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Recognizing Amazonian tree species in the field using bark tissues spectra

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Abstract

The identification of tree species in the field is often a subjective process and misidentifications cause many problems for forest management in the Amazon Forest. Near infrared spectra from dried leaves of herbarium specimens are able to distinguish species in tropical forests. However, toolsto improve species identification directly in the field are needed. In this study, we tested whether spectral reflectance of bark tissues (rhytidome and phloem) collected with a portable spectrometer in the field can be used for the discrimination of tree species. Spectral data was collected for 254 trees of 8 families, 10 genera and 11 species from terra firme forests in Central Amazon with an ASD field spectrometer. Data consisted of reflectance values within 350–2500 nm wavelengths. We compared the rate of correct species recognition for different datasets using linear discriminant models. The rate of correct species assignment using this technique was 98% when using spectra from the inner bark (phloem) and 94% with outer bark (rhytidome) spectra. We suggest that the application of this technique can improve the quality of species identification directly during field inventories, fostering better forest management practices.

Graphical abstract
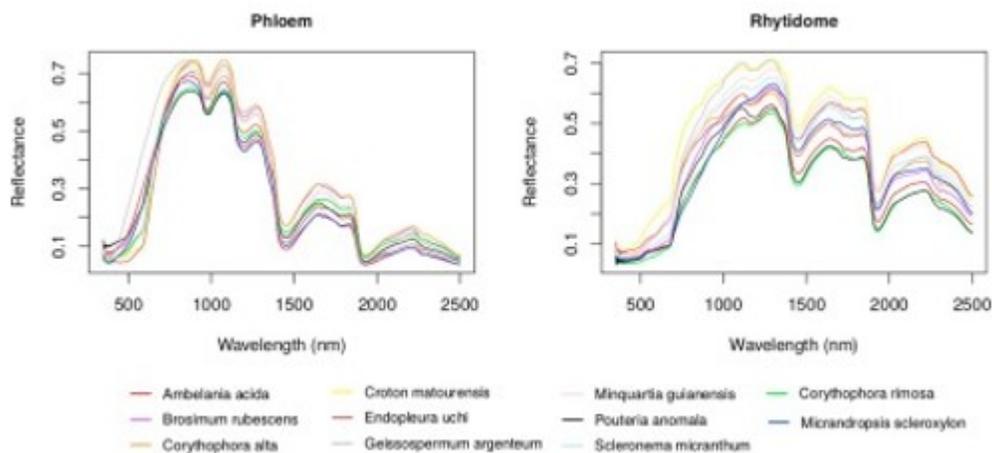
Keywords: Forest management, Species identification, VIS-NIRs, Inner bark, Outer bark, Reflectance data

1. Introduction

The identification of tree species in forest inventories is a key activity in maintaining high quality of forest management. In the field, trees are frequently identified to species by para-botanists ('mateiros') and voucher specimens are only rarely collected for comparison with herbaria or

consultation with plant specialists. In the Amazon region, high tree species diversity (Steege et al., 2016, Cardoso et al., 2017) and poor sampling make it difficult to recognize species with confidence (Nelson et al., 1990, Hopkins, 2007, Gomes et al., 2013). Problems relate to both finding scientific names for species, and also to the grouping of trees into the same species at local scales (Gomes et al., 2013). While the first problem is complex, as the Amazon has many undescribed species (62% of the estimates of ter Steege et al., 2013) and taxonomic uncertainty abound, the second problem, crucial to the sound management of plant populations, can be more easily tackled. Recent studies suggest that near-infrared spectroscopy may increase the quality of grouping trees into local species and, given a robust model, to also obtain a scientific name for them (Durgante et al., 2013, Lang et al., 2015). Hence, near-infrared spectroscopy is a promising technique for increasing the accuracy of local species delimitation and also for their taxonomic assignment, a problem that impairs our understanding of basic diversity patterns for the Amazon (Cardoso et al., 2017).

It is common practice in forest inventories, particularly for forest management, to identify trees only in the field without the collection of herbarium specimens for plant identity confirmation. This activity is most frequently conducted by para-botanists, lacking formal training, who use morphological characteristics, such as trunk shape, bark type and texture, wood color, the presence or absence of exudates, odor and other vegetative traits to identify trees into local species in the field. This is conducted in a cognitive way and the actual characters used are hard to untangle (Procópio and Secco, 2008, Gomes et al., 2013). These field observations allow varying identification of trees to family, genus and species, with uncertain scientific names frequently extracted from vernaculars used during fieldwork. Vernacular names also vary among regions and para-botanists, causing the same species to have different vernaculars, and the same vernaculars are used for different species (Martins-da-Silva et al., 2003, Procópio and Secco, 2008). Hence, the extraction of scientific names from vernaculars is prone to large errors, with negative consequences to the management of plant populations, and this is likely the most frequent method by which inventories for timber exploitation are conducted. The clustering of different species with the same vernacular causes overexploitation of rare species, overestimation of the size of populations of commercial species, and jeopardizes the integrity of commercial transactions of forest products (Procópio and Secco, 2008, Gaui, 2013).

A more reliable process to recognize species is conducted when herbarium samples are collected for morphological comparison with specimens identified and deposited in herbaria, or with the aid of the botanical literature. Rarely all individuals are collected, so the problem of grouping trees into local species may persists if this process is conducted in the field without caution to difficulties caused by poor taxonomy, cryptic variation and ontogeny in local species assignment (Gomes et al., 2013). Moreover, in

many plant groups it is frequently necessary to have reproductive material to identify taxonomically a local species from an inventory, and most samples collected in inventories are sterile due to irregular phenological patterns (Newstrom and Frankie, 1994), and also due the low importance that the problem of local species and taxonomic assignments receives in forest inventories in general (Gomes et al., 2013;Procópio and Secco, 2008;Martins-da-Silva et al., 2003).

The problem of species identification in the Amazon is also increased by the taxonomic quality of the reference collections. Herbaria with plants from the Amazon are the most important reference to obtain a taxonomic name for a local species from a forest inventory. However, different plant taxonomists and specialists may identify plants in a single herbarium collection. Hence, different people may have different levels of training and see different things (Gomes et al., 2013), and even specialists in a single plant group may differ in their taxonomic concepts. This is exemplified by the fact that duplicates of the same samples in different herbaria have distinct taxonomy, amounting to more than 50% of incongruence for the name of some tropical species (Hopkins, 2007, Goodwin et al., 2015).

Therefore, it is necessary to improve the processes by which trees are assigned into local species in forest inventories, and also the quality of reference collections with better sampling and taxonomic definitions. Some new technologies promise to greatly change the way plant species are defined and may be recognized. Molecular data is causing a revolution in taxonomic practice (Hollingsworth et al., 2009), and is likely to be in the near future the main reference for plant identification despite current limitations of plant molecular barcoding (Kress et al., 2009, Gonzalez et al., 2009). However, the use of molecular data for assigning trees into species at local scales, a non-trivial problem in Amazonian forests (Gomes et al., 2013), is difficult to operationalize. For this task, when species taxonomic definition is not at stake, technologies using chemometrics data, like visible and infrared spectra (NIR and SWIR) of plant tissues, are showing excellent results in the discrimination of plant species in tropical forests (Pastore et al., 2011, Durgante et al., 2013). Visible and infrared spectroscopy is a fast and non-destructive tool (Pasquini, 2003) that together with multivariate techniques identifies, quantifies and characterizes organic samples (Tsuchikawa, 2007). This tool has been used to discriminate plant species using absorbanceextracted from dried leaves and wood. In the Amazon, recent studies show high predictive power of NIR spectra from dried leaves to discriminate closely related species in Lecythidaceae (Durgante et al., 2013), 111 species of 34 families from a large-scale plot (Curty, 2014), species of Burseraceae at different stages of development (Lang et al., 2015), and species with samples from different Amazon regions (Botelho, 2017). Absorbance data from wood has been used to discriminate endangered species from other species with similar wood characteristics, and to assist in the monitoring of illegal logging (Pigozzo, 2011, Braga, et al., 2011, Pastore

et al., 2011, Bergo et al., 2016). NIRs technology is on the list of new forensic methodologies for detecting illegal logging (Dormontt et al., 2015).

The majority of studies on leaf and wood spectroscopy were performed under laboratory conditions. The development of field methods with portable equipment is necessary to improve the recognition of tree species in forest inventories with high reliability, a problem that can be tackled otherwise only by extensive botanical sampling (Gomes et al., 2013). Portable devices have been used in remote sensing to study forest canopy vegetationthrough leaf reflectance (Asner, 1998), as well as to discriminate species in tropical forests by spectra from fresh leaves (Clark and Roberts, 2012, Asner et al., 2014). Local species assignment during forest inventories would greatly benefit from the possibility of using information from the bark of trees, lowering costs and improving accuracy. The trunk is easier to access in forest inventories than leaves and the possibility of using spectral data from the bark would diminish the need for herbarium samples once a spectral model is build, greatly facilitating studies and inventories that conduct frequent censuses of trees in permanent plots. Here, we ask whether spectra obtained from the external or internal bark of trees, collected with a portable spectrometer in the field, would permit to discrimination of 11 selected species in an Amazonian forest.

2. Material and methods

2.1. Study area

The study was carried out at the ZF-2 Experimental Station of the National Institute for Amazonian Research (INPA), located 90 km north of Manaus - AM, Brazil (2°38′38″S and 60°09′49.9″W). The station has a total area of 21,000 ha, covered by tropical rainforest(Higuchi and Santos, 1997), characterized by high floristic diversity (Higuchi et al., 1998, Carneiro, 2004, Gaui, 2013). The trees were selected in the 12 permanent plots of 1 ha of BIONTE Project that has been monitored since 1989 (Higuchi and Santos, 1997). Herbariumspecimens were collected for all trees for taxonomic identification by comparing this material with specimens deposited at the INPA herbarium references and with the aid of specialized literature (Gaui, 2013).

2.2. Species sampling

We selected 254 individuals with DBH ≥ 10 cm of 8 families, 10 genus and 11 species, choosing the most abundant species in the plots, capturing broad phylogenetic variation, including different types of external bark (rhytidome) and species with and without exudates (Table 1). This study is a first step to recognize tree species in field by bark tissues. To test the method to recognize species with bark spectra we used species that are considered easy to identify in the field, as to better separate the power of bark spectra to recognize species with species delimitation problems.

Table 1. Number of individuals per species used to obtain reflectance data.

Family	Species	Vernacular	N
Apocynaceae	*Geissospermum argenteum* Woodson	acariquara-branca	20
Apocynaceae	*Ambelania acida Aubl.*	pepino-da-mata	15
Lecythidaceae	*Corythophora rimosa* W.A. Rodrigues	castanha-jacaré	24
Lecythidaceae	*Corythophora alta R.Knuth*	ripeiro-vermelho	28
Euphorbiaceae	*Croton matourensis* Aubl.	dima	23
Euphorbiaceae	*Micrandropsis scleroxylon* (W.A.Rodrigues) W.A.Rodrigues	piaozinho	44
Sapotaceae	*Pouteria anomala* (Pires) T.D.Penn.	abiurana-olho-de-veado	21
Moraceae	*Brosimum rubescens* Taub.	pau-rainha	22
Malvaceae	*Scleronema micranthum* (Ducke) Ducke	cardeiro	25
Coulaceae	*Minquartia guianensis* Aubl.	acariquara-roxa	21
Humiriaceae	*Endopleura uchi* (Huber) Cuatrec.	uxi-amarelo	11

2.3. Spectral data

Spectral reflectance measurements were obtained directly from the bark of living trees in the field with a portable spectrometer (Field Spec 3, ASD inc., 2010). The data collection time was 0.1 s per spectrum. Each obtained spectrum consists of 2151 reflectance values spanning the 350–2500 nm wavelength region. The resolution was 3 nm for the visible region (350–700 nm) and 10 nm for the NIR and SWIR region (700–2100 nm). Spectral data were collected through a glass slide, placed between the optical reader and the bark tissue (inner or outer bark). This was used to preserve the optical reader from resins, latex and other exudates. The slide was cleaned with ethanol (98%) between measurements. Before collecting the spectra of each individual tree, a white panel (Spectralon, Labsphere, Inc.) was used for background calibration, to convert radiance values to reflectance. The inner and outer bark had unique spectral reflectance curves. Occasionally the spectrometer collected erroneous spectra that was possible to recognize looking at the curve during data collection. In these cases the measurement was repeated.

Tree bark can be subdivided into non-living outer bark (rhytidome) and live inner bark (phloem). The inner bark includes all tissues of the vascular cambium to the periderm, formed by three layers: suber, phellogen and phelloderm (Esau and de Morretes, 1974, Ferri, 1999). Therefore, six spectra were collected per individual tree, three for the rhytidome and three for the inner bark, collected at different points around the tree trunk to capture

intra-individual variation. For the rhytidome, measurements were placed where lichens or bryophytes did not cover the bark and, if necessary, macro-lichens and bryophytes were removed with a glove or machete prior to the reads. To access the inner bark, we carefully removed the dead tissue of the rhytidome with the aid of a machete, until the live tissue was exposed.

2.4. Analyses

Principal Component Analyses (PCA) were used to explore the spectral variation of bark tissues (rhytidome and phloem) and species. The averages of the three spectra collected per tissue were used to represent the individual trees.

Linear discriminant analyses (LDA) were used to determine the potential of bark spectra to recognize species. Discriminant functions were generated having the 11 species as the dependent variables and the 2151 values of reflectance obtained per individual as independent variables. These LDA analyses were conducted for both tissues separately and individuals represented either by a single spectrum randomly selected among the three measured, or the average spectrum of the three measurements per tissue. To assess the predictive power of bark spectra, we used two different cross-validation techniques with LDA: (1) a leave-one-out cross-validation (LOOCV), where each individual tree was identified by a model built with the spectra of the remaining individuals; and, (2) a Holdout cross-validation (HOCA), by which the model (training set) was build with 70% of the individuals of each species, and the test set was composed of the remaining 30% (Kohavi, 1995). The holdout cross-validation was repeated 100 times with the model and test sets randomly selected each time. These results were averaged over the 100 permutations.

To minimize type I errors due to co-linearity in the spectral data (Xiaobo et al., 2010), the analyses above were repeated with selected variables. Variable selection was conducted using a stepwise approach to define the best subset of variables for species discrimination. The number of variables selected was limited to 1/3 the number of samples, following the recommendation of Williams and Titus (1988). In this way, a new discriminant function was generated only with the characters selected and tested by the same cross-validation techniques of the previous analyses.

3. Results

Rhytidome and phloem have different spectral signals and are clearly separated in a reduced space of two dimensions for the majority of the tested species. These ordinations also indicated that spectra from the rhytidome present greater variation than those collected from the phloem (Fig. 1). Therefore, we considered two separate datasets, rhytidome and phloem, to test the utility of each data type in species discrimination.
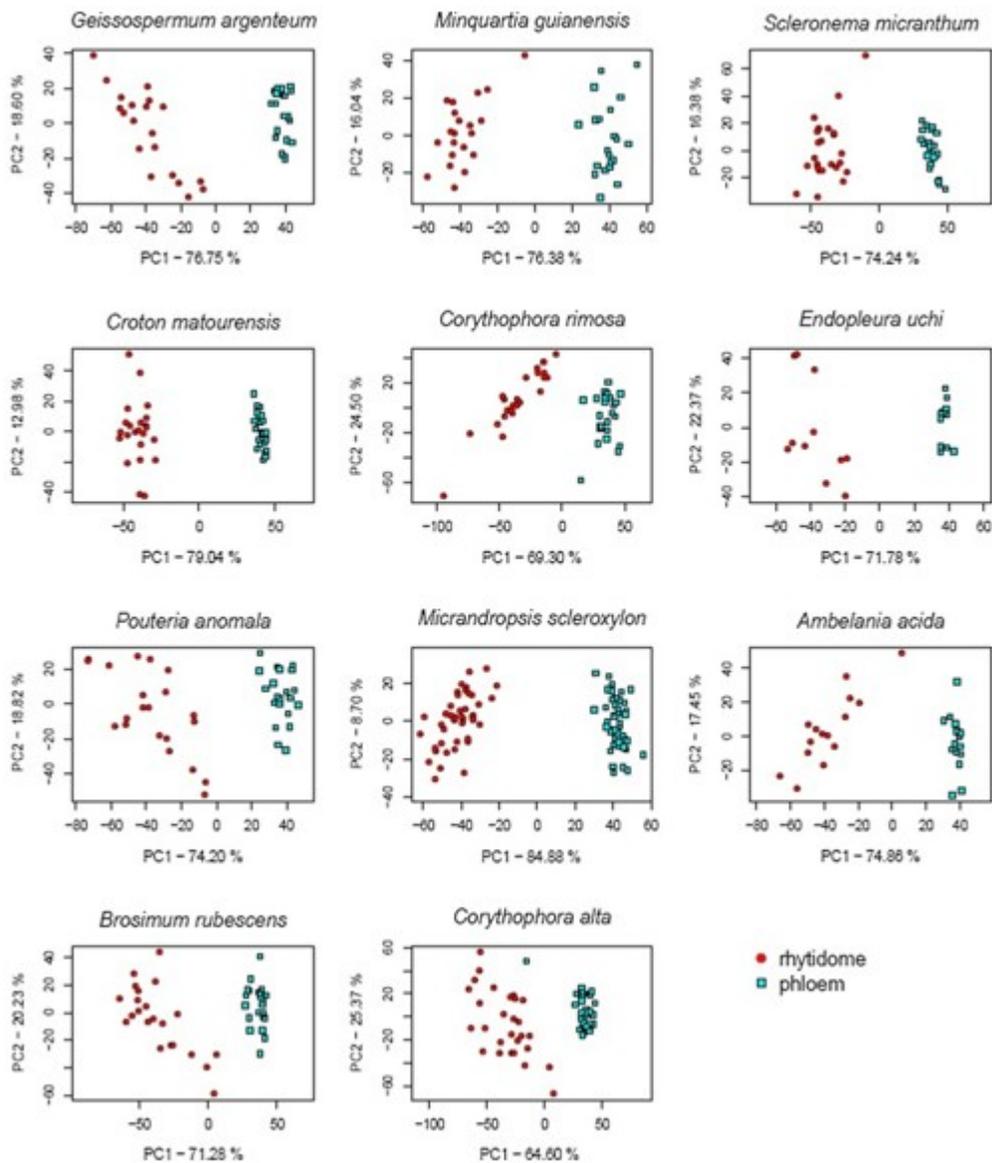
Fig. 1. Principal Components Analysis with the average of the three spectral readings of the rhytidome and the three phloem readings, collected per individual per species in the wavelengths from 350 to 2500 nm.

Both datasets indicated high potential for discriminating species in the field, with the rate of correct tree assignment varying by 77% to 98% among the different analyses conducted (Table 2). The lowest percentage of correct species identification (77%) was obtained for the rhytidome dataset when using a single randomly selected spectrum per tree. However, when the average spectra were used, the rate of species discrimination using rhytidome spectra increased to 94%. The best prediction was obtained with the average spectra of the phloem (98%).

Table 2. Percentage of correct species prediction by Linear Discriminant Analyses for different datasets and validation methods. Analyzes for all species tested with the random and mean spectra and the

selection of stepwise variables with 85 variables, for the cross-validations "leave one out" and "holdout" with uncertainty.

Bark tissue	Predictive models	Number of variables	Leave one out validation	Holdout validation
Rhytidome	single random spectrum	2151	77%	73,00% ± 4,15%
	mean spectrum	2151	94%	89,08% ± 3,11%
	stepwise	85	90%	89,47% ± 2,86%
Phloem	single random spectrum	2151	94%	91,63% ± 2,00%
	mean spectrum	2151	98%	97,24% ± 1,52%
	stepwise	85	94%	93,10% ± 2,07%

The stepwise variable selection indicated 85 wavelengths as the best variables in species discrimination (Fig. 2). Using only these selected variables, the power to discriminate species decrease but were still above 89% for both datasets (rhytidome and phloem; Table 2). These 85 wavelengths may point to chemical compounds that are related to species discrimination. Comparing the more informative characters with the literature indicated that the selected wavelengths for the rhytidome are associated with lignin, hemicellulose and aromatic compounds (Workman and Weyer, 2008;Schimeck and Evans, 2004;Jones et al., 2006;Schwanninger et al., 2011, Finley et al., 2016 – see S.1). This means that these compounds were more important to characterize the outer bark. In the phloem, selected wavelengths were indicative of cellulose (Schwanninger et al., 2011).
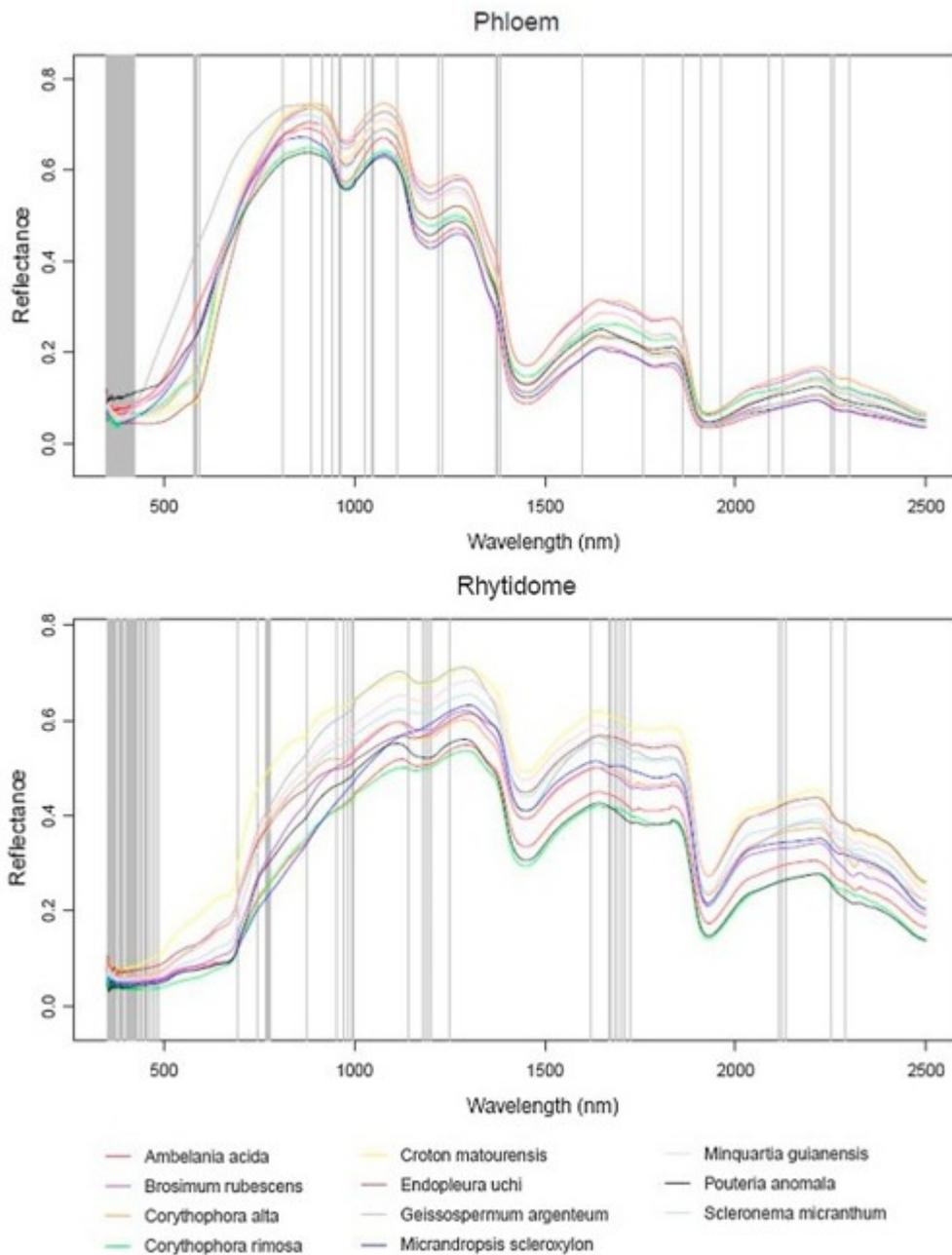
Fig. 2. Average spectra per species studied and most informative regions (grey bars), for the phloem data (above) and the rhytidome (below). The wider gray region is the visible region.

The few misclassified trees for the LOO of both datasets and with average spectra (best results), showed no clear pattern in relation to error assignments, both in relation to traits or phylogeny, although there was confusion between two individuals of *Corythophora*(Lecythidaceae; Fig. 3, Fig. 4). The presence of exudates such as latex, as well as the different types of rhytidome (Table 3 and Fig. 5) did not influence the prediction of species. It was possible to recognize what tree was misclassified and to what species

that tree was predicted using LOO validation. The rhytidome dataset has 13 misclassified trees among 254 samples for six species (Fig. 3). For the phloem, of 254 trees tested, only four trees of three species were misclassified (Fig. 4).
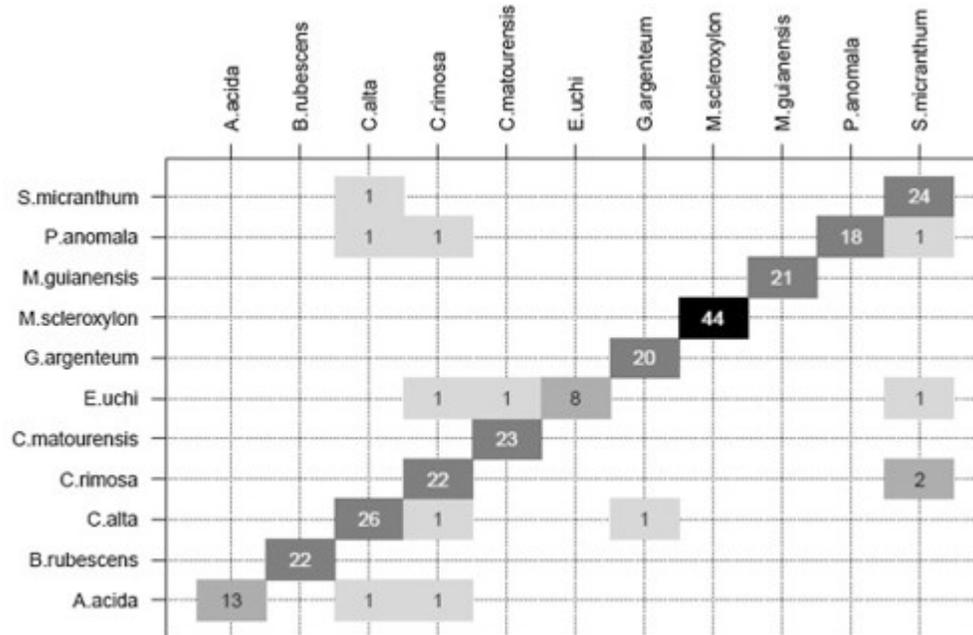

Fig. 3. Matrice with the results of a Leave-one-out (LOO) discriminant analysis for the rhytidome with mean spectra. The diagonal line represents the correct predictions of species identify. Rows represent tests, the cells show the number of trees predicted by model (columns).
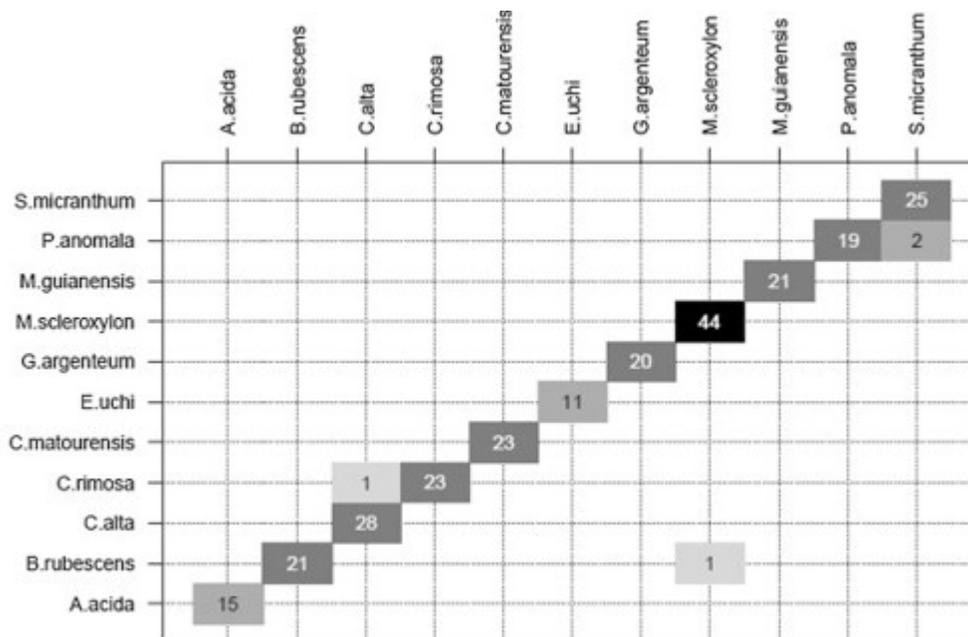

Fig. 4. Matrice with the results of a Leave-one-out (LOO) discriminant analysis for the phloem with mean spectra. The diagonal line represents the correct predictions of species identify. Rows represent tests and the cells show the number of trees predicted by model (columns).

Table 3. Traits of species observed in field for the outer bark and the inner bark.

Species	Traits
Geissospermum argenteum	Trunk fenestrated, rhytidome with fine fissures and soft bark. Phloem without exudates
Ambelania acida	Thick rhytidome, hard consistency and rough texture. Phloem with abundant white latex
Corythophora rimosa	Rhytidome with deep grooves and without evident detachment. Phloem with resin
Corythophora alta	Rhytidome with irregular depressions with plaque detachment. Phloem without exudates
Croton matourensis	Rhytidome longitudinally striated, whitish bark and soft bark. Phloem with reddish resin
Micrandropsis scleroxylon	Thick rhytidome and hard consistency. Phloem without exudates
Pouteria anomala	Thick rhytidome, longitudinally fissured. Phloem with latex
Minquartia guianensis	Grooved trunk, longitudinally fissured and soft bark. Phloem with sparse white latex
Scleronema micranthum	Rhytidome fissured and striated. Phloem without exudates
Brosimum rubescens	Rhytidome with lenticels and smalls bumps horizontally. Phloem with latex
Endopleura uchi	Rhytidome fissures, lenticels masked, detachment in elongated plaques, thick bark. Phloem with resin

Fig. 5. Morphological representation of the bark tissues (phloem and rhytidome) of all the species tested, and ilustration of how the measurement were performed in the field.

4. Discussion

Here we asked whether VIS-NIR-SWIR spectral data collected from the bark of trees in the field would permit the discrimination of species. Both inner (phloem) and outer bark (rhytidome) spectral data permitted the discrimination of the 11 selected tree species with high accuracy (>=94% of correct tree assignment when using the average of three spectra per tree per tissue). Although our taxonomic sampling was small and further tests are still required, our results indicate a great potential of this technique to both improve the quality of local species delimitation during forest inventories, and also for the taxonomic identification of local species.

The bark spectra collected from live trees in this study indicate similar results for species discrimination as studies using NIR spectra from dry leaves (Durgante et al., 2013;Lang et al., 2015), xylem spectra (Bergo et al., 2016), and from leaves and branches (Clark and Roberts, 2012;Lang et al., 2017). Our study is the first to show this technique as an important tool for species identification by bark in the field. This approach can improve the quality of plant identification in forest inventories, reducing costs, increasing speed and minimizing the need for climbing trees to collect material. Given a robust reference spectral model, either for the local species themselves, or a yet to build comprehensive public spectral repository linked to herbarium collections, the quality of local species and of taxonomic identification would be greatly increased. During forest inventories spectral data can be collected for all trees, and in addition to species discrimination, bark spectra may also inform on about chemical and physical characteristics of the wood (So and Eberhardt, 2006, Wu et al., 2011, Toscano et al., 2017), tree health (Finley et al., 2016) and functional traits (Asner et al., 2014).

Bark spectra were different between the inner and outer bark for the eleven species tested and may reflect different chemical characteristics between these two tissues (So and Eberhardt, 2006). In addition, outer bark spectra presented greater intraspecific spectral variation and lower discriminant power. The bark thickness, environmental effects, plant age/diameter may be related to greater spectral variation observed for rhytidome. Bark thickness is highly variable between and within species in tropical forests and functions such as defense from herbivory, fire, biomechanical support and respiration are performed by the rhytidome tissue (Paine et al., 2010). In our study, the 11 species sampled presented wide variation in bark thickness. However, when each individual was represented by the average of the three collected spectra per rhytidome, the noise caused by greater variation in rhytidome spectra was reduced, and the power of species discrimination increased. For the inner bark (phloem) we obtained good results (94%) when using a single spectrum per tree, so the intraspecific spectral variation in the phloem was low, possibly due to the small environmental influence suffered by this tissue.

The most informative wavelengths selected by a stepwise variable selection process indicated that few variables are needed to distinguish species. The most informative variables responsible for species distinction point to chemical compounds that are part of the anatomical or structural characteristics of the bark tissues. Conventional studies on cell wall and chemistry of the inner and outer barks have shown significant differences between these tissues (Freire et al., 2002, Hafizoglu et al., 1997). However, few studies have analyzed the differences between inner and outer barks using NIR spectroscopy, and most evaluated xylem spectra. Here selected wavelengths in the NIR-SWIR range are from regions attributed to wood constituents, such as cellulose (Schimleck and Evans, 2004, Jones et al., 2006) hemicellulose (Schwanninger et al. 2011), lignin and aromatic compounds (Jones et al., 2006). Although this interpretation must be taken with caution, the data suggest that the outer bark has low levels of polysaccharides and are more lignified (Eberhardt and So, 2005;Hafizoglu et al., 1997), while the contents of holocellulose and cellulose are higher in the inner bark (phloem) than in the outer bark (rhytidome) (Hafizoglu et al., 1997). Wavelengths associated with water absorption were also selected for the rhytidome at 971 nm; 982 nm and 1200 nm. Water creates near infrared (NIR) absorption characteristics at 970 nm and 1,200 nm, respectively (Clark and Roberts, 2012, Workman and Weyer, 2008), but apparently had little effects in species discrimination.

Current limitations of this spectral technique for ample use in forest inventories are mostly related to lack of a robust reference model that can be used to predict species identities from a bark or a leaf spectrum. This requires obtaining spectra from bark tissues in the field for well-identified trees, preferably with a voucher specimen in a herbarium, since taxonomic definitions are largely unstable in the Amazon (Hopkins, 2007, Steege et al., 2016). Nonetheless, this technique may be used already at local/regional contexts, and may help at such contexts to better define local species even if their taxonomic names remain uncertain. Spectral models for local studies, for bark, leaves or both, could be collected by all permanent plots currently monitored in the Amazon for improving local species definitions, enabling a collectively built repository of spectral data that could be used to inventory new and unexplored areas. Our results indicate that bark VIS-NIR-SWIR spectra have a strong potential to improve the quality of forest inventories and tree population management.

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