an index of bark damage (% of area)

	Area (%) pH value[1]						
	>7	7–4	4	4–2	2	Moss area (%)	Index of bark damage
Start pH 2							
T4	56.28	40.36	1.87	1.34	0.16	91.82	0.46
T7	37.96	40.49	18.12	3.12	0.31	37.43	0.60
T11	46.98	35.18	14.62	3.13	0.09	52.24	0.48
T13	71.51	14.80	8.69	4.78	0.22	8.98	0.24
T14	80.61	13.11	4.76	1.42	0.10	36.40	
T19	4.21	36.39	43.88	15.36	0.16	50.58	0.00
T20	48.53	30.12	15.43	5.53	0.39	34.79	0.22
T21	11.18	22.17	48.42	18.19	0.05	8.48	0.60
Start pH 9							
T1	95.73	2.87	0.78	0.56	0.06	71.09	0.38
T5	93.52	4.47	0.95	0.87	0.19	76.3	0.28
T8	90.10	7.2	1.78	0.8	0.12	63.81	0.5
T9	95.77	2.87	0.74	0.49	0.13	2.54	0

[1]Please note that the summation of some pH area percentages (for a given subpicture) may not exactly equal 100 due to rounding.

proportion of their bark surfaces with a pH > 7 (Table 3). Cacao trees T19 and T21 were notable exceptions since the largest proportion of the bark surface of these trees had a pH of 4 (Table 3). Starting with an agar-agar panel with pH 9, the vast majority of the bark surfaces had a pH > 7 (Table 3), although the areas mapped are of interest because they reveal details about bark pH that are not evident with an agar-agar panel with a starting point of pH 2.

It is important to note that the quantification and subsequent mapping of pH by the agar-agar panels is dependent on the ionic strength of H^+ and OH^- and the sensitivity of the pH-indicator to the difference in pH. For agar-agar panels starting at a pH of 9, the indicator is insensitive to pH reactions and distinctions between pH of 7 and 9 cannot be assessed. This is demonstrated by overlapping peaks for pH values of 7 and 9 (Figure 3). In contrast to the neutral and very basic pH values, our method is highly sensitive to changes in pH between 2 and 7 where small changes in pH can be detected (Figures 4a–e). The focus on lower pH values is, of course, justified since bark tends to be more acidic. If one were interested in higher pH values, bromphenol blue or Tymolphtalein could be used as the pH indicator instead of bromocresol purple. Thus, for the chosen pH indicator (bromocresol purple), the *in situ* bark pH measurements are robust, being on the order of ~ 0.1 pH units in our experiments for the 4–7 pH range, based on our calibration and validation procedures.

Comparison with Conventional Bark pH Methods

Figure 5 illustrates the pH of the extracted water or KCl solution from samples of bark, bark with mosses, bark with lichens, and bark with mosses removed that were excised from cacao trees at the study site. For all bark samples extracted in water, the pH of the solution increased with time and appeared to reach an equilibrium state after two hours (Figure 5), whereas the bark-covered lichen samples extracted in the KCl solution had a pH that moved in opposite directions (Figure 5). Only the replications of the KCl extract for the bark-covered moss samples and bark samples with the moss removed were similar and showed the same pH over time (Figure 5); the other samples exhibited marked variability, partially dependent upon whether protons were on the bark surface or mobilized from within the bark over time. One drawback of this conventional method is that only one pH value is detected at a particu-

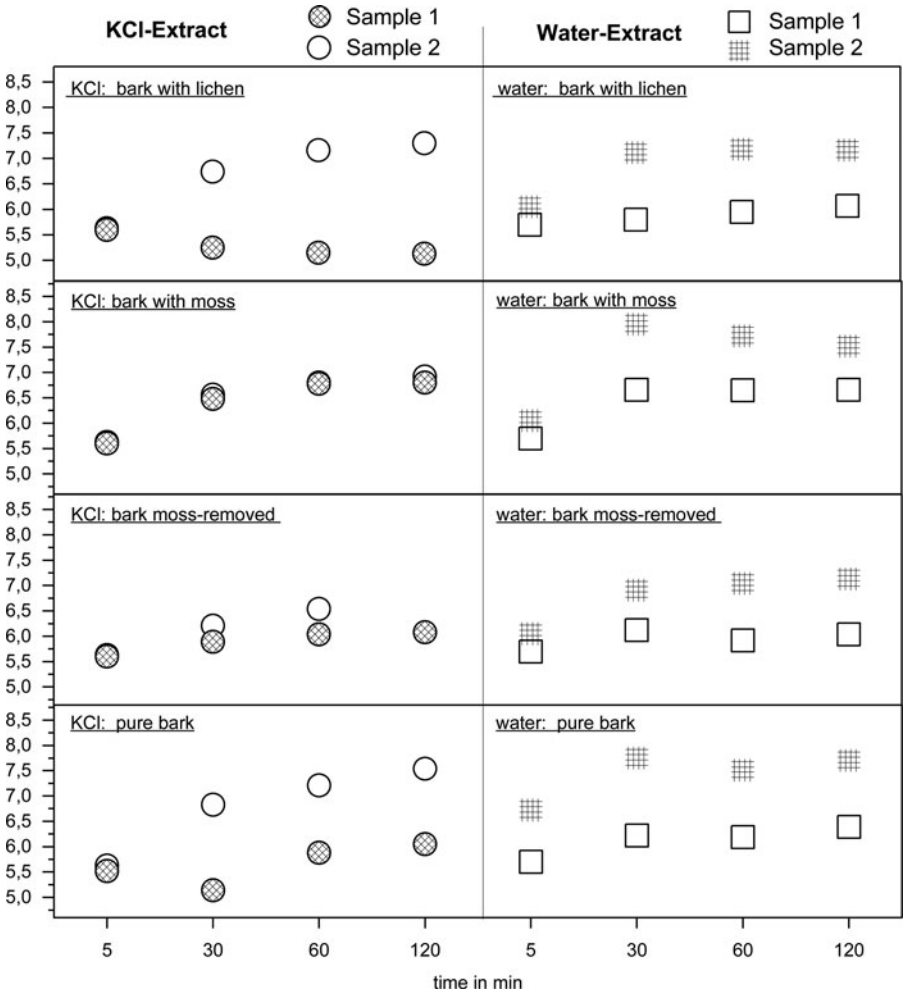

FIGURE 5. A time-series of bark pH as inferred from extracts in pure deionized water and KCl solutions for bark samples harvested from selected cacao trees.

lar time and is assumed to represent the whole bark sample (Figure 5). Thus, fine-scale changes in pH on the bark surface could not be detected with this method. Moreover, with extracted solutions, pH equilibrates over time and one is unable to distinguish between bark pH and pH influenced by other organisms (e.g., mosses, insects). Thus, with traditional extraction methods, the average of the whole bark sample is assessed and one cannot trace the ions in solution to the bark surface or deeper within the bark. This is to say that extraction methods treat bark pH as a "black box," whereas our bark pH measurement and mapping method permits one to examine the spatial variability of bark pH and the effects of other organisms on bark pH. Our method also permits one to examine the variability of bark pH over space (and time) to connect pH with epiphytic coverage, bark microrelief, and stemflow paths, among other factors.

CONCLUDING DISCUSSION

Some Caveats of Bark pH Measurement

As stated earlier, the measurement of H^+ and/or OH^- ions is necessary to determine the pH of bark surfaces. For agar-agar panels, the detection of pH is determined by the mobilization of H^+ and/or OH^- ions from the bark sur-

face which, in turn, influences the pH-potential of the agar-agar panel. Because the agar-agar panel is in direct contact with the bark surface, it equilibrates to the pH of the bark as H^+ ions move from the surface of the bark to the agar-agar panel and its indicators. It is important to note that the agar-agar panels only measure the freely available H^+ or OH^- ions at or near the bark surface, and not those located within the bark (as opposed to extract solutions), since there is no water or other solution to elute ions from deeper within the bark. This is one significant advantage of our method compared to extract solutions. The fine-scale measurement of bark pH over the bark surface (as H^+ and/or OH^- ions are mobilized within the agar-agar panel) renders a detailed map of the local spatial heterogeneity of bark pH (Figure 4a–e), which is particularly useful for ecological research, as described later. For the sake of clarity, it also is important to highlight the fact that the pH level revealed by the agar-agar panels does not represent an absolute pH value at the bark surface, but rather an approximation, since the actual pH is based upon the net balance between H^+ and OH^- ions. This is to say that the bark may be unable to deliver enough OH^- ions to raise the pH potential at the agar-agar panel to a certain pH threshold value and, thus, the agar-agar panel may reflect a slightly lower pH value than the actual bark pH.

The agar-agar panels demonstrate the best results, with clear and distinct differences in bark pH, when the difference of H^+ concentration between the bark and agar-agar panel is larger. In our study, usually <10% of the agar-agar panel showed pH values greater than pH 7 (Table 2). As such, the pH indicator could indicate clear areas of the bark with pH values between 4 and 7 because a wide gradient of H^+ was present and initiated an equilibration with the agar-agar panel. For the agar-agar panels with a starting at pH 9, the pH pattern showed smaller pH fields with a pH gradient lower than pH 9 because there was a smaller gradient of OH^-concentration from bark surface to the agar-agar panel. Secondly, the agar-agar panels show a clear change in color for pH values between 5 and 7 with less sensitivity for pH values > 7. If sensitivity to smaller differences between pH levels of the bark is desired, then other pH indicators with a smaller spectrum should be used to produce sharper and more precise results.

Measurement of Bark pH with Agar-Agar Panels: Sample Applications

Bark microrelief is an important factor partly governing stemflow production and stemflow chemistry.[15,23] Wavelet analysis has been employed successfully to quantify bark microrelief.[24] Coupling wavelet analysis with the measurement of bark surface pH using agar-agar panels would permit a better understanding between bark microrelief and bark pH. Figure 4a–e demonstrated that our bark pH measurement method is capable of identifying areas where pH is altered by irregularities in the bark. Thus, with the coupling of wavelet analysis and our bark pH measurement method, it would be possible to develop a predictive relationship between bark microrelief and bark surface pH. Such a relationship would likely be of importance to hydrologists and biogeochemists examining hot moments of biogeochemical reactivity in relation to stemflow production within particular events,[25] as well as lichenologists and bryologists examining the spatial variation of epiphyte colonization around and along the bole of trees. In fact, a seminal paper by Yarranton[6] found a relationship between lichen species colonization of the bark surface and bark microrelief. Moreover, bark pH is a primary determinant for lichen colonization on trees.[26]

Pathogens can alter the pH of bark. Stem rot pathogens on Japanese cedar, for instance, have been found to lower pH in comparison with uninfested portions of the tree trunk.[27] Given the fact that bark pH[28] and bark water-storage capacity[29] have been documented to change along the vertical profile of a canopy, it would be informative to investigate the relationship between bark water-storage capacity and bark pH with our method. Such an exercise could yield insights into the influence of stem pathogens on bark pH. Are stem pathogens

more affected by pH or moisture status of the bark? At what scale are changes in bark water storage and bark pH important for the recruitment, colonization, and infestation by pathogens? Answers to these and other relevant questions have the potential to develop better strategies to combat tree disease. It is important to note that some corticolous organisms anchor below the bark surface. In such cases, if the bark is wetted at depth, it also would be important to quantify any differences in bark pH at depth compared to the bark surface. Longer durations of bark wetting, or lesser concentrated extract solutions, would likely diminish any differences between pH within the bark and the bark surface as ion exchange would tend to equilibrate with time[30] and stabilize pH.

The bark pH measurement method developed, described, and validated in this article is inexpensive and straightforward. As such, our method has the potential for wide adoption by scientists across disciplines who are interested in the influence and effects of bark pH on life in the cortisphere. Unlike conventional methods to measure bark pH in deionized water or KCl extracts, our method is able to identify fine-scale spatial changes in bark pH that are relevant for the colonization of bark by organisms. This is a distinct advantage which has broad implications for transformative science to uncover the dynamic interrelationships between bark pH, bark microrelief, and epiphyte colonization and/or stemflow inputs to forest soils.

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