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2 Acoustic indices perform better when applied at ecologically meaningful time and frequency scales

3 Oliver C. Metcalf<sup>1</sup>, Jos Barlow<sup>2,3,4</sup>, Christian Devenish<sup>1</sup>, Stuart Marsden<sup>1</sup>, Erika Berenguer,<sup>3,5</sup>

4 Alexander C. Lees<sup>1,6</sup>

5 1. Division of Biology and Conservation Ecology, Department of Natural Sciences, Manchester

6 Metropolitan University, Manchester, UK

7 2. Departamento de Biologia, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil

8 3. Lancaster Environment Centre, Lancaster University, Lancaster, Lancashire, UK

9 4. MCTIC/Museu Paraense Emílio Goeldi, Belém, Pará, Brazil

10 5. Environmental Change Institute, School of Geography and the Environment, University of

11 Oxford, OX1 3QY, Oxford, UK

12 6. Cornell Lab of Ornithology, Cornell University, Ithaca, NY 14850, USA

13 Contact author:

14 Oliver C. Metcalf<sup>1</sup>

15 [o.metcalf@mmu.ac.uk](mailto:o.metcalf@mmu.ac.uk)

16 Division of Biology and Conservation Ecology, Department of Natural Sciences, Manchester

17 Metropolitan University, Manchester, UK

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20 Running headline: Acoustic indices impacted by signal masking

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23 Abstract:

- 24 1. Acoustic indices are increasingly employed in the analysis of soundscapes to ascertain  
25 biodiversity value. However, conflicting results and lack of consensus on best practices for  
26 their usage has hindered their application in conservation and land-use management  
27 contexts. Here we propose that the sensitivity of acoustic indices to ecological change and  
28 fidelity of acoustic indices to ecological communities are negatively impacted by signal  
29 masking. Signal masking can occur when acoustic responses of taxa sensitive to the effect of  
30 interest are masked by less sensitive acoustic groups, or target taxa sonification is masked by  
31 non-target noise. We argue that by calculating acoustic indices at ecologically appropriate  
32 time and frequency bins, masking effects can be reduced and the efficacy of indices  
33 increased.
- 34 2. We test this on a large acoustic dataset collected in Eastern Amazonia spanning a  
35 disturbance gradient of undisturbed, logged, burned, logged-and-burned, and secondary  
36 forests. We calculated values for two acoustic indices: the Acoustic Complexity Index and the  
37 Bioacoustic Index, across the entire frequency spectrum (0-22.1 kHz), and four narrower  
38 subsets of the frequency spectrum; at dawn, day, dusk and night.
- 39 3. We show that signal masking has a large impact on the sensitivity of acoustic indices to  
40 forest disturbance classes. Calculating acoustic indices at a range of narrower time-  
41 frequency bins substantially increases the classification accuracy of forest classes by random  
42 forest models. Furthermore, signal masking led to misleading correlations, including  
43 spurious inverse correlations, between biodiversity indicator metrics and acoustic index  
44 values compared to correlations derived from manual sampling of the audio data.
- 45 4. Consequently, we recommend that acoustic indices are calculated either at a range of time  
46 and frequency bins, or at a single narrow bin, predetermined by *a priori* ecological  
47 understanding of the soundscape.

48

49 1 – Índices acústicos são cada vez mais utilizados em análises de paisagens sonoras para entender  
50 padrões de biodiversidade. Entretanto, sua aplicação em biologia da conservação e em contextos de  
51 manejo do uso do solo têm sido atrasada devido a resultados conflitantes e a uma falta de consenso  
52 sobre as melhores práticas a serem empregadas. Aqui nós propomos que a sensibilidade de índices  
53 acústicos em capturar mudanças ecológicas, assim como a fidelidade com que índices acústicos  
54 capturam comunidades ecológicas, são severamente impactados por mascaramento do sinal. O  
55 mascaramento do sinal pode ocorrer quando respostas acústicas sensíveis aos efeitos que estão  
56 sendo monitorados são mascaradas por outros grupos menos sensíveis ou quando a vocalização do  
57 taxa alvo dos estudos é mascarado por barulho de outros taxa. Nós argumentamos que ao calcular  
58 índices acústicos em intervalos apropriados de tempo e frequência, efeitos mascaradores podem ser  
59 reduzidos e a eficácia dos índices acústicos aumentada.

60

61 2 – Nós testamos isso em um vasto grupo de dados acústicos coletados na Amazônia oriental,  
62 abrangendo um gradiente de distúrbios antrópicos, incluindo florestas primárias não perturbadas e  
63 aquelas afetadas por extração madeireira, incêndios florestais, extração madeireira e incêndios,  
64 assim como florestas secundárias. Nós calculamos os valores de dois índices acústicos, o Índice de  
65 Complexidade Acústica e o Índice Bioacústico. Para isso, empregamos todo o espectro de  
66 frequências (0-22.1kHz) e quatro subgrupos menores do espectro de frequências: o amanhecer, o  
67 dia, o anoitecer e a noite.

68

69 3 – Nós mostramos que o mascaramento do sinal tem um grande impacto na sensibilidade dos  
70 índices acústicos a distúrbios florestais. Calculando índices acústicos em um intervalo menor de  
71 tempo-frequência aumentou substancialmente a acurácia da classificação das classes florestais por  
72 modelos do tipo Random Forest. Além disso, o mascaramento do sinal levou a correlações errôneas,  
73 incluindo correlações negativas espúrias entre métricas de biodiversidade e valores de índices

74 acústicos, quando comparados com correlações geradas a partir de amostragem manual dos dados  
75 de áudio.

76

77 4 – Consequentemente, nós recomendamos que índices acústicos sejam calculados em intervalos de  
78 tempo e frequência menores, pré-determinados por conhecimento ecológico a priori da paisagem  
79 sonora.

80

81 Key words:

82 Acoustic indices, ecoacoustics, remote sensing, bioacoustics, biodiversity, Amazonia, soundscape,  
83 tropical ecology

84

## 85 **Introduction**

86 Acoustic monitoring is rapidly becoming a key tool to measure biodiversity, with strident calls for  
87 broader uptake (Burivalova et al., 2019; Deichmann et al., 2018; Wagner Ribeiro Jr et al., 2017).

88 Despite increasing ease of data collection, there remain significant obstacles to the analysis of  
89 acoustic data, with species-level classification limited by the expertise and effort required to train  
90 machine-learning models, and the limited availability of both open source software and large audio  
91 libraries (Gibb et al., 2019; Priyadarshani et al., 2018). Consequentially, the use of acoustic indices  
92 has grown in popularity, often used as proxies for more traditional biodiversity metrics like species  
93 richness and composition, and presented as alternative effective tools for rapid biodiversity  
94 assessments (Sueur et al., 2008). There are a wide range of acoustic indices, but most involve  
95 calculating and comparing acoustic power within temporal and frequency bins (Farina, 2014; Sueur,  
96 Farina, Gasc, Pieretti, & Pavoine, 2014; Buxton et al., 2018; Gibb et al., 2019). These are, in turn,  
97 used to assess soundscape qualities such as evenness, entropy and complexity. Acoustic indices infer

98 community level information from entire soundscapes, in contrast to species-level classification  
99 approaches that require time-consuming complex model-training techniques necessitating large  
100 training libraries, indices are relatively simple and readily available on a range of open-source  
101 platforms.

102

103 Despite their increasing popularity, acoustic indices are not always effective at answering key  
104 questions related to conservation or natural resource management. The first issue relates to their  
105 *sensitivity* to changes in environmental conditions. Acoustic indices have been shown to effectively  
106 distinguish between disparate land uses (Bradfer-Lawrence et al., 2019; Carruthers-Jones et al.,  
107 2019; Depraetere et al., 2012). However, they are less successful in distinguishing differences  
108 between similar land uses; for example between different types of forest (Bormpoudakis et al.,  
109 2013, Eldridge et al., 2018, Do Nascimento et al., 2020), or require a very large number of spatial  
110 replications to do so (Mitchell et al., 2020). The second issue relates to their *fidelity* as indicators of  
111 biodiversity, as they can be inconsistent predictors of traditionally-used biodiversity metrics such as  
112 species richness (Eldridge et al., 2018; Fuller et al., 2015; Jorge et al., 2018; Mammides et al., 2017).

113 The problems of low sensitivity and inconsistent fidelity are potentially caused by signal  
114 masking – whilst certain vocalising taxa or taxonomic groups may respond strongly to  
115 changes in environmental condition, others may not. By measuring acoustic indices at  
116 intervals that measure across multiple taxonomic groups, sensitivity to these varied  
117 responses is lost, which may not be the case if indices were measured with multiple  
118 intervals. Similarly, fidelity to a single taxonomic group is lost by the use of broad time and  
119 frequency intervals, which may be improved by the use of narrower, tailored intervals.

120 There are two key ways in which signal masking can occur in acoustic indices. The first,  
121 temporal masking, can occur when acoustic indices are measured over time periods that  
122 are too long, so that sounds from sensitive time periods may be confounded by a lack of

123 change or contrasting responses in other time periods. For example, the vocal community  
124 at dawn may respond to a disturbance event very differently from the dusk community  
125 (Deichmann et al., 2017), so that measuring both together masks overall community  
126 responses. To avoid this, the analysis of acoustic indices often involves temporally limiting  
127 or splitting the data analysed into discrete periods, such as dawn and dusk (Bradfer-  
128 Lawrence et al. 2020, Deichmann et al., 2017; Fuller et al., 2015, Eldridge et al., 2018;  
129 Machado et al., 2017), selecting time periods that coincide with the peak communication  
130 time for certain groups.

131 The second form of signal masking, frequency masking, can occur when acoustic indices are  
132 measured at frequency bins that are too broad, so that sounds at sensitive frequencies are swamped  
133 by contrasting or null responses at other frequencies. Although the importance of frequency  
134 masking has not been explicitly considered in relation to acoustic index functioning, there is strong *a*  
135 *priori* reason to believe it may be important, and has been postulated by others (Eldridge et al.,  
136 2018). There is a broad negative relationship between body size and the frequency at which animals  
137 vocalize (Gillooly and Ophir, 2010; Ryan and Brenowitz, 1985; Seddon, 2005; Wilkins et al., 2013),  
138 meaning that the largest species, predominantly mammals, vocalize at the lowest frequencies, whilst  
139 orders composed of smaller species such as orthopterans predominate at higher frequencies. In  
140 addition, neotropical bird vocalisations exhibit both temporal and frequency partitioning to avoid  
141 signal masking from cicadas and other loud insects (Aide et al., 2017, Hart et al., 2015). At its  
142 simplest, this should result in different frequency bins being dominated by sounds from different  
143 broad taxonomic groupings.

144

145 Here, we use a data set from one of the world's most speciose ecosystems - the Brazilian Amazon –  
146 to explore how the use of time and frequency bins (henceforth TFBs) can improve the sensitivity and  
147 fidelity of acoustic indices. By calculating acoustic index values within restricted frequency bands,

148 the potential masking effect could be reduced, and correlations with specific taxonomic groups  
149 increased. Initially, we establish whether measuring indices at broad time and frequency scales, as is  
150 standard practice, masks variation in acoustic responses across narrower TFBs. Next, we look at the  
151 impact of signal masking on the efficacy of acoustic indices as a proxy for biodiversity and test the  
152 suitability of using TFBs as a solution by asking two questions of high relevance to practitioners and  
153 policy makers. First, do TFBs improve the sensitivity of acoustic indices to changes in forest condition  
154 (e.g. disturbance)? This is key to monitoring forest recovery following disturbances such as selective  
155 logging or wildfire, analyses which underpin many applied ecology questions. Second, do TFBs  
156 improve the fidelity of acoustic indices as proxies for traditional field surveys aimed at establishing  
157 species richness and composition? These field surveys can be expensive and inefficient for a range of  
158 taxonomic groups (Gardner et al., 2008), and if acoustic indices can be shown to be a reliable  
159 replacement for traditional survey methods, such as point count bird surveys, then they may offer a  
160 significant cost-saving.

161

## 162 Methods

### 163 Study area and data collection:

164 We collected acoustic data in the eastern Brazilian Amazon in the municipalities of Santarém-  
165 Belterra-Mojuí dos Campos (latitude -3.046, longitude -54.947, hereafter Santarém) in Pará state,  
166 between 12 June 2018 and 16 August 2018. We used the permanent transects of the Sustainable  
167 Amazon Network (Gardner et al. 2013) distributed in *terra firme* forest habitats. We sampled 28 300-  
168 m transects distributed into five forest classes: undisturbed primary forests (n = 4), logged primary  
169 forests (n = 4), burned primary forests (n= 5), logged-and-burned primary forests (n = 12), and  
170 secondary forests (n = 3); forests recovering after being completely felled). We installed Frontier  
171 Labs Bioacoustic Recording Units with a 16 bit 44.1 kHz sampling rate at points halfway along each  
172 transect. Recorders were placed in trees at a height of 7-10 m, with the microphone placed in a

173 downward facing position, at a distance of 10-20 m from the transect to reduce the chance of  
 174 recorder theft. Recording units were placed away from immediately overhanging dense vegetation  
 175 to avoid sound being blocked and to limit geophony from leaves and branches. The microphones  
 176 used have 80 dB signal to noise ratio and 14dBA self-noise, a fixed gain pre-amp of 20dB, a flat  
 177 frequency response ( $\pm 2$ dB) from 80Hz to 20kHz and an 80Hz high-pass filter to filter out low-  
 178 frequency wind noise (Frontier Labs, 2015). All files were recorded in wav format. Recordings were  
 179 made continuously (Frontier Labs software writes a new file every ~6 hrs) over multiple discrete time  
 180 periods of differing length at each point with discrete time periods ranged in duration between 3  
 181 and 20 days. Total recording duration and first and last recording dates are included in SOM 1  
 182 Appendix 1. The inaccessibility of some transects used in previous studies meant that a balanced  
 183 survey design was impossible across the disturbance categories (Table 1).

184 *Table 1: Audio sampling by forest class after automated removal of recordings containing heavy*  
 185 *rainfall*

Forest Class	Sampled points (n)	Total sampling time (minutes)
Primary	4	90,600
Logged primary	4	89,540
Burned primary	5	139,720
Logged-and-burned primary	12	238,130
Secondary	3	60,970

186

187 Data Analysis:

188 We selected two acoustic indices, the Acoustic Complexity Index and the Biodiversity Index as they  
 189 are two of the commonest indices used in ecoacoustic studies. However the Acoustic Complexity  
 190 Index is commonly applied across broad frequency ranges, and the Bioacoustic Index is typically



191 applied at restricted frequency ranges, making an ideal comparison for this study as in combination,  
192 they are likely to be representative of how many acoustic indices will be affected by the use of  
193 narrower time and frequency bins. The Acoustic Complexity Index is intended to quantify biotic  
194 sound whilst being robust to non-target noise (Duarte et al., 2015; Fairbrass et al., 2017; Pieretti et  
195 al., 2011), and is commonly applied across broad frequency bins. Acoustic Complexity Index  
196 measures the irregularity in amplitude across time samples by frequency bin, relative to the total  
197 amplitude of the frequency bin. The Acoustic Complexity Index has been found to significantly  
198 correlate with species richness for some taxa (Bertucci et al., 2016, Bradfer-Lawrence et al. 2020,  
199 Eldridge et al., 2018, Mitchell et al., 2020), whilst in others it showed little or no correlation (Fuller et  
200 al., 2015; Mammides et al., 2017; Moreno-Gomez et al., 2019) although this may be due to  
201 limitations in methodology and small sample sizes. In contrast, the Bioacoustic Index (BI) is generally  
202 applied to narrower frequency bins, and is intended to provide relative abundance of avian  
203 community within a frequency range that contains most bird sound (Boelman et al., 2007). It  
204 measures the disparity between the quietest and loudest 1 kHz frequency bins. Again, the  
205 Bioacoustic Index has been found to be a good predictor of diversity in some studies (Eldridge et al.,  
206 2018, Hilje et al., 2017, Gasc et al., 2017, Mitchell et al., 2020) whilst others have found it to be poor  
207 (Fuller et al., 2015, Moreno-Gomez et al., 2019), although concerns about the limitations of the  
208 methodologies used in these studies apply here too. We expect both indices to increase with  
209 increasing species richness and species abundance, and for correlations between both abundance  
210 and richness with the indices to be strongest in the frequency and time bins that are most  
211 dominated by the target taxa (Table 2), particularly diurnal bird species at dawn between 0.3-12 kHz  
212 and nocturnal taxa at night between 0.3-4 kHz.

213

214 We calculated the indices using the soundecology package (Villanueva-Rivera et al., 2011, v1.3.3) in  
215 R (R Core Team, 2019) which includes minimum and maximum frequency limits for both the Acoustic

216 Complexity Index and Bioacoustic Index, allowing easy and consistent index calculation at a range of  
217 frequency bins. To limit microphone self-noise the lowest frequency included in analysis was 300 Hz.  
218 We then calculated the mean index value per 10 minute interval of data collected for each acoustic  
219 index and each of the 20 TFBs (Fig.1a), having first screened out recording periods containing heavy  
220 rainfall (n = 527) using the hardRain package in R Studio (Metcalf et al., 2020, v0.1.1).

221 We selected TFBs with the objective of capturing periods of time and frequency bands that  
222 are most taxonomically homogenous. TFBs were not quantitatively optimised, but rather  
223 subjective approximations that aimed to effectively capture broad taxonomic groupings in  
224 tropical forest landscapes. Temporal limits were determined by patterns in animal  
225 communication in the diel cycle, to encapsulate dawn, dusk, daytime and night-time  
226 periods (Pieretti et al., 2015; Rodriguez et al., 2014); commonly used sampling periods in  
227 acoustic recording (Sugai et al., 2019). 'Dawn' was assigned to the period from 30 minutes  
228 prior to sunrise and for the following 2 hours, whilst 'Dusk' was the 2 hour period ending  
229 at 30 minutes after sunset. 'Day' and 'Night' are the respective intervening periods.

230 Frequency limits were determined by a review of the literature and our own experience of  
231 manually analysing 100s of hours of acoustic data from the region. The taxonomic  
232 groupings we hypothesize dominate each TFB are illustrated in Figure 1a. Table 2 contains  
233 some of the TFBs likely to contain particularly high activity from particularly homogenous  
234 groupings. 0.3-22.1 kHz frequency bin (hereafter 'baseline') was used as baseline data,  
235 representative of how most terrestrial acoustic indices are currently calculated across the  
236 spectrum of human hearing or the common sampling rate of 44.1 kHz. However, it is  
237 worth noting that the Bioacoustic Index is commonly calculated with a narrower frequency  
238 bin than the baseline, typically from 2 to 8 or 11 Khz (Boelman et al., 2007, Bradfer-  
239 Lawrence et al., 2019, Villanueva-Rivera et al., 2011). We have used the same baseline as  
240 the Acoustic Complexity Index for ease of comparison, and because the mechanisms

241 causing masking between ecologically relevant and non-relevant frequency bins is the  
 242 same regardless of absolute frequency. Of course, macro frequency bands will never solely  
 243 encompass single taxonomic groups, and boundaries will always be somewhat arbitrary  
 244 due to variations in acoustic communication at species, temporal and even individual  
 245 levels.

246 *Table 2: Selected time-frequency bins and the taxonomic groups expected to dominate each sample.*

Frequency Band (kHz)	Time Period	Taxonomic group	References
0.3-4	Night	Terrestrial/arboreal mammals, anuran and birds	Chek, Bogart, & Loughheed, 2003; Lima, Pederassi, Pineschi, & Barbosa, 2019
4-12	Day	Hemiptera/Orthoptera	Hart et al., 2015, Schmidt et al., 2013
4-12	Night	Hemiptera/Orthoptera	Hart et al., Schmidt et al., 2013
0.3-12	Dawn	Diurnal/crepuscular birds	Tobias, Planqué, Cram, & Seddon, 2014
12-22.1	Dusk	Insects, bats and frogs	Lima, Pederassi, Pineschi, & Barbosa, 2019, Schmidt et al., 2013
12-22.1	Night	Insects	Schmidt et al., 2013

247

248 Signal Masking

249 To investigate whether the soundscape responds differently to human-driven disturbance across  
250 time and frequency, we looked at the variation in response of each disturbance class for each TFB.  
251 Having removed periods with extreme outlying index values, we took a random sample of acoustic  
252 index values for each acoustic index and TFB from each forest class (n=500), giving a total sample  
253 size of n=2500 per TFB/index. For each TFB and acoustic index we conducted a Kruskal-Wallis  
254 (Kruskal and Wallis, 1952) test between the five forest classes, and calculated the effect size ( $\epsilon^2$ ).  
255 When significant differences between the classes were found, we used a Dunn's test (Dunn, 1964) to  
256 establish how many of the ten forest class pairs were significantly different from each other.

257

258 The sensitivity of acoustic indices to habitat

259 To assess whether the use of TFBs increased acoustic index sensitivity to forest classes, we built  
260 distributed random forest models from the 'h2o' R package (LeDell et al., 2020 v3.30.0.1), varying  
261 the number of TFBs used as predictors. Firstly, we tested if the use of TFBs improved classification  
262 accuracy between the two most ecologically distinct sampled habitats; undisturbed primary forest  
263 and secondary forest (Moura et al., 2013). To do so, we built two binomial random forest models,  
264 the first using training data only from the baseline frequency bin across all time periods, the second  
265 using training data from all frequency bins and time periods. Next, models were trained and tested  
266 on data from all five forest classes, which previous studies (e.g. Moura et al. 2013) suggest would  
267 provide a more challenging classification problem.

268 We used each combination of index and TFB as a separate predictor. The training datasets required  
269 subsampling to obtain predictors of equal length, as not all time periods were of the same duration,  
270 and forest classes had unequal survey effort. We used the same subsample as above (see Signal  
271 Masking), so that each TFB predictor had n=2,500 samples, with 500 samples from each forest class.

272 This resulted in a greatly reduced dataset for training the models with 100,000 acoustic indices  
273 values compared to 1,277,560 in the original dataset. Prior to model training, the dataset was split  
274 with 75% of observations used for training and 25% as a test dataset. Model parameters were kept  
275 constant across all models (SOM 1 Appendix 2). We used balanced accuracy (Fielding and Bell, 1997),  
276 F1 scores (Chinchor, 1993) and Matthew's Correlation Coefficient (Guilford., 1954) as accuracy  
277 metrics (Table 3), which were calculated per forest class based on predictions of the test dataset and  
278 are presented here as an unweighted mean across all forest classes included in the respective  
279 model.

280

#### 281 Fidelity of acoustic indices to taxonomic measures of biodiversity

282 We assessed correlations between acoustic index scores and biodiversity indicator metrics, to see  
283 how representative the indices were of commonly used indicators of diversity. Data on the  
284 presence/absence of three sets of species were generated from two subsets of the audio data. Each  
285 audio subset consisted of 28 hrs of sound recordings, in the form of 240 15 s recordings from each  
286 point. The first data set was restricted to the dawn period (hereafter dawn birds), in which all  
287 identifiable avian vocalisations were assigned to species by an ornithologist (Nárgila Gomes De  
288 Moura) with extensive field experience of point counts in the same sites (e.g. Moura et al. 2013).  
289 This method of species detection is likely to produce comparable results to traditional point count  
290 surveys as several papers have shown that experienced observers reviewing recordings and  
291 spectrograms can be more or equally effective at detecting species than field-based surveys (Darras  
292 et al., 2019; Shonfield et al., 2018). The second set of data was restricted to the nocturnal period  
293 (hereafter nocturnal birds), and again all identifiable avian vocalisations were assigned to species by  
294 an experienced ornithologist (OCM). The third set was generated from the nocturnal data subset  
295 again (hereafter nocturnal taxa), but comprises all biophony below 4kHz, identified (by OCM) where

296 possible or sonotyped if not. It is worth noting that all of the bird species identified at night vocalized  
297 below 4 kHz, so that the nocturnal bird set is wholly a subset of the nocturnal taxa set.

298

299 For each of these matrices (i.e. dawn birds, nocturnal birds and nocturnal taxa), five metrics were  
300 calculated; total number of encounters (the sum of the number of 15 s recordings each species was  
301 present in), species richness, Shannon diversity, Pielou's evenness, and the first axis from a  
302 nonmetric multidimensional scaling ordination (hereafter MDS1) using the Jaccard method from the  
303 vegan package (Oksanen et al., 2019). Total encounters was included as a proxy for the abundance  
304 of sounds, to test if indices responded more strongly to more sources of noise, regardless of  
305 composition. Estimated species richness from the dawn matrix was calculated for each point at  
306 98.5% coverage based on rarefaction/extrapolation using the iNEXT package (Hsieh et al., 2020,  
307 v2.0.20), as some of the survey files were removed as they contained periods of heavy rain which  
308 affected the number of vocalizing species. Observed species richness was used for metrics from the  
309 nocturnal matrix, as the data were pre-screened for rain. Shannon diversity, Pielou's evenness and  
310 species richness were included as standard measures of ecological diversity (Oksanen et al., 2019).  
311 MDS1 was included to reflect turnover mediated by disturbance, as high values correspond with less  
312 disturbed habitats, whilst lower values have communities associated with more disturbed habitats.  
313 Correlations between these metrics are available in SOM 1 Appendix 3. Median values of each  
314 acoustic index were calculated for each point and TFB, and Spearman's rank order correlations ( $\alpha =$   
315 0.05) were calculated between these and the biodiversity metrics. Significant differences between  
316 each correlation and the respective baseline correlation were calculated using Zou's confidence  
317 interval test (Zou, 2007) in the 'cocor' package (Diedenhofen and Musch, 2015 v.1.1-3).

318

319 Results

320 Sensitivity: forest disturbance

321 The Kruskal-Wallis and Dunn's test revealed strong evidence that acoustic masking affects the  
322 sensitivity of acoustic indices, both temporally and by frequency. All of the Kruskal-Wallis tests were  
323 significant ( $p < 0.05$ ), showing that acoustic indices are sensitive to at least some disturbance events  
324 regardless of frequency band or time period. There were significant differences between all ten  
325 forest class pairs in every time period and with both indices when considering all frequency-  
326 restricted TFBs together. In contrast, there were no time periods with significant differences  
327 between all forest class pairs when using only the baseline TFBs, but Acoustic Complexity did have  
328 significant differences between nine forest class pairs in three time periods, and Bioacoustic Index  
329 once. Twelve TFBs showed significant difference ( $p < 0.05$ ) between more forest classes than the  
330 corresponding baseline, and 21 TFBs had higher effect sizes than the corresponding baseline,  
331 suggesting that in many cases stronger responses to disturbance events at narrower frequency bins  
332 are masked by the use of broad frequency bins (Fig. 2). No baseline TFB achieved perfect separation  
333 between all ten forest-class pairs but this was achieved by three of the non-baseline TFBs.  
334 Furthermore, Acoustic Complexity Index at dusk and the baseline frequency bin produced the lowest  
335 number of significantly different forest class pairs, just two, suggesting that using only the broadest  
336 frequency bin can result in relatively poor differentiation between forest disturbance classes. No  
337 one frequency bin or time period had a consistently larger effect size, or consistently differentiated  
338 between more forest classes. There were several occasions in which effect size increased in  
339 comparison to the baseline, whilst the number of different forest classes decreased (e.g. Acoustic  
340 Complexity Index at dawn, 0.3-4 kHz, Bioacoustic Index at night, 0.3-4 kHz). This suggests that the  
341 soundscape at this frequency bin is showing a particularly strong response to disturbance in one or  
342 more of the forest classes (in SOM 1 Appendix 4)."

343

344 Sensitivity: Forest class differentiation

345 The random forest models generated using all of the TFBs as predictors were able to classify forest  
346 classes with a high degree of accuracy, with 99.6% balanced accuracy between secondary and  
347 undisturbed forest and 88.2% between the five forest classes (Fig. 3). The models using all TFBs as  
348 predictors outperformed the corresponding baseline models in both tests, but as expected the  
349 baseline models performed particularly poorly when classifying between all five forest classes,  
350 achieving just 62.1%. The confusion matrix for the random forest model using all TFBs across all five  
351 classes suggest that acoustic indices do respond to soundscapes in ecologically meaningful ways, as  
352 both burned forest classes had comparatively high error between them, as did the two most  
353 disturbed classes, logged and burned vs secondary forest (SOM 1 Appendix 5).

354

#### 355 Fidelity: Biodiversity Correlations

356 Correlations with traditional biodiversity metrics revealed complex patterns, underpinned by strong  
357 variation across index, frequency bins and time periods. For simplicity, we have focussed on time  
358 periods in which acoustic index values most directly reflect variation in manually reviewed datasets -  
359 dawn and day time for the dawn bird dataset, and night for the nocturnal datasets (Fig. 4),  
360 Correlations at other time periods are presented in SOM 2 Appendix 6. The two strongest  
361 correlations, were at night between Bioacoustic Index and diurnal avian MDS1 at 4-12 kHz ( $r_s = 0.74$ )  
362 and dusk at 0.3-12 kHz ( $r_s = 0.72$ ).

363 The correlation scores provide strong evidence that the use of TFBs increase the fidelity of  
364 correlations. We found that correlation directions of the frequency/ bins differed from the  
365 corresponding baseline frequency bin for at least one of the metrics in every time period in both  
366 indices. The strongest examples of this were between Bioacoustic Index at dawn with dawn birds,  
367 which saw predominantly significant positive correlations at frequency bands at which dawn birds  
368 vocalize, but negative correlations in the baseline and 12-22.1 kHz frequency bins. We also found 28



369 instances in which correlations were significantly different to the corresponding baseline frequency  
370 bin.

371 Overall, the Acoustic Complexity Index was inconsistently correlated with biodiversity indicator  
372 metrics with predominantly negative and significant correlations with diversity metrics at dawn, but  
373 mostly positive correlations during the day and at night for most frequency bands, with far fewer  
374 significant correlations. In contrast, the Bioacoustic Index showed predominantly positive  
375 correlations with most diversity metrics except MDS1 across all three time periods and all three  
376 frequency bins only including sound below 12 kHz. For the community metrics most likely to be  
377 useful to ecologists, species richness and Shannon diversity, there were 19 significant correlations.  
378 However, where we found significant correlations with these metrics, there were still strong reasons  
379 for doubting the fidelity of acoustic indices as proxies. At dawn, correlations for both indices were  
380 weaker than the respective correlations with total encounters or MDS1, suggesting the indices were  
381 more sensitive to the number of individual sounds or the overall community. At night, nocturnal  
382 birds and taxa correlations with the Bioacoustic Index showed conflicting patterns with correlations  
383 at frequency bins more likely to be relevant to the relevant taxonomic group, suggesting a strong  
384 masking effect by vocalisations of non-target taxa. The exception to this is the correlation between  
385 dawn birds and BI during the day at 0.3-4 kHz, which shows strong correlations with species richness  
386 and Shannon diversity ( $r_s = 0.44$  and  $0.40$ ), a similar correlation with total encounters ( $r_s = 0.40$ ) and  
387 no significant correlation with MDS1.

388

### 389 Discussion

390 We found that calculating acoustic indices at narrower TFBs results in large increases in the  
391 sensitivity of acoustic indices to the soundscape response of different forest classes. Calculating  
392 acoustic indices across a single broad frequency bin, as is commonplace in the ecoacoustic literature  
393 (Sueur et al., 2014; Buxton et al., 2018; Gibb et al., 2019) can mask varied responses across time

394 periods and frequency bins, reducing the sensitivity of acoustic indices. Furthermore, when acoustic  
395 indices are used as proxies for biodiversity indicator metrics, masking can have a serious impact on  
396 the fidelity of the correlations. Correlating broad frequency bins with biodiversity metrics generated  
397 from taxa whose vocalisations do not occur across the entire frequency range is likely to be highly  
398 misleading. It not only misrepresents the magnitude of correlations, but potentially results in  
399 spurious inverse correlations caused by the responses of acoustically dominant species or patterns  
400 from acoustic space that are not biologically relevant.

401

402 The sensitivity gains of this new methodology are particularly apparent when using acoustic indices  
403 to differentiate between environments with classification accuracy greatly increased. Accuracy  
404 scores are as good or better than many in the literature (Bormpoudakis et al., 2013; Bradfer-  
405 Lawrence et al., 2019; Eldridge et al., 2018, Do Nascimento et al., 2020), especially considering that  
406 model hyperparameters are not optimised, fewer acoustic indices were used, and that the forest  
407 classes considered here are all of the same land cover (tropical forest) and within the same  
408 landscape. In addition, these results have been achieved whilst using only approximately one sixth of  
409 the training data compared to the models using only a single baseline frequency band, suggesting  
410 that by using TFBs, large efficiency savings can be made in terms of data collection. We therefore  
411 recommend that acoustic indices are calculated across a range of frequency bins and temporal  
412 periods in any study using acoustic index values to characterise and identify land use.

413 The impact of signal masking, and the benefits of using narrower time-frequency bins to avoid it, are  
414 equally apparent when correlating acoustic indices with biodiversity metrics. However, despite the  
415 increased fidelity of the correlations, the use of single acoustic indices as direct proxies for  
416 biodiversity indicator metrics is still problematic. Whilst we found the predicted positive correlations  
417 between diversity, richness and the Bioacoustic Index at the most relevant TFBs, we found a  
418 negative correlation between the Acoustic Complexity Index at dawn with bird species richness and

419 abundance. This contrasts with other similar studies in comparable habitats that found positive  
420 relationships (Bradfer-Lawrence et al., 2020, Eldridge et al., 2018, Mitchell et al., 2020). However,  
421 Mitchell et al., (2020) found high Acoustic Complexity values in oil-palm plantations where diversity  
422 was low, and noted that the significant relationships they found were within habitat types, but not  
423 across different habitats. Furthermore, the complex mechanisms determining abundance and  
424 species richness in tropical forests remain poorly understood, particularly in relation to the impacts  
425 of disturbance (Barlow et al., 2016, Terborgh et al., 1990). It is possible that idiosyncratic responses  
426 of single or a few taxa to disturbance could create such a negative correlation (Moura et al., 2016),  
427 especially if the taxa are acoustically dominant. In general, the strongest correlations we found were  
428 with total encounters and MDS1 – metrics that would only be of ecological interest if the  
429 underpinning species were well understood, requiring extensive manual surveys and undermining  
430 the purpose of acoustic indices. Despite this, the strong positive correlation between Bioacoustic  
431 Index and dawn bird species richness and diversity in the day at 0.3-4 kHz is interesting. It is plausible  
432 that this time-frequency bin contains the least vocalization from non-target taxa, insects sonify  
433 predominantly around >4 kHz and after the dawn chorusing of acoustically dominant vertebrates,  
434 particularly red-handed howler monkey *Alouatta belzebul* (Sekulic, 1982). Furthermore, it is after the  
435 end of the bird dawn chorus, during which it is possible that intense vocal activity of a few species  
436 may mask underlying richness and diversity.

437 We have deliberately chosen to use subjective frequency bins determined by *a priori* knowledge of  
438 acoustic space use in our study system, to demonstrate both the wide applicability of this method,  
439 and that frequency bin selection need not be onerous to generate substantial benefits. However,  
440 choosing narrower or different frequency bins and time periods based on prior quantification of  
441 acoustic space use could provide substantial further benefits in understanding the effects of signal  
442 masking on correlations. Several existing methods exist to do so, either comprehensively through  
443 the multiscalar fractal approach (Monacchi and Farina, 2019), or more broadly using measures of  
444 acoustic space use or biophonic density (Aide et al., 2017, Eldridge et al., 2018) Quantifying TFB

445 dominance by even broad acoustic clades could be highly informative and could provide quantifiable  
446 data on the relative effect size of the impact of disturbance types on those clades. Additionally,  
447 variation in the granularity of TFBs may well reveal further unknown ecological patterns. Whilst we  
448 have focused primarily on masking in the frequency domain, and across the diel cycle in the  
449 temporal domain, it is entirely plausible that analysis of acoustic indices at both greater and finer  
450 temporal scales, and broad frequency ranges, could reveal other patterns. For instance, within dawn  
451 choruses where we already know bird species can hold very specific temporal niches in the tropics  
452 (Fjeldså et al, 2020), or across seasonal scales such as the winter midday chorus in temperate forests  
453 (Farina and Gage, 2017).”

454 We found that acoustic indices are sensitive to soundscapes modified by habitat disturbance and can  
455 therefore be highly cost-effective tools for assessing forest condition and monitoring changes in  
456 conservation value in response to management interventions or other environmental changes.  
457 Acoustic indices are however highly susceptible to signal masking, where divergent responses across  
458 temporal and frequency spectrums are masked by calculating indices at inappropriate scales. We  
459 therefore recommend that acoustic indices are calculated either at a range of time and frequency  
460 bins when used to characterise a landscape, or a narrow bin predetermined by *a priori* ecological  
461 understanding of the soundscape when used as a proxy for the biodiversity of a specific taxonomic  
462 group.

463

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475 Author's contributions:

476 OCM designed the study, undertook data collection, analysed the data and drafted the article. ACL,  
477 JB and SM made significant contributions to the study conception and design, aided in data analysis  
478 and made substantial revisions to manuscript drafts. CD contributed significantly to the data analysis  
479 and made substantial revisions to manuscript drafts. EB contributed greatly to the study design,  
480 collection of the data, and made substantial revisions to manuscript drafts.

481 Data Availability:

482 The following datasets are available on the Dryad repository:

- 483 1. All of the indices values used in this research
- 484 2. The subset of indices values used for the sensitivity analysis
- 485 3. The full set of results from the sensitivity analysis
- 486 4. All of the diversity metrics used in the correlation analysis

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