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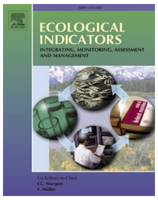

Original Articles

Shannon tree diversity is a surrogate for mineland rehabilitation status

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ABSTRACT

Mineland rehabilitation is performed to reduce the overall impacts of mining operations. Thus, statistically validated and easily measurable indicators are necessary to monitor the environmental status along time, enhance the rehabilitation process, and increase institutional tractability. The objective of this study is to derive an effective indicator to assess the environmental quality of iron mining waste piles undergoing rehabilitation in the Carajás National Forest, eastern Amazon, Brazil, from a curated set of field-surveyed environmental variables related to vegetation structure, invertebrate and vegetation compositions, diversity, and ecological processes. Data were collected from a chronosequence that included non-revegetated areas, areas in different rehabilitation stages and natural reference sites. All variables were integrated to produce a single estimate of rehabilitation status using a multivariate approach. Individual variables largely differed in their response ratios; nevertheless, the data integration showed that more than 50% of the predisturbance ecosystem structure, diversity and functioning were restituted after only seven years, which highlights the potential of rehabilitation activities to effectively reduce mining impacts. Among all 27 variables, the Shannon index of tree diversity had the highest predictive power for overall rehabilitation status, qualifying this metric as the most effective indicator for the use in future comprehensive monitoring activities in waste piles undergoing rehabilitation in the Carajás National Forest. The positive relationship between tree diversity and mineland rehabilitation status in the examined areas emphasizes the importance of diverse tree communities in increasing rehabilitation success and ecosystem and soil functioning over short time periods.

1. Introduction

Ecological restoration, i.e., the restitution of biodiversity and ecosystem structures, functions and services of original ecosystems, and rehabilitation, i.e., partial restoration, including novel ecosystems (Gastauer et al. 2018a), are performed to reduce the overall impacts of mining operations (Maiti, 2013; Perring et al., 2015; Gann et al., 2019; Guerra et al., 2020). To guarantee corporate tractability of mineland rehabilitation activities, refine rehabilitation practices, and outline environmental advances over time, monitoring is indispensable (Lamb et al., 2015; Lechner et al., 2018; Mazón et al., 2019). In this sense, multidisciplinar and multivariate approaches have been proposed (e.g., Mukhopadhyay et al., 2014; Kollmann et al., 2016; Gastauer et al., 2018b; Bandyopadhyay et al., 2020) in order to understand the full complexity of ecosystems under rehabilitation (Prach et al., 2019). Accordingly, the number of monitored ecosystem characteristics has increased, although the identification and validation of easily measurable, effective indicators able to reduce the costs and labor efforts of environmental monitoring programs are required in practice (Gastauer et al., 2020a).

Specific ecosystem properties, such as structural parameters, may recover faster than others, e.g., plant diversity (Laughlin et al., 2017; Yuan et al., 2018), but initiatives specifying variables to survey in environmental assessments, e.g., the Atlantic Forest Pact monitoring protocol (Viani et al., 2017), are scarce. International standards for environmental monitoring require evaluations of the vegetation structure, community diversity and ecological processes (Wortley et al., 2013; Gann et al., 2019) without details regarding which and how many

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ecosystem properties to survey. Consequently, monitoring programs differ in the number and types of monitored characteristics (Vickers et al., 2012; Derhé et al., 2016; Kollmann et al., 2016), hindering comparisons among different projects (Carabassa et al., 2019; Gann et al., 2019) and reducing institutional tractability of the rehabilitation process (Gastauer et al., 2018a).

Statistical approaches to integrate empirical, field-survey data can validate whether a sufficient number of ecosystem characteristics have been used to reliably assess the rehabilitation status. A previous approach examined biases associated with surveyed variables/variable groups and used the bootstrapping method to identify the minimum required number of environmental variables to reliably estimate rehabilitation status (Gastauer et al., 2020a), but the impacts of additional, not-yet measured ecosystem properties on the rehabilitation status and derivation of indicators were not assessed. Since environmental variables differ in their rehabilitation trajectories and response ratios, not-yet-surveyed ecosystem properties may include the following:

1. environmental variables unrelated to degradation/rehabilitation, i.e., those with no difference among degraded, rehabilitating and reference sites, such as the important seed bank characteristics identified in Medeiros-Sarmento et al. (2020);
2. non-rehabilitating ecosystem properties such as similarity of rehabilitating communities to reference sites in Laughlin et al. (2017) that does not enhance with revegetation and/or rehabilitation time;
3. ecosystem properties that increase over the rehabilitation time but achieve different response ratios compared to the predisturbance ecosystems, including variables that exceed predisturbance levels, such as soil enzyme activities in some cases (Feng et al. 2019);
4. variables that increase with the launch of rehabilitation activities (revegetation) to different levels compared with those at the reference sites but do not increase further, e.g., soil respiration in Gastauer et al. (2019); and
5. variables that decline with rehabilitation, e.g., the richness of microbial communities in Gastauer et al. (2020a).

Simulations are essential tools for understanding ecological complexity (Green et al., 2020) and are frequently used to estimate climate change effects on land use (Monteiro Junior et al., 2019) and species and disease distributions (Santika, 2011), but they have not been applied to estimate the completeness and reliability of environmental assessments. Systematic approaches may involve simulating variables that follow hypothetical trajectories to identify the effects of the additional, not-yet-surveyed variables on rehabilitation status. Such approaches may provide additional tools to validate the actual variables applied to estimate the rehabilitation status and reliability of environmental indicators to scale up monitoring activities.

The objective of this study was to select potential indicators of environmental quality of iron mining waste piles undergoing rehabilitation in the Carajás National Forest, eastern Amazon, Brazil. To do so, (i) we integrated a curated set of 27 environmental variables collected across a mineland rehabilitation chronosequence into a single rehabilitation status estimate using a multivariate approach. The chronosequence comprised non-revegetated minelands and land in different rehabilitation stages as well as undisturbed evergreen Amazonian forest as the reference ecosystem. Then, we validated the estimate by checking for (ii) biases associated with single variables or variable groups, (iii) the minimum number of environmental variables for reliable assessments, and (iv) the influence of simulated variables following hypothetical trajectories, representing not (yet) monitored ecosystem properties. Finally, (v) we derived the indicator that best described the overall rehabilitation status among all the environmental variables by statistical modeling.

2. Methods

2.1. Study site

The Carajás National Forest, eastern Amazon, Brazil (Fig. 1), harbors the largest iron ore reserves in the world. Extraction of these mineral reserves occurs by open pit mining. To extract the ore, original vegetation cover and overburden are removed. The overburden, which is also known as mining waste, is deposited next to the mining pits, forming substantial waste piles. To stabilize the slope and reduce the impacts on biodiversity and ecosystem services, these piles are rehabilitated by hydroseeding a standardized mixture of fertilizers, organic compost and seeds of mainly nonnative, noninvasive, fast-growing grasses (e.g., *Avena strigosa* Schreb., *Pennisetum glaucum* (L.) R. Br., both Poaceae), sunflower (*Helianthus annuus* L., Asteraceae), and nitrogen-fixing legumes (*Crotalaria spectabilis* Roth., *Stylosanthes macrocephala* M.B. Ferreira & S. Costa, *Canavalia ensiformis* (L.) DC. and *Cajanus cajan* (L.) Huth., Fabaceae).

To encourage the long-term self-sustainability of these areas, approximately 15% of the seed mixture is composed of native species. The seeds are collected from natural ecosystems in the Carajás National Forest by a seed-collecting cooperative. Since seed availability shows large seasonal and interannual variability, the applied seed mixtures may vary among projects and years.

2.2. Sampling design

In total, we installed 54 permanent 10 × 20-m^2 plots along one- to seven-year-old rehabilitation chronosequences associated with three distinct waste piles (Fig. 1). The chronosequences contained six plots in two non-rehabilitated areas, i.e., areas that had undergone topographic reformulation immediately before hydroseeding, and 15 reference plots at three distinct sites covered by undisturbed, natural vegetation (with evergreen tropical forest that represented the rehabilitation target). Nine of these reference plots were established near waste piles WP NW2 and WP S5, but an additional six plots were established in the forest interior at a location called “*Arenito*” to minimize eventual edge effects.

For sampling of the rehabilitating ecosystems, we used annual reports and maps from the mining company to identify the start of rehabilitation activities. After evaluating the continuity of the on-site vegetation using satellite imagery (Google Earth, Landsat), we installed three permanent plots in different-aged stands identified on each waste pile for a total of nine rehabilitation plots in WP NW2, nine in WP West and 15 in WP S5. We grouped the benches that represented different stand ages into initial (up to three years after hydroseeding; nine plots), intermediate (three-six years; 15 plots) and advanced (seven years after the beginning of rehabilitation activities; nine plots) stages. We established this classification system because the vegetation was herbaceous in the initial stage and bushy with individual trees in the intermediate stage, while the seven-year-old stands in the advanced stage formed a continuous canopy. The number of plots differed among stages because the classification after field surveys grouped more than one age class from the same waste pile in the same rehabilitation stage. All plots were installed in areas with representative vegetation and without signs of disturbance after revegetation activities.

After georeferencing, vegetation, invertebrate fauna and soil were sampled from each plot to generate data on 27 environmental variables related to the attributes of vegetation structure, community diversity and ecological processes (Table 1). To characterize the vegetation structure and diversity, all trees with a diameter at breast height equal to or larger than 3 cm were tagged and identified to the species level. Samples of the species not recognized during the field campaigns were collected and identified by comparison with herbarium specimens from the Museu Paraense Emílio Goeldi (MPEG) and the help of specialists. Species nomenclature and classification follow that of the Missouri Botanical Garden (MoBot) and Angiosperm Phylogeny Group (APG) IV

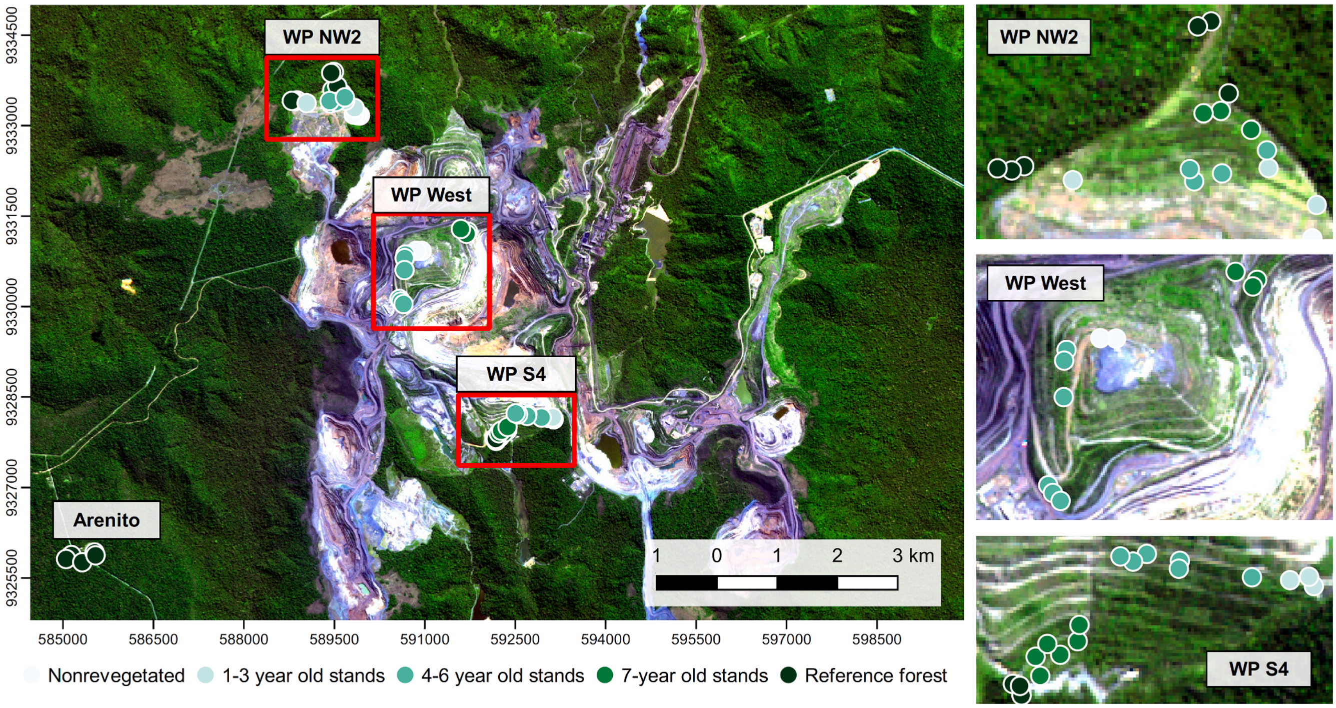


Fig. 1. Sentinel 2A image of 4 study sites in the Carajás mining project (image date: June 30, 2017); the points indicate the sampled plots. WP represents waste pile.

(The Angiosperm Phylogeny Group, 2016). The pooled cover values of all native species (excluding bare soil areas and areas covered by invasive grasses, such as molasses grass, *Melinis minutiflora* P. Beauv., and signal grass, *Brachiaria decumbens* Stapf), were estimated in 1x1-m subplots at the vertices of the plots. Additionally, the leaf area index (LAI), as a surrogate for canopy closure, was measured during the field campaigns. Vegetation sampling was performed during the rainy season.

Invertebrate fauna were sampled using four pitfall traps (commercial plastic cups; diameter 10 cm; height 15 cm) placed in a 10x10-m quadrat in each plot. The plastic cups were buried in the soil so that their upper edge was at the soil surface level (Fig. S1). The traps were filled with 50 ml of commercial ethanol (70% m/v) and maintained in place for 24 h. Two field campaigns were performed for invertebrate sampling: one during the rainy season and one during the dry season. The samples collected from each sampling point were pooled. The collected invertebrates were counted, classified as morphospecies and identified to the order, family or species level, depending on the taxonomic group.

Soil respiration was measured during the field campaign performed during the dry season (for details on measurements, please see Table 1). Composite soil samples were collected to detect organic matter and other biological and biochemical soil attributes. Soil was sampled to a depth of 10 cm with an auger (diameter 10 cm) at five sampling points after removing the litter layer; the sampling points were homogeneously distributed in the plots. Then, the soil samples were stored in a cooler for transport to the laboratory.

2.3. *Rehabilitation status and its indicators*

We integrated all the variables in Table 1 into a single estimate of rehabilitation status as proposed by Gastauer et al. (2020a) using principal coordinate analysis (PCoA) to address multicollinearity of the variables. Then, we computed the Euclidean distances between the principal coordinates of each plot and obtained the rehabilitation status for each plot based on the shortest distance between the focal plot and a reference plot (ΔReh-Ref) in relation to the shortest non-revegetated-reference distance (ΔNR-Ref), as shown in equation (1).

$$Rehabilitation\ \ status = \left(1 - \frac{\Delta Reh - Ref}{\Delta NR - Ref}\right)*100 \tag{1}$$

The quotient of the nearest reference site distance and the nearest non-rehabilitated-reference site distance represents the proportion of environmental advances still necessary to achieve predisturbance levels (in relation to the overall trajectory). Thus, the rehabilitation status represents the proportion of achieved environmental success, compared to overall rehabilitation trajectory necessary to restitute reference ecosystems. By definition, the mean rehabilitation status of all non-rehabilitated sites is 0, and the value of the reference plots is equal to 100%.

To determine the best indicator of environmental rehabilitation status, we individually modeled rehabilitation status with all 27 environmental variables using linear models. The Akaike information criterion (AIC) was applied to rank different models and select the variable that showed the best model fit and best represented the rehabilitation status (Burnham and Anderson, 2002).

To evaluate the field-surveyed environmental variables to determine the rehabilitation status, we carried out three distinct analyses. First, we checked whether single variables or different combinations of variables affected the assessment of the rehabilitation status since such bias would make the variable/variable combination mandatory in the assessments. Then, we identified the minimum number of required variables to reliably estimate the rehabilitation status using the bootstrap method to confirm sample sufficiency when smaller variable sets than those actually surveyed estimate rehabilitation status to the same magnitude (Gastauer et al., 2020a). Third, we simulated variables to assess how additional, not-yet-surveyed variables might affect the overall rehabilitation status.

To check for bias in terms of single variables or variable groups, we computed the rehabilitation status without focal variable(s) for each plot as described above and correlated this value with the status computed from the entire set of variables by linear regression. Pearson correlation coefficients and maximum residual values, i.e., discrepancies caused by the focal variable(s) for single plots, were used to estimate the overall bias. Additionally, we compared the mean rehabilitation status of advanced-stage plots derived from computations with and without

Table 1

Variables, ecological significance and methodological details related to the measurement of the rehabilitation status of waste piles in the Carajás National Forest, eastern Amazon, Brazil.

Attribute	Variable	Ecological significance	Methodological details
Vegetation structure	Tree density [trees ha^{-1}]	Important characteristic used to evaluate the advance of succession (Chazdon, 2003).	Number of trees per plot
	Basal area [m^2 ha^{-1}]	Measure of the aboveground tree biomass (Chave et al., 2014)	Cross-sectional area of the tree stems at breast height
	Leaf area index	The one-sided green leaf area per unit ground surface area in broadleaf canopies; measure for canopy closure, primary productivity and evapotranspiration	Field measurements using LAI-2200C sensors (LI-COR Inc., Lincoln, NE, USA) following the manufacturer's instructions; sky conditions were continuously monitored by a sensor at a site free of vegetation, and a second sensor was used to capture two below-canopy readings at each corner and at the center of each plot, totaling 10 below-canopy readings for each plot
Community composition and diversity	Tree species richness	Measure of tree species diversity	Number of tree species per plot
	Tree Shannon diversity (H')	Measure of tree species diversity	$H' = -\sum_{i=1}^{S} p_i * lnp_i$, where p_i is the relative abundance of species i
	Tree evenness (J')	Measure of biodiversity that quantifies the equality of species abundance in the community	$J' = \frac{H'}{lnS}$, where S is the number of species so that ln S is the maximum Shannon diversity given that all species are equally abundant
	Phylogenetic diversity of the tree community [Myr]	Measure of feature diversity (Forest et al., 2007)	Pruning and dating the megatree R20160415.new to sampled species using the Community Tree Optimizer (Gastauer et al., 2018c) followed by computation of the lengths of the evolutionary paths (in million years) that connect the sampled taxa from each plot
	Tree dissimilarity to reference sites	Measure of the differences in tree community composition (Purschke et al., 2013)	Mean Jaccard dissimilarity (1-Jaccard similarity) in relation to all reference plots
	Proportion of native trees [%]	Relative abundance of native tree species	Pooled relative abundance of native tree species
	Proportion of native species cover [% of total cover]	Proportion of native species in overall vegetation	Pooled relative cover of native species excluding base soils and exotic grasses *Melinis minutiflora* P. Beauv. and *Brachiaria decumbens* Stapf (Poaceae)
	Invertebrate species richness	Measure of invertebrate species diversity	Number of invertebrate species detected in each plot
	Number of sampled invertebrate specimens	Measure of invertebrate density	Number of invertebrate specimens captured in each plot
	Invertebrate dissimilarity to reference sites	Measure of differences in invertebrate community composition (Purschke et al., 2013)	Mean Jaccard dissimilarities (1-Jaccard similarity) of invertebrate communities in relation to those in reference plots
Ecological processes	Soil respiration [μmol m^{-2} s^{-1}]	Measure of the biological activity in soils (Phillips and Nickerson, 2015)	Field measurements of CO_2 effluxes using an LI-6400XTR (LI-COR Inc., Lincoln, NE, USA); in each plot, three (distance 2 m from one another and at least 0.5 m away from tree trunks to minimize their effects on measurements) polyvinyl chloride (PVC) collars (10.2 cm diameter, 6 cm height) were inserted 3 cm into the soil as support for the soil CO_2 flux chamber; the PVC collars were stabilized for 24 h before measurements; soil respiration measurements were performed between 9:00 and 12:00 h
	Basal respiration [mg CO_2 $g^{-1}h^{-1}$]	Measure of microbial activity, microbial-mediated carbon loss from soil to atmosphere	Estimated according to the CO_2 released from soil samples incubated with 0.05 mol L^{-1} NaOH for three days (Alef and Nannipieri, 1995)
	Carbon in microbial biomass in soil [μg g^{-1}]	Measure of the active part of soil organic matter to provide information about decomposition and transformation processes	Determined based on the fumigation-extraction method (Vance et al., 1987)
	Soil organic matter [dag kg^{-1}]	Exerts numerous positive effects on soil physical and chemical properties and the soil's capacity to provide regulatory ecosystem services (Lal, 2009)	Soil organic carbon was determined using the Walkley–Black method (Walkley and Black, 1934) and adjusted for soil organic matter according to (Teixeira et al., 2017)
	Glucosidase activity [μg g^{-1}]	Measure of cellulose degradation; indicator of soil health (Adetunji et al., 2017)	Spectrophotometrically quantified at wavelengths of 490 and 410 nm (Dick et al., 1996)
	Phosphatase activity [μg g^{-1}]	Phosphatases catalyze the hydrolysis of phosphate esters; indicator of soil health (Adetunji et al., 2017)	
	Hydrolysis of fluorescein diacetate (FDA) [mg g^{-1}]	Estimate of soil/litter microbial activity; indicator of soil health (Parihar et al., 2020)	
	Urease activity [μg g^{-1}]	Catalyzes the hydrolysis of urea to CO_2 and NH_3 with a reaction mechanism based on the formation of carbamate as an intermediate; a measure for N cycling in the soil (Adetunji et al., 2017)	Quantified according to the amount of ammonia released after incubation of the soil with a urea solution for 2 h at 37 °C (Tabatabai and Bremner, 1972)
	Functional diversity	Different measures of functional diversity link taxonomic diversity with ecosystem functioning (Cadotte et al., 2011; Lavorel et al., 2013)	Computed from data on 15 functional traits* retrieved from the literature and measurements of herbarium specimens using the 'dbFD' function in the FD package in R (Villéger et al., 2008; Mouchet et al., 2010)
	Rao's entropy		

(*continued on next page*)

Table 1 (*continued*)

Attribute	Variable	Ecological significance	Methodological details
		Measure of the average functional dissimilarity among species, correcting raw functional diversity with species richness (Botta-Dukát, 2005)	
	Functional dispersion	Range of functional traits in a given community; represents the amplitude of niches (Díaz et al., 2007)	
	Functional richness	Number of functionally distinct species (Legras et al., 2018)	
	Functional evenness	Functional evenness captures the distribution of functional trait relative abundance (Mason et al., 2005)	
	Glomalin activity [μg g-1]	Indicates root colonization by mycorrhizal fungi (Vasconcellos et al., 2016)	Quantified as proposed by (Wright and Upadhyaya, 1998)

*We functionally characterized all species found in this study. Information about the maximum height, maximum diameter (Oliveira-Filho, 2017), wood density (Zanne et al., 2009), growth form (Jardim Botânico de Rio de Janeiro, 2020), fruit type, and dispersal and pollination syndrome (Silva et al., 2003; Ferraz et al., 2004; Amaral et al., 2009; Vásquez and Webber, 2010; Barretto and Catharino, 2015; Gastauer et al., 2015) was retrieved from public databases or the literature as indicated. Additionally, we measured the petiole length, leaf length, leaf width, fruit length and fruit width from herbarium specimens (MPEG) and computed the ratios between height and diameter, petiole and leaf lengths, and fruit length and fruit width.

the focal variables to determine whether their removal affects the estimation of the environmental quality of the areas. To assess whether excluding variables or variable groups affects indicator selection, the status computed without the focal variable(s) was remodeled with all 27 environmental variables as described above.

To evaluate whether the number of environmental variables for the estimation was sufficient, we used the bootstrapping method. The status estimation procedure is considered reliable if the status tends to stabilize with much fewer variables than were actually used (Gastauer et al., 2020a). To perform this evaluation, we computed the status for random subsamples that contained a smaller number of randomly selected variables than that in the full set (1000 randomizations for one, two, three, … 27 variables); evaluated the correlation coefficients, maximum residuals, and mean estimated rehabilitation status of advanced-stage sites; and derived the best indicator for each randomization.

To determine the theoretical impacts of additional, not-(yet)-monitored ecosystem properties on the overall estimation of the rehabilitation status and indicator selection, we simulated variables following eight empirical rehabilitation trajectories (Fig. 2): i) random variables without differences among rehabilitation stages; ii) variables that do not show enhancement over rehabilitation time and have higher values at the reference sites (two to five times higher than those at non-revegetated and rehabilitation sites); variables that increase by one to eight times per year and reach iii) 100%, iv) 20–80% or v) 140% of the reference level after seven years; vi) variables with values that increase by a factor of one to eight per year in relation to the reference values within five years but subsequently decline to non-revegetated levels; vii) variables that reach 20–100% of the reference values, with revegetation independent of stand age; and viii) variables that decline with revegetation and rehabilitation activities (Fig. 2). We added one, two or three simulated variables of the same variable type (corresponding to an additional sampling effort of 3.7, 7.4 and 11.1%, respectively) or one, two, three, five, or ten variables in random combinations of different variable types to the field-surveyed variable set. In each case, we carried out 1000 simulations, computed the rehabilitation status and derived the best indicators (additional sampling effort of up to 37%).

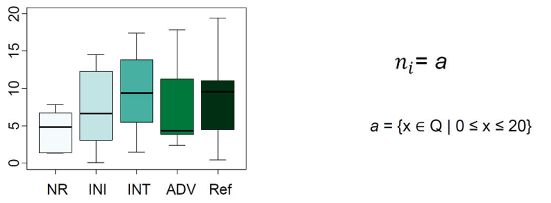
3. Results

3.1. *Vegetation inventories*

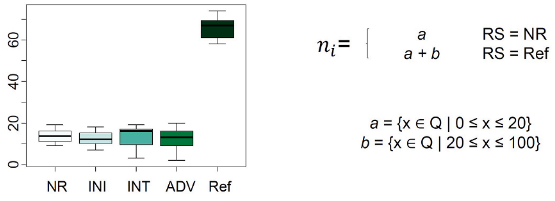
In total, 1,505 trees belonging to 197 species, 121 genera and 41 families were sampled. All three variables related to the vegetation structure increased over the course of rehabilitation, but only the tree density reached predisturbance levels in the advanced stages (Fig. S2). The taxonomic and phylogenetic tree diversity increased with rehabilitation time, and Pielou's evenness reached the reference levels in the intermediate-stage (three- to six-year-old stands), where it was higher than those in the non-rehabilitated, initial and advanced stages (Fig. S2). Different metrics of vegetation functional diversity were higher in the older stages than in the younger stages, and the intermediate and advanced stages did not differ from the reference sites in terms of functional diversity, functional dispersion or Rao's entropy (Fig. S3).

In total, 153 tree species were recorded at the reference sites, and 51 species were recorded at the sites undergoing rehabilitation; only seven species were found at both the reference and rehabilitating sites. This result indicates large floristic differences between rehabilitating and reference sites and explains the low similarity among the sites at all rehabilitation stages and reference sites (Fig. S2). The percentage of native trees at the sites in different rehabilitation stages did not statistically differ from that at the reference sites, but some plots showed an increased relative abundance of Singapore cherry (*Muntingia calabura* L., Muntingiaceae); additionally, some rehabilitating plots showed decreased native species coverage, mainly in the initial and intermediate stages (Fig. S2).

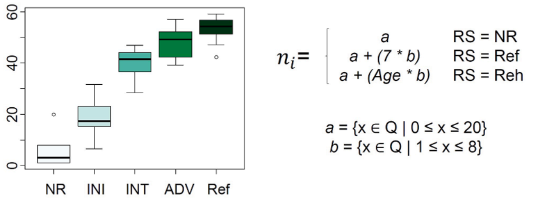
i) Variable unrelated to degradation and rehabilitation



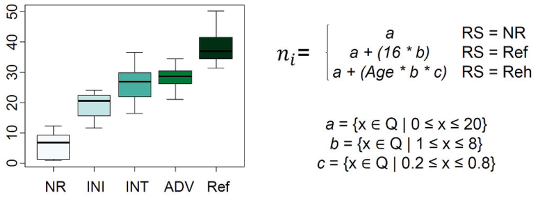
ii) Variable indiferente to revegetation and rehabilitation



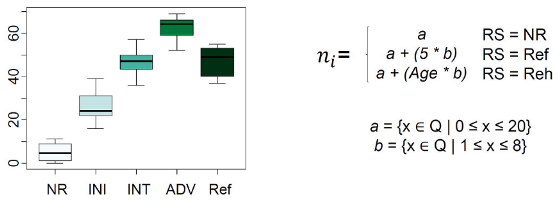
iii) Variable increasing to reference level within 7 years



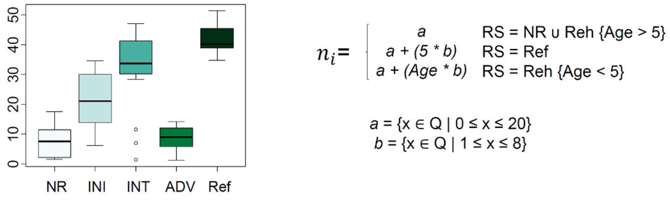
iv) Variable reaching 20-80% of reference levels within 7 years



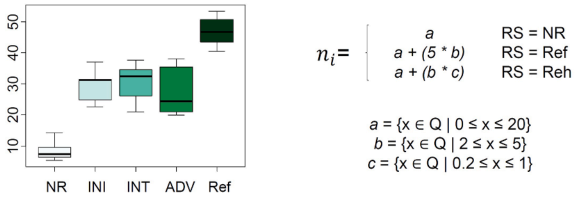
v) Variable increasing to 140% of reference level within 7 years



vi) Variable increasing to reference level, then declining



vii) Variable achieving 20-100% of reference level in revegetated sites



viii) Variable degrading with rehabilitation time

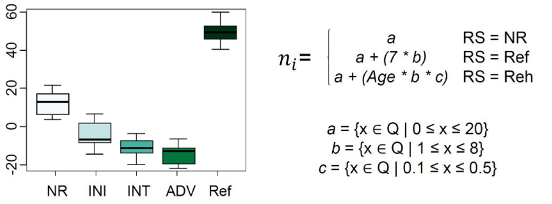


Fig. 2. Illustrations and mathematical formulae to simulate different variable types. a, b and c are randomly drawn numbers; a is individually drawn for each plot, and b and c (if necessary) are drawn once for each simulated variable. RS: rehabilitation stage; NR: non-revegetated sites; Reh: rehabilitation sites; INI: initial stage (up to three years of rehabilitation activities); INT: intermediate stage (three- to six-year-old stands); ADV: advanced stage (seven-year-old stands); Ref: reference sites covered by natural, undisturbed evergreen tropical forest.

3.2. *Invertebrate sampling*

In total, we collected 8,856 invertebrates, with 218 species belonging to 20 orders. The most abundant orders were Hymenoptera (1,125 individuals, of which 1,117 belonged to the Formicidae family), Collembola (315 individuals), Diptera (293 individuals) and Coleoptera (234 individuals). The invertebrate density and species richness were lowest at non-rehabilitated sites, but the rehabilitation sites did not differ from the reference sites in terms of these variables (Fig. S2). The rehabilitation sites had greater similarity to the reference sites than the non-rehabilitated sites, but the rehabilitation sites did not reach the reference levels (Fig. S2).

3.3. *Soil attributes*

All biological soil attributes except carbon in the microbial biomass increased with increasing rehabilitation time and reached the reference levels in the intermediate and advanced stages (Fig. S3). For example, glucosidase activity increased with rehabilitation time. Phosphatase activity was higher in the plots at the initial, intermediate and advanced stages than in the non-revegetated areas, but it was lower than that in the reference plots (Fig. S3). Urease activity was lower in non-revegetated plots and in the initial and intermediate stages than in the reference plots, but the sites in the advanced stage did not significantly differ from those in the other stages. Fluorescein diacetate (FDA) hydrolysis was higher at the reference sites than in the non-revegetated plots, while the sites in the various rehabilitation stages achieved intermediate scores, without significant differences. Finally, glomalin activity showed no increase along the rehabilitation chronosequence and was statistically lower than that at the reference sites (Fig. S3).

3.4. *Rehabilitation status and indicators*

When all variables were integrated via the PCoA, the mean rehabilitation status value at all sites undergoing environmental rehabilitation was 47.0%; the values ranged between 20.9 ± 2.6% for one-year-old WP S5, which was in the initial rehabilitation stage, and 66.0 ± 7.08% for seven-year-old WP NW2, which was in the advanced rehabilitation stage (Fig. 3). The rehabilitation status increased with increasing rehabilitation time (t-value = 9.711, p-value < 0.001) and was higher in the advanced stage (55.8 ± 14.3%) than in the intermediate (50.6 ± 10.5%) and initial stages (32.4 ± 14.9%). The Shannon index of tree diversity (H') was the best predictor of the rehabilitation status (Table 2), followed by the LAI, phylogenetic diversity, species richness and phosphatase activity, which performed statistically worse than the H' (ΔAIC greater than 10).

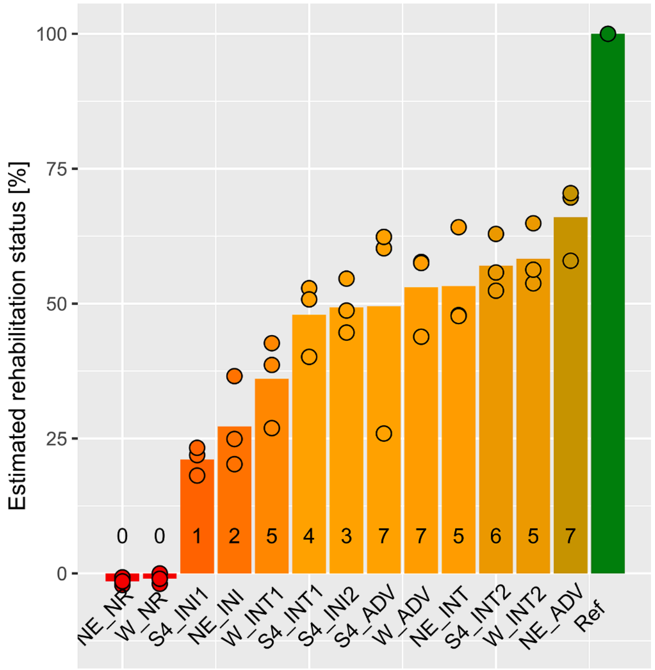


Fig. 3. Estimated environmental rehabilitation status along a chronosequence of waste pile rehabilitation in the Carajás National Forest, eastern Amazon. NR: non-revegetated sites; INI: initial stage (up to three years of rehabilitation activities); INT: intermediate stage (three- to six-year-old stands); ADV: advanced stage (seven-year-old stands); Ref: reference sites covered by natural, undisturbed evergreen tropical forest; NE: Northeast waste pile (WP); W: Western WP; S5: South 4 WP. The points indicate the rehabilitation status of individual plots. Numbers in the columns of the rehabilitation sites indicate the stand age at these sites.

Table 2

Validation of environmental variables as indicators of rehabilitation status using likelihood ratio tests and the Akaike information criterion (AIC) with linear mixed-effect models. R^2 is the coefficient of determination of the model and indicates the predictive power of the indicator for the rehabilitation status estimation; it was not a criterion for model selection.

Environmental variable	log-Likelihood	AIC	R^2
Shannon index of tree diversity (H')	44.920	−83.84	0.955
Leaf area index (LAI)	33.119	−60.23	0.893
Phylogenetic tree diversity	30.669	−55.34	0.916
Tree species richness	25.623	−45.28	0.882
Phosphatase activity	22.205	−38.41	0.877
Taxonomic evenness	17.588	−29.18	0.858
Functional evenness	17.179	−28.35	0.854
Tree dissimilarity to reference sites	13.577	−21.15	0.807
Functional richness	12.783	−19.56	0.816
Carbon in microbial biomass	12.604	−19.21	0.811
Basal area	11.788	−17.58	0.799
Glucosidase activity	10.613	−15.22	0.799
Glomalin activity	9.699	−13.39	0.784
% native species cover	7.548	−9.09	0.784
Invertebrate dissimilarity to reference sites	5.697	−5.39	0.749
Functional dispersion	3.171	−0.34	0.741
Functional diversity	0.921	4.15	0.709
Tree density	−2.489	10.98	0.651
% native trees	−3.138	12.39	0.641
Soil respiration	−3.547	13.09	0.631
Soil organic carbon	−4.651	15.30	0.609
Rao's functional entropy	−5.716	17.43	0.602
Urease activity	−6.384	18.77	0.565
Invertebrate species richness	−9.153	24.31	0.505
Number of sampled invertebrate specimen	−11.177	28.35	0.443
FDA	−11.826	29.65	0.417
Basal respiration	−15.256	36.51	0.254

With maximum modulus residual values below 3.5% for all 27 environmental variables, correlation coefficients above 0.99, and only marginal alterations of the rehabilitation status of advanced sites, no significant bias associated with any single variable affecting the rehabilitation status was detected (Fig. S4). The H' remained the best indicator, even when it was not used to compute the rehabilitation status (data not shown). When entire variable groups were removed, the residuals increased and eventually surpassed 5%; correlation coefficients below 0.99 were obtained when variables related to tree functional diversity (five variables), invertebrate fauna (three variables), overall diversity (ten variables) or ecological processes (14 variables) were not included, indicating the importance of these ecosystem properties during environmental assessments (Fig. S5). Again, the H' was the best indicator in all cases, and the mean rehabilitation status value for the sites at the advanced stage differed only slightly from the estimate derived from the full variable set (Fig. S5).

An increase in the number of variables enhanced the strength of the mean correlation with the estimate of the rehabilitation status using all variables. A random subset of seven field-surveyed variables was necessary to achieve a mean R^2 above 0.95. The use of more than 15 randomly selected variables guaranteed a mean R^2 value above 0.99 (Fig. 4), and the mean rehabilitation status value of advanced-rehabilitation-stage sites stabilized near the observed value. In all randomizations using more than 15 randomly selected variables, the H' was ranked as the best indicator of the rehabilitation status (Fig. 4).

The addition of up to three simulated variables of the same type only marginally affected the estimated rehabilitation status. Correlations between simulated and observed rehabilitation status values showed excellent correspondence, with a mean R^2 above 0.99 in all cases (Table 3); however, the mean rehabilitation status value decreased slightly when type-i, -ii, -iv or -vi variables were added. The addition of type-viii variables increased the rehabilitation status estimate in the advanced stage. The further addition of randomly selected variables reduced the rehabilitation status value. Increasing the number of simulated variables increased the effects on the overall estimated rehabilitation status. Only one ($n = \text{two}$) and two ($n = \text{three}$), respectively, of 1,000 randomizations ranked one of the type-iv simulated variables as being better rehabilitation status indicator than the H'.

4. Discussion

4.1. Mineland rehabilitation status of the examined waste piles

Here, we applied a recently described statistical approach to integrate a set of environmental variables to estimate the environmental quality of minelands in the eastern Amazon. By integrating information about the soil, vegetation and invertebrate fauna into a single measure, the present procedure to estimate the environmental rehabilitation status comprises the main components of terrestrial ecosystems and is consistent with international monitoring standards (Gann et al., 2019; Bandyopadhyay et al., 2020). We tested the variable set for sufficiency and eventual biases and provided simulations to outline the effects of additional, not-yet-surveyed environmental variables on the dataset and concluded that the estimate of the overall rehabilitation status in relation to the non-rehabilitated and reference sites was statistically sound and reliable.

The field-surveyed environmental variables differed greatly in terms of response ratios, ranging from no rehabilitation (e.g., similarity of the tree community to that at the reference sites) to complete restitution to predisturbance levels for some ecosystem properties (e.g., invertebrate species richness and functional diversity of tree communities). The vegetation structure is expected to recover faster than diversity and ecological processes (Suganuma and Durigan, 2014; Laughlin et al., 2017), but no such tendency was found in our study since variables in all three attributes showed increased variability. Despite these differences, we found higher response ratios for most individual variables and a

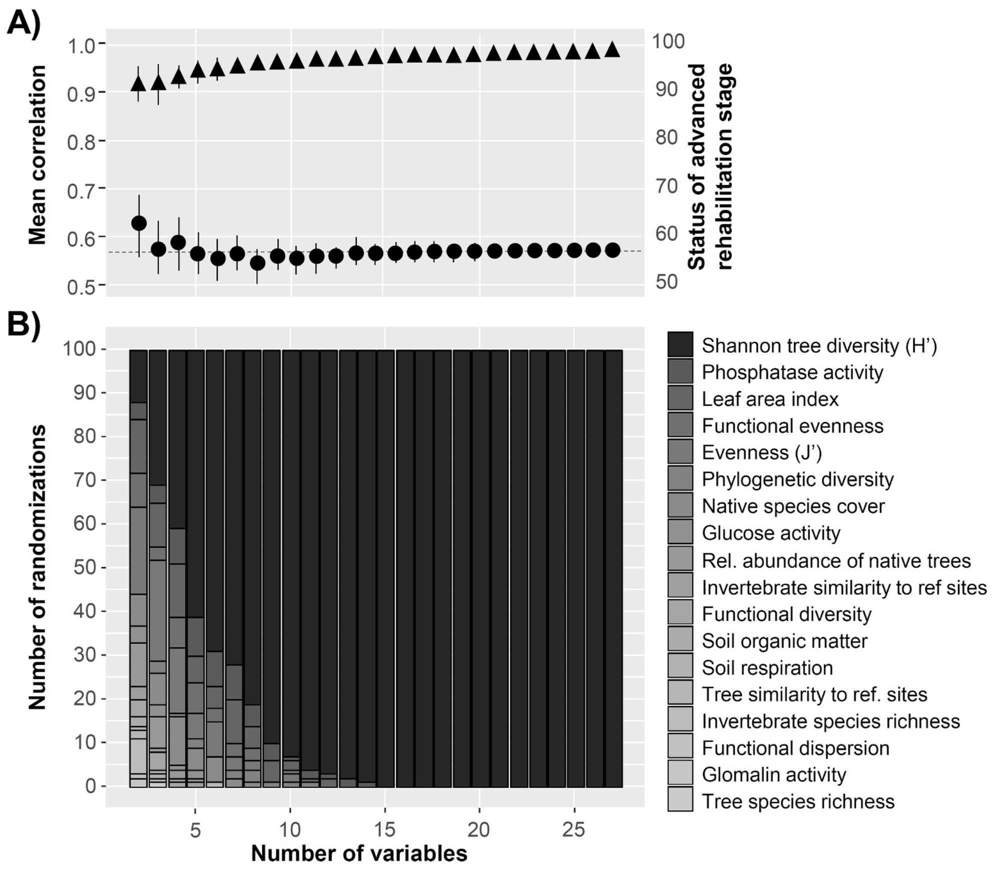


Fig. 4. Effect of variable bootstrapping on rehabilitation status and indicator selection. (A) Mean rehabilitation status of advanced stages (black circles) from 1000 randomizations based on fewer variables and mean coefficients of determination (black triangles) of the correlation between rehabilitation status estimated from the entire set and randomly reduced set of variables; the error bars indicate standard deviations. The dashed horizontal line represents the rehabilitation status of advanced sites computed from all 27 variables. See the methods for details. (B) Selected indicators for each randomization.

better rehabilitation status of older rehabilitation sites, in accordance with previous findings (Londe et al., 2020). Although only space-for-time substitutions, i.e., chronosequences instead of time series, were analyzed in this study, these data indicated overall environmental enhancement with rehabilitation time among the analyzed waste piles. Thus, the rehabilitation activities set the trajectories of these areas on a desired course, and rehabilitation areas tended to converge with pre-disturbance ecosystems (Gastauer et al., 2019), although full ecosystem rehabilitation requires longer periods than that actually observed (Ahirwal and Maiti, 2018).

The simulation of additional variables slightly affected the overall rehabilitation status. Not surprisingly, the simulation of type-ii (no

Table 3

Effects of additional simulated variables on rehabilitation status and indicator selection. The mean rehabilitation status of sites in the advanced stage (Adv Reh), mean correlation coefficient (R^2) values between simulated and observed rehabilitation status and number of simulations (out of 1,000), where the Shannon index of tree diversity (H') was ranked as the best indicator of the simulated rehabilitation status.

	n = 1			n = 2			n = 3			n = 5			n = 10		
Variable type	Adv Reh	R^2	H'	Adv Reh	R^2	H'	Adv Reh	R^2	H'	Adv Reh	R^2	H'	Adv Reh	R^2	H'
i) random variables	54.74 ± 0.61	0.9993	1000	53.91 ± 0.86	0.9987	1000	53.59 ± 1.19	0.9981	1000	–	–	–	–	–	–
ii) no advance	53.52 ± 0.68	0.9993	1000	51.38 ± 0.94	0.9979	1000	50.03 ± 0.95	0.9961	1000	–	–	–	–	–	–
iii) increase to reference level	55.76 ± 0.79	0.9997	1000	55.64 ± 1.08	0.9993	1000	55.63 ± 1.24	0.9989	1000	–	–	–	–	–	–
iv) increase to 20–80% of reference level	55.37 ± 0.76	0.9996	1000	54.97 ± 1.03	0.9992	999	54.65 ± 1.19	0.9988	998	–	–	–	–	–	–
v) increase to 140% of reference level	55.79 ± 0.76	0.9997	1000	55.67 ± 1.10	0.9993	1000	55.54 ± 1.27	0.9989	1000	–	–	–	–	–	–
vi) increase to reference level, then decline	53.94 ± 0.84	0.9992	1000	52.21 ± 1.17	0.9993	1000	50.83 ± 1.41	0.9966	1000	–	–	–	–	–	–
vii) advance (20–100%) with revegetation	55.85 ± 0.93	0.9997	1000	55.85 ± 1.22	0.9992	1000	55.89 ± 1.52	0.9986	1000	–	–	–	–	–	–
viii) declining	54.28 ± 0.67	0.9996	1000	53.21 ± 1.01	0.0001	1000	50.71 ± 1.23	0.9977	1000	–	–	–	–	–	–
Random selection	55.11 ± 1.24	0.9995	1000	54.34 ± 1.64	0.9989	1000	53.95 ± 2.03	0.9984	1000	52.92 ± 2.44	0.9973	1000	51.28 ± 3.04	0.9946	1000

enhancement along the rehabilitation chronosequence) or type-viii variables (further degradation) tended to reduce the estimate of the rehabilitation status. In contrast, the simulation of variables with higher response ratio values (e.g., type-vii variables) increased the estimated rehabilitation status in the advanced stage. The impacts on the overall rehabilitation status increased with the number of added variables, but moderate increases (up to three variables of a single type or up to ten variables of different types) only marginally affect the status since all the simulations estimated the rehabilitation status in the advanced stage as above 50% (compared to 55.8 ± 14.3% for the 27 field-surveyed variables).

Checking for biases regarding variables and variable bootstrapping revealed that the environmental rehabilitation status was only slightly affected by single variables or variable groups and remained stable, even when fewer variables were included in the statistical computation; this validates the use of the set of variables applied here. The aforementioned information validates the objectivity and confidence in our procedure for the estimation of the rehabilitation status and the derived indicator for further monitoring. Despite the need for further environmental enhancements at the studied sites, we conclude that a mean environmental status value above 50% after less than a decade of rehabilitation represents a very satisfactory result in these substantially impacted areas. To promote further improvements in the return of diversity (vegetation and fauna), recovery of vegetation structure and gradual restitution of ecosystem and soil functions in these areas, further monitoring activities are necessary.

4.2. The H' is the best surrogate for mineland rehabilitation status

In the presented dataset, the H', with intermediate response ratios (approximately 50% of reference levels at sites in intermediate and advanced stages), was the best predictor of mineland rehabilitation status among all the field-surveyed variables. This variable outperformed easily measurable variables including structural parameters (Viani et al. 2017), tree species richness (Londe et al., 2017), organic matter content (Bandyopadhyay and Maiti, 2019), and invertebrate community-related variables (Menta and Remelli, 2020), which were previously recommended for the measurement of mineland rehabilitation quality. Structural parameters were outperformed because stands with low tree diversity might show high performance in terms of vegetation structure, but reduced tree diversity reduces overall ecosystem functioning. Furthermore, the biological soil attributes of the analyzed waste piles and invertebrate communities quickly recovered after revegetation, and these variables indicate only the transition of exposed to revegetated soils. Since successional processes to reestablish ecosystem functions require larger time spans, the H' with intermediate response ratios was considered the most effective indicator for further monitoring activities in the region.

Interestingly, the H' was found to perform significantly better than other indices of diversity, such as species richness or metrics of functional or phylogenetic diversity. This phenomenon may result from the lower sensitivity of the H' to changes in rare species populations compared to species richness (and phylogenetic diversity, which is highly correlated with species richness) (Hill, 1973). Functional diversity performed poorly as an indicator because no differences between the sites in different rehabilitation stages and the reference sites were detected in this study. This result confirms the conclusions of previous studies (Gastauer et al., 2019) and highlights that the principal plant functional types are present in plots undergoing rehabilitation even though the taxonomic and phylogenetic plant diversity, vegetation structure and ecological processes have not completely recovered. Increases in taxonomic and phylogenetic diversity with increasing rehabilitation time and higher levels at reference sites are expected to principally contribute to functional redundancy and not the amplitude of ecological functions, which guarantees greater stability and community resilience (Kang et al., 2015).

Statistical support for the use of the H' for further monitoring activities remained high, even when the number of field-surveyed variables was decreased. Furthermore, additional new simulated variables did not alter the status of the H' as the best available indicator of the overall ecosystem and soil function. This statistical support and high predictive power of H' in terms of the overall rehabilitation status validates the use of this environmental variable as an indicator in future monitoring activities in the region. Expected increases in the H' during recurring surveys can indicate environmental enhancements in these areas over time, while any reduction in its value may indicate system degradation and requires further investigation into the underlying causes. As outlined above, the incorporation of the H' as an indicator for further monitoring activities may simplify and reduce the cost of further environmental monitoring of the rehabilitation of minelands in the evaluated region.

Finally, its positive relationship with overall rehabilitation status highlights the importance of tree diversity in environmental quality and overall rehabilitation, as more diverse tree communities are associated with better environmental quality and better performance of ecological processes and structural parameters. Tree diversity results from the colonization of spontaneously arriving propagules, but it may be directly affected by the successful establishment of additional species during active mineland rehabilitation (Ahirwal et al., 2017). Thus, the removal of dispersal barriers and the identification and development of propagation technologies for additional species, e.g., using functional approaches (Gastauer et al., 2020b), in future rehabilitation programs may contribute to higher overall rehabilitation success.

5. Conclusion

Despite large differences in the response ratios of individual variables, the proposed framework reliably estimated the rehabilitation status and complies with international monitoring standards. The detected environmental enhancements along the analyzed chronosequence highlight that the rehabilitation trajectories of the analyzed areas were on a desired course. Specifically, the restitution of more than 50% of predisturbance environmental quality in less than a decade in the examined areas highlights the importance of rehabilitation activities for the effective reduction in mining impacts.

The positive relationship between tree diversity and environmental status in the examined areas confirms the Shannon index as the most effective indicator to track further development in these areas; moreover, it will simplify and reduce the cost of more comprehensive monitoring activities in minelands undergoing rehabilitation in the Carajás National Forest in the future. The correlation further highlights the importance of diverse tree communities since the successful establishment of additional tree species during rehabilitation is expected to contribute to the faster return of biodiversity and soil functioning in minelands and reduce the overall impact of mining.

Statistically sound analyses to validate the selection of environmental variables for environmental assessments encourage similar approaches in cases without binding standards regarding which or how many environmental variables are required monitor rehabilitation or restoration activities. The identification of effective indicators to monitor rehabilitation activities may further contribute to more efficient environmental assessments in future monitoring projects.

CRediT authorship contribution statement

Markus Gastauer: Conceptualization, Methodology, Supervision, Writing - original draft, Writing - review & editing. **Priscila Sanjuan de Medeiros Sarmento:** Data curation, Formal analysis, Validation, Writing - review & editing. **Cecílio Frois Caldeira:** Conceptualization, Formal analysis, Writing - review & editing. **Arianne Flexa Castro:** Data curation, Validation, Visualization, Writing - review & editing. **Silvio Junio Ramos:** Conceptualization, Writing - review & editing. **Leonardo**

Carreira Trevelin: Formal analysis, Writing - review & editing. **Rodolfo Jaffé:** Formal analysis, Investigation, Writing - review & editing. **Gilliana Almeida Rosa:** Investigation. **Marco Aurélio Carbone Carneiro:** Investigation. **Rafael Borges da Silva Valadares:** Writing - review & editing. **Guilherme Oliveira:** Writing - review & editing. **Pedro Walfir Martins Souza Filho:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.108100.

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