Contributed Paper

Use of ecoacoustics to determine biodiversity patterns across ecological gradients

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Abstract: *The variety of local animal sounds characterizes a landscape. We used ecoacoustics to noninvasively assess the species richness of various biotopes typical of an ecofriendly forest plantation with diverse ecological gradients and both nonnative and indigenous vegetation. The reference area was an adjacent large World Heritage Site protected area (PA). All sites were in a global biodiversity hotspot. Our results showed how taxa segregated into various biotopes. We identified 65 singing species, including birds, frogs, crickets, and katydids. Large, natural, protected grassland sites in the PA had the highest mean acoustic diversity (14.1 species/site). Areas covered in nonnative timber or grass species were devoid of acoustic species. Sites grazed by native and domestic megaherbivores were fairly rich (5.1) in acoustic species but none were unique to this habitat type, where acoustic diversity was greater than in intensively managed grassland sites (0.04). Natural vegetation patches inside the plantation mosaic supported high mean acoustic diversity (indigenous forests 7.6, grasslands 8.0, wetlands 9.1), which increased as plant heterogeneity and patch size increased. Indigenous forest patches within the plantation mosaic contained a highly characteristic acoustic species assemblage, emphasizing their complementary contribution to local biodiversity. Overall, acoustic signals determined spatial biodiversity patterns and can be a useful tool for guiding conservation.*

Keywords: biodiversity assessment, ecological networks, insect conservation, landscape mosaics, protected areas, soundscape ecology

El Uso de la Eco-Acústica para Determinar los Patrones de Biodiversidad a lo largo de Gradientes Ecológicos

Resumen: *La variedad de los sonidos de los animales locales caracteriza a un paisaje. Utilizamos eco-acústicas para valorar de manera no invasiva la riqueza de especies de varios biotipos típicos de una plantación de bosque amigable con el ambiente y con diversos gradientes ecológicos y vegetación nativa y no nativa. El área de referencia fue una gran área protegida (AP) adyacente considerada sitio de Patrimonio Mundial. Todos los sitios se encontraban dentro de un punto caliente de biodiversidad global. Nuestros resultados mostraron cómo los taxones se segregaban en varios biotipos. Identificamos a 65 especies canoras, incluyendo aves, ranas, grillos y cigarras. Los sitios de pastizales grandes, naturales y protegidos dentro del AP tuvieron la media más alta de diversidad acústica (14.1 especies/sitio). Las áreas cubiertas por árboles no nativos o por especies de pasto estuvieron desprovistas de especies acústicas. Los sitios pastados por mega-herbívoros nativos y domésticos tuvieron bastante riqueza (5.1) de especies acústicas pero ninguna de estas especies era única en este tipo de hábitat, mientras que la diversidad acústica fue mayor que en los sitios de pastizal con un manejo intensivo (0.04). Los fragmentos de vegetación natural dentro del mosaico de la plantación permitían una media alta de diversidad acústica (bosques nativos 7.6, pastizales 8.0, humedales 9.1), la cual incrementaba conforme la heterogeneidad de las plantas y el tamaño de los fragmentos incrementaban. Los fragmentos de bosque nativo dentro del mosaico de la plantación contenían un ensamblaje de especies acústicas altamente característico, lo que enfatiza su contribución complementaria a la biodiversidad local. En general, las señales acústicas determinaron los patrones espaciales de la biodiversidad y puede ser una herramienta útil para guiar a la conservación.*

Introduction

Environments are infused with sound produced by animals, humans, and other natural and anthropogenic sources to form soundscapes (Pijanowski et al. 2011). At a species level, acoustic communication allows conspecifics to identify, locate, and assess the fitness of potential mates (Greenfield 2002). When one characterizes and classifies these species-specific signals, signaling species can be identified. Even though a species may not be taxonomically identifiable, classification of signal parameters allows one to define recognizable taxonomic units (Riede 1993; Tishechkin 2014). Acoustic surveys can sometimes provide similar results as visual point-count surveys (Sedláček et al. 2015) and are frequently used for estimating species richness for bats (e.g., MacSwiney et al. 2008), frogs (e.g., Villanueva-Rivera 2007), birds (e.g., Farina et al. 2011), and insects (e.g., Riede 1993), including katydids (Orthoptera: Tettigoniidae) (e.g., Riede 1998; Penone et al. 2013). But many of these sound-emitting animals are hidden from view in vegetation or in darkness; acoustic recognition can reveal their presence. This means that detection of sound provides a noninvasive proxy for investigating diversity patterns across ecological gradients, an approach known as ecoacoustics (Sueur & Farina 2015), and that ecoacoustics is useful for conservation (Laiolo 2010; Tucker et al. 2014).

Ecoacoustics is a powerful yet underused tool for assessing the biodiversity value of the landscape mosaic relative to reference sites in protected areas (PAs). Large-scale landscape transformation and associated habitat loss cause major disruptions to biodiversity and functional ecosystem integrity. In South Africa, managed landscapes, such as plantation forestry, occur primarily within threatened grassland. Blocks of nonnative trees are in extreme contrast to indigenous biomes and support little indigenous biodiversity (Samways & Moore 1991; Pryke & Samways 2011, 2012). Timber plantations are also barriers or filters to movement between grassland remnants (Bazelet & Samways 2010), cause landscape fragmentation (Lindenmayer & Fischer 2006), and have hard edges that negatively impact adjacent biotopes (Pryke & Samways 2011). We defined *biotopes* as a combination of the physical environment (habitat) and its distinctive assemblage of conspicuous species (Olenin & Ducrotoy 2006), these being the dominant plant types in this terrestrial system.

The South African timber industry uses nonnative *Eucalyptus* and *Pinus* species. About one-third of the plantation landscape is left unplanted and is maintained

as conservation ecological networks (ENs) of remnant indigenous protected grassland, wetlands, and native forest patches, as well as firebreaks, power line servitudes, and service roads (Samways & Pryke 2016). Ecological networks are interconnected conservation corridors and nodes that improve connectivity between remnant patches and extensive natural areas to maintain structural, compositional, and functional biodiversity (Samways et al. 2009). Ecological networks adjacent to PAs effectively extend the size of PAs (Pryke & Samways 2012). Improvement to EN design includes selectively planting or removing timber stands in strategic areas so as to alter their size or shape to include areas with special landscape features or to enlarge corridors in key locations. To do this, managers must assess remnant areas to identify those of high conservation value. This means there is a need to conduct reliable, rapid assessment surveys of remnant areas across the forestry-plantation and protected-area mosaic. Thus, we sought to use ecoacoustics as a tool to improve assessment and thus conservation of these mosaics.

Methods

Study Area and Design

The focal area was 3 commercial plantations in KwaZulu-Natal, South Africa (20–90 m asl): Nyalazi (28°12'S; 32°22'E), DukuDuku (28°19'S; 32°22'E), and Kwambonambi (28°39'S; 32°10'E) (Fig. 1). These plantations had mainly nonnative *Eucalyptus* and were all in threatened Maputaland Wooded Grassland or Maputaland Coastal Belt biomes in the Maputaland–Pondoland–Albany global biodiversity hotspot (Mittermeier et al. 2005). Declining levels of natural vegetation in hotspots underscore the need for conservation initiatives within these biologically crucial areas (Sloan et al. 2014). In this area, the plantations also border the iSimangaliso Wetland Park (a World Heritage Site), but there is no fence between them so large animals, including the African elephant (*Loxodonta africana*), white rhinoceros (*Ceratotherium simum*), African buffalo (*Syncerus caffer*), and leopard (*Panthera pardus pardus*), move freely between the ENs and PA. Cattle also grazed the ENs but not the PA.

We selected 210 sites of 7 types within the plantation mosaic for sampling: naturally formed indigenous forest patches ($n = 34$); small wetlands ($n = 48$); grassland patches of various sizes (see below) ($n = 43$); *Eucalyptus* timber stands with no understory vegetation ($n = 15$); managed sites in ENs (areas under powerline servitudes and firebreaks mowed biweekly) ($n = 21$); grazed
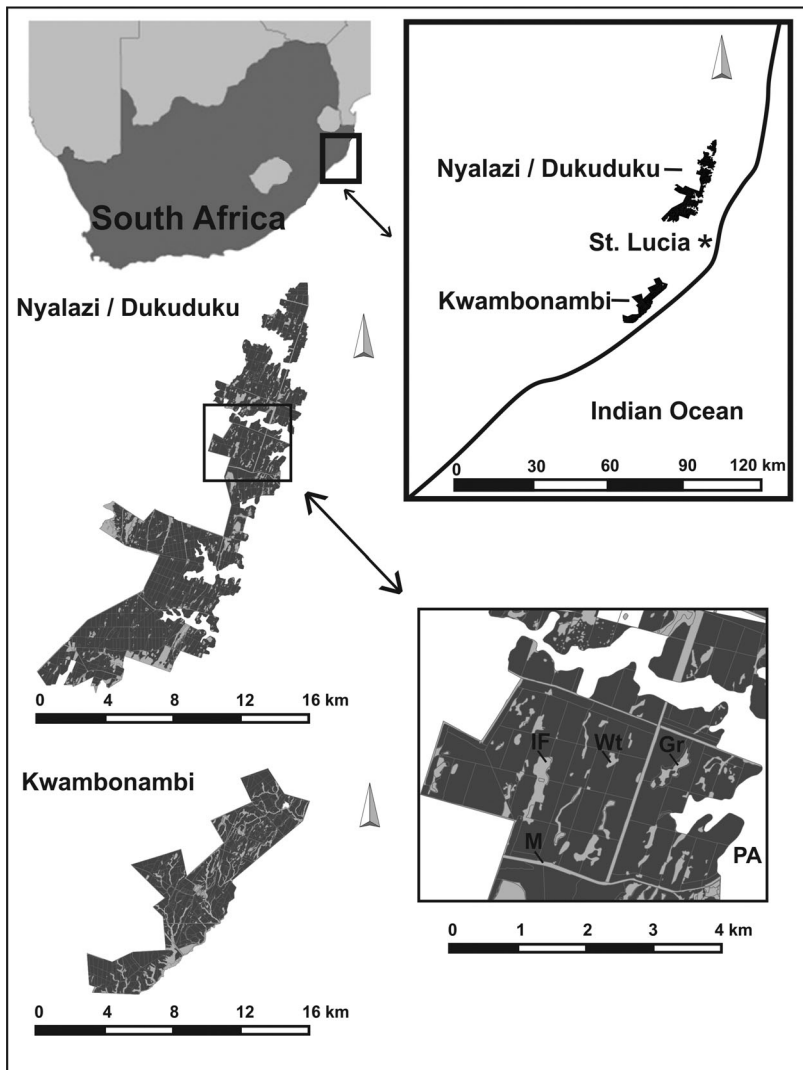
Figure 1. Location of commercial plantations in this study: Nyalazi, DukuDuku, and Kwambonambi in KwaZulu-Natal, South Africa. Bottom right shows mosaic of agroforestry and protected area (dark gray, timber stands; light grey, remnant patches of indigenous vegetation; IF, indegenious forest; W, wetland; Gr, grassland; M, managed area; white, protected area).

patches in ENs (grassland and wetland patches grazed by indigenous megaherbivores) ($n = 11$); and margins of dirt roads in ENs (narrow margins of tall grass [> 30 cm] between *Eucalyptus* stands and road) ($n = 21$). The PA reference sites were large (>150 ha) areas of natural grassland ($n = 17$). PA sites were >1 km apart and > 32 m away from timber stands to avoid edge effects (Pryke & Samways 2011).

Environmental variables, including site category, such as wetland or grassland patch and size (perimeter and area) were determined with Manifold System version 8.0. Shape of sites was measured by using the shape index, an area-independent measure where a perfectly circular site has a value of 1 and the value increases as a site's circularity decreases. Grassland-patch size classes were exponentially chosen to determine effects of small patches on biodiversity: 1, <1 ha ($n = 24$); 2, 1–3 ha ($n = 29$); 3, 3–10 ha ($n = 59$); 4, 10–150 ha ($n = 71$); 5, >150 ha ($n = 27$).

Invasive species are a major threat to many indigenous and endemic species (Clavero & García-Berthou 2005) and can reduce biodiversity of acoustic species (Standish 2004). Therefore, at each site we recorded percent cover of invasive, nonnative crow's foot grass (*Dactyloctenium australe*). This species is structurally different from native grasses and readily identifiable.

Because heterogeneity of vegetation structure and cover can affect species diversity (Costanza et al. 2011), we ranked it on a scale of 1–10 (1, uniform cover of one structural vegetation type; 2, 2 structural vegetation types, etc. for ranks 3–9; 10, ≥10 structural vegetation types [highly diverse cover]).

Acoustic Sampling

We conducted nocturnal acoustic surveys in late summer (February 2010), which corresponds with peak acoustic activity of katydids (Tettigoniidae), crickets (Gryllidae), and frogs. Many acoustic species sing more intently during dawn and dusk (Riede 1998; Sueur et al. 2008; Diwakar & Balakrishnan 2007). Therefore, to avoid the influence of dawn and dusk choruses on site comparisons,

acoustic recordings were made from 1 h after sunset and to 1 h before dawn (2100–0500). We surveyed for only nocturnal species because these species mostly use different dominant frequencies rather than temporal partitioning patterns to communicate (Diwakar & Balakrishnan 2007; Schmidt et al. 2012). Within each site, 3 ambient acoustic recordings, each 1 min long were recorded within minutes of each other. Each of the 3 ambient recordings within sites were separated by 100 m to increase signal-detection coverage, but small sites occasionally limited this distance to 50–100 m.

The time acoustic surveys were conducted was analyzed categorically. Site sampling events were assigned to 8 hourly categories (i.e., 1, 2100–2200 to 8, 0400–0500). Recordings were made with a Sennheiser ME67/K6 microphone (Sennheiser, Germany) and a Marantz PD660 (Marantz, USA) solid-state digital recorder. The Sennheiser microphone has a frequency response up to 20 kHz, ideal for recording call structure of various acoustic species even at some distance from the source. It minimizes background noise without severe attenuation of the very high frequencies. Because it is a directional microphone, it was aligned horizontally by hand and rotated 360° across the site over the 1 min of recording time so as to capture all acoustic signals. A single observer recorded all data. The Marantz PD660 recording parameters were the same for all sites and were set to a sampling rate of 48 kHz with an uncompressed file format (.wav). During acoustic sampling, the time, temperature, wind speed, and relative humidity were recorded at each site.

We also located the source of certain acoustic signals to confirm the taxon of the signaler and to record the species-specific call of that signaler. For Orthoptera (katydids and crickets) voucher specimens were collected and deposited in the entomology collection at Stellenbosch University. Katydids were identified to species level. The taxonomy of South African crickets (Gryllidae) is poorly known, so calling individuals could be assigned only to the family and the recognizable acoustic species (Riede 1993). In the case of frogs, photographs of calling individuals were taken for identification.

Signal Analyses

Acoustic recordings were analyzed by the same observer who collected recordings with a Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY, USA). Spectrograms created for all recordings were used to graphically display and analyze signals with Hann windows. Sample size was 256 (5.33 milliseconds), time grid had 50% overlap, hop size was 128 samples (2.67 milliseconds), frequency grid spacing was 188 Hz, and discrete Fourier transformation was 256 samples. Each 1 kHz frequency band within spectrograms was isolated and examined in detail for species-specific signals (Fig. 2). Signals from acoustic species
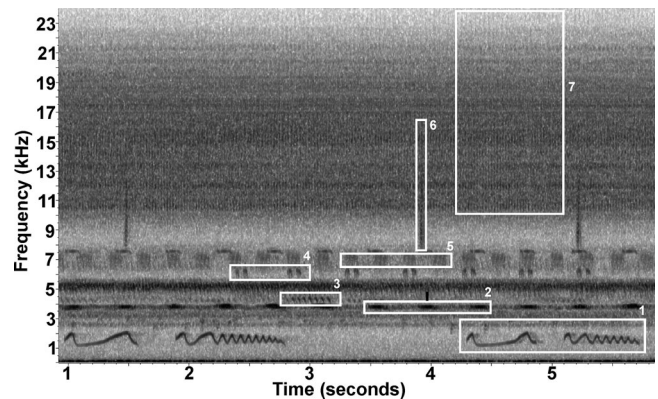

Figure 2. Spectrogram of a large, natural, protected grassland showing frequency versus time (the darker the shading, the higher the decibels). Rectangles (1–7) demarcate particular species' signals within this spectrogram and show the variation in frequency and pulse rates species use to avoid acoustic interference. Rectangles 7 and 6 show high-frequency broadband signals of katydids (Ruspolia sp. 2 [Eurycorypha proserpinae]). Rectangles 2–5 show low-frequency narrowband signals of crickets (Gryllidae sp. 1; Gryllidae sp. 13; Gryllidae sp. 12; Gryllidae sp. 22). Rectangle 1 shows a low-frequency signal with complex amplitude modulations characteristic of the Fiery-necked Nightjar.

were characterized according to their signal structure based on center frequency (measured in kHz, center frequency is the smallest discrete frequency that contains 50% of total energy) and pulse rate (number of pulses per second). Pulses are the smallest discrete amplitude modulations within signals and are grouped together as chirps (multiple pluses) or trills (continuous pulses). The gaps between pulses, chirps, and trills are referred to as intervals.

Classification of species based on pulse rate and center frequency allows for the definition of recognizable taxonomic units (Riede 1998; Tishechkin 2014). Once acoustic species are defined, signals can be used as sensitive indicators of biodiversity and habitat quality. Within South Africa, acoustic signals for many taxonomic groups are already well known, including frogs (du Preez & Carruthers 2009) and birds (http://www.xeno-canto.org), which allows identification of the acoustic species within recordings. Bird acoustic signals are recognizable through complex fluctuating intensity across lower-frequency ranges <3 kHz (Riede 1993); few species are nocturnal (Fig. 2). Comparatively, frogs in South Africa are mostly nocturnally active and have relatively simple call structures that are easily classified by frequency range and pulse rates (Malkmus & Riede 1996). Frog acoustic signals are generally <3 kHz, although some

species have signals <5 kHz (du Preez & Carruthers 2009).

A large portion of acoustic space both in number of species signaling and nocturnal acoustic energy is generated by crickets (Othoptera: Gryllidae); their signals are simple, repetitive, and in a narrowband frequency range of 1–11 kHz (Riede 1998). Pulse repetition rate and frequency range generally permit easy acoustic classification of cricket species. However, in some regions, there is the potential to confuse cricket and frog signals that have simple call structures and are within lower frequency. Although frog calls in South Africa are well known, we avoided potential misidentification of taxa by identifying signalers at sites, which also provided a means of verifying signals in recordings.

Katydid (Orthoptera: Tettigoniidae) acoustic signals were identified in ambient recordings by recording, collecting, and identifying species of signaler. Katydids are nocturnal insects that usually produce species-specific signals with broadband frequency ranges of 6–130 kHz (Montealegre-Z et al. 2006) and considerable temporal amplitude modulations (Greenfield 2002).

Data Analyses

We used the Shannon–Wiener index (H') to calculate species diversity across sites. With this diversity index, values approaching zero equate to lower diversity. Because the H' index is sensitive to rare species, it is recommended for landscape diversity assessments (Nagendra 2002).

Generalized linear models (GLMs) were used to determine the relationship between environmental variables and species richness in Statistica 12 (Dell 2015). These models are mathematical extensions of linear models that allow for nonlinearity and nonconstant variance structures in the data (Guisan et al. 2002). They are based on a relationship (link function) between the mean of the response variable and the explanatory variable (Guisan et al. 2002). Data can be from a variety of probability distributions (e.g., normal, binomial, Poisson, negative binomial, or gamma), which better fit nonnormal error structures often present in ecological data (Guisan et al. 2002). Therefore, GLMs provide a flexible, robust approach for analyzing ecological relationships that can be represented by both categorical and continuous data that are nonnormal when using probability distributions with link functions.

To ensure data were comparable across the 3 plantations, variables in the model included plantation categories, time categories, and continuous meteorological variables (temperature, wind speed, and relative humidity). The model also included size categories, site categories (timber stands, managed areas, grazed areas, road margins, indigenous forest, grassland patches, wetlands and PA), heterogeneity categories, and continuous data

on percentage cover of nonnative vegetation to determine influence on species richness and the Shannon–Wiener index. No anthropogenic noise was present at any site; therefore, potential influence of anthropogenic noise was not relevant to our analyses.

GLMs were calculated with Poisson distribution and log-link functions for all data. Because these analyses showed no overdispersion of the variances compared with the models, Wald chi-square statistics (χ^2) were calculated using the penalized quasi-likelihood (Bolker et al. 2009). Significance level was set at $P \leq 0.05$.

Results

Among the 3 plantations, there were no significant differences in species richness ($\chi^2 = 1.46$, $P = 0.48$) or their Shannon–Wiener index values ($\chi^2 = 5.95$, $P = 0.51$), which allowed sites across all plantations to be pooled for analyses.

All species were detected across sites during all eight 1-h categories from 2100 to 0500. Time of sampling therefore did not affect total species richness across sites ($\chi^2 = 6.31$, $P = 0.38$). Meteorological variables did not vary significantly across sites: mean temperature of 25.6°C (SD 1.3) ($\chi^2 = 2.11$, $P = 0.14$), wind speed of 0.26 m/s (0.34) ($\chi^2 = 2.19$, $P = 0.13$), and relative humidity of 74.9% (4.7) ($\chi^2 = 0.04$, $P = 0.83$). Species richness across sites consequently did not vary with temperature ($\chi^2 = 2.35$, $P = 0.12$), wind speed ($\chi^2 = 6.19$, $P = 0.12$), or relative humidity ($\chi^2 = 2.22$, $P = 0.13$). This allowed for unbiased assessment of acoustic species richness across sites without time or weather conditions being a factor.

In total, 65 acoustic species were identified across all sites (Table 1), including bird ($n = 1$), frog ($n = 11$), katydid ($n = 21$), and cricket ($n = 32$) species. Although some overlap occurred, acoustic taxa separated out based on pulse rate and center frequency ($\chi^2 = 18.81$, $P = 0.001$) (Fig. 3). Katydid acoustic signals had the highest pulse rates and frequency ranges, followed by crickets, with birds and frogs occupying the lowest frequency ranges and pulse rates. All acoustic species were also verified to taxon at sites, with the exception of 6 out of 7 arboreal katydids found only in indigenous forest. These 6 species were classified to taxon based on classification of their signals, which were characteristic of this group.

The various site categories (timber stands, managed areas, grazed areas, road margins, indigenous forest, grassland patches, wetlands, and PA) had differences in vegetation structure, with consequent significant differences in mean species richness ($\chi^2 = 179.50$, $P = 0.001$) (Fig. 4). Shannon–Wiener Index scores also showed a corresponding increase with mean species diversity (Fig. 4). Timber stands contained no acoustic species. Managed sites supported a single cricket species

Table 1. Acoustic species detected across sites in 3 commercial plantations in KwaZulu-Natal, South Africa.[a]

	Family	Acoustic species	PA	Wt	Gr	IF	Gz	Rd	M	RA	PR	kHz
1	Caprimulgidae	*Caprimulgus pectoralis*	41	15	2	9				8.6	0.2	1.7
2	Arthroleptidae	*Leptopelis mossambicus*	35	20	35	12	36			18.6	16.8	1.4
3	Bufonidae	*Amietophrynus gutturalis*	6	2	5					1.9	6.6	1.3
4	Hemisotidae	*Hemisus guttatus*		1						1.0	15.9	2.1
5	Hyperoliidae	*Afrixalus aureus*		4	2					1.4	6.2	4.3
6	Hyperoliidae	*Hyperolius marmoratus*		4						1.0	0.9	3.1
7	Hyperoliidae	*Hyperolius pusillius*	6	4	2					1.9	2.7	4.9
8	Hyperoliidae	*Hyperolius semidiscus*	18	10	2		9			4.8	3.0	2.7
9	Hyperoliidae	*Kassina maculata*			2		9			1.0	1.3	1.7
10	Hyperoliidae	*Kassina senegalensis*	12	6			9			2.9	0.9	1.0
11	Ptychadenidae	*Ptychadena mossambica*		4						1.0	29.0	1.9
12	Ptychadenidae	*Ptychadena oxyrhynchus*	18	6	2	6				4.3	10.7	2.4
13	Gryllidae	Oecanthinae sp. 1	18	25	37	29	9	29		22.9	41.1	2.5
14	Gryllidae	Oecanthinae sp. 2	35	29	16		27	14		15.7	44.8	3.4
15	Gryllidae	Oecanthinae sp. 3	24	40	37		45	5		21.4	51.1	6.6
16	Gryllidae	Gryllidae sp. 1	76	42	47	85		14		40.5	1.0	3.6
17	Gryllidae	Gryllidae sp. 2	35	23	19		18	5		13.3	14.0	5.7
18	Gryllidae	Gryllidae sp. 3	6	19						4.8	25.2	6.5
19	Gryllidae	Gryllidae sp. 4	35	17	28	71				23.8	38.9	5.8
20	Gryllidae	Gryllidae sp. 5	59	54	49	21	27	5		32.4	34.9	4.9
21	Gryllidae	Gryllidae sp. 6	18	6	2	6				4.3	7.6	4.6
22	Gryllidae	Gryllidae sp. 7	12	21	23					1.9	29.7	10.7
23	Gryllidae	Gryllidae sp. 8	29	38	47	50	45	24		33.3	8.9	3.4
24	Gryllidae	Gryllidae sp. 9	29	27	23	50	18	14		23.8	13.6	3.1
25	Gryllidae	Gryllidae sp. 10	29	2	12					5.2	64.5	6.0
26	Gryllidae	Gryllidae sp. 11	29	4	21					7.6	32.4	6.4
27	Gryllidae	Gryllidae sp. 12	24	10	5	6				6.2	7.8	5.8
28	Gryllidae	Gryllidae sp. 13	59	38	33	29			5	25.2	12.9	4.2
29	Gryllidae	Gryllidae sp. 14	6	6	2					2.4	30.1	4.2
30	Gryllidae	Gryllidae sp. 15	24	8	9	44				12.9	20.4	4.8
31	Gryllidae	Gryllidae sp. 16	41	13	2	6	9			8.1	1.9	6.9
32	Gryllidae	Gryllidae sp. 17	12	2						1.4	22.4	6.0
33	Gryllidae	Gryllidae sp. 18	12							1.0	7.1	4.6
34	Gryllidae	Gryllidae sp. 19	53	17	19	6	46			15.2	3.1	3.9
35	Gryllidae	Gryllidae sp. 20	18							1.4	18.7	6.6
36	Gryllidae	Gryllidae sp. 21	12	2	9					3.3	76.1	7.5
37	Gryllidae	Gryllidae sp. 22	6		2					1.0	81.6	7.1
38	Gryllidae	Gryllidae sp. 23	24	27	2					8.6	2.9	6.2
39	Gryllidae	Gryllidae sp. 24	12	4	2	3				2.9	0.9	5.5
40	Gryllidae	Gryllidae sp. 25		6						1.4	19.9	7.1
41	Gryllidae	Gryllidae sp. 26	12	27	5					8.1	8.7	3.2
42	Gryllidae	Gryllidae sp. 27	47	8	26	6				11.9	1.0	6.0
43	Gryllidae	Gryllidae sp. 28				32				5.2	0.8	3.7
44	Gryllidae	Gryllidae sp. 29				56				9.0	26.3	5.8
45	Tettigoniidae	Tettigoniidae sp. 1				56				9.0	0.9	8.7
46	Tettigoniidae	Tettigoniidae sp. 2				35				5.7	1.8	14.0
47	Tettigoniidae	Tettigoniidae sp. 3				18				2.9	2.9	11.1
48	Tettigoniidae	Tettigoniidae sp. 4				18				2.9	23.5	10.1
49	Tettigoniidae	Tettigoniidae sp. 5				12				1.9	94.7	7.6
50	Tettigoniidae	Tettigoniidae sp. 6				12				1.9	15.8	13.7
51	Tettigoniidae	*Conchotopoda belcki*				35				5.7	46.7	7.3
52	Tettigoniidae	*Conocephalus caudalis*	12							1.0	198	4.2
53	Tettigoniidae	*Conocephalus iris*	24	17	5					6.7	27.5	14.2
54	Tettigoniidae	*Conocephalus maculatus*	65	21	26	3				15.7	48.2	20.0
55	Tettigoniidae	*Eulioptera atkinsonae*	24	8	9					5.7	1.0	9.8
56	Tettigoniidae	*Eurycorypha proserpinae*	12	6	9	3				4.8	0.9	12.1
57	Tettigoniidae	*Plangia graminea*	52	40	30	6	27			21.9	1.1	9.6
58	Tettigoniidae	*Ruspolia* sp. 1	82	44	53		73	14		32.9	38.1	14.3
59	Tettigoniidae	*Ruspolia* sp. 2	88	67	77	18	45	38		47.1	39.2	12.0
60	Tettigoniidae	*Ruspolia* sp. 3	18	23	12	3		19		11.4	196	12.9
61	Tettigoniidae	*Ruspolia* sp. 4	6	2	9	9		33		7.6	18.8	12.3
62	Tettigoniidae	*Ruspolia* sp. 5						48		4.8	8.6	8.6
63	Tettigoniidae	*Thoracistus viridifer*	12	44	5		27			13.3	380	14.3
64	Tettigoniidae	*Tylopsis continua*	41	29	26	15	9			18.1	2.2	15.4
65	Tettigoniidae	*Zuludectes modestus*	53	35	30		18			19.5	209	19.9

[a]*Abbreviations: PA, protected area; Wt, wetland; Gr, grassland; IF, indigenous forest; Gz, grazed; Rd, road margin; M, managed; RA, percent relative abundance across all 210 sites; PR, pulse rate; kHz, center frequency (kHz) of signals. The frequency with which species were detected (%) is listed under each site category (PA, Wt, Gr, IF, Gz, Rd, and M).*
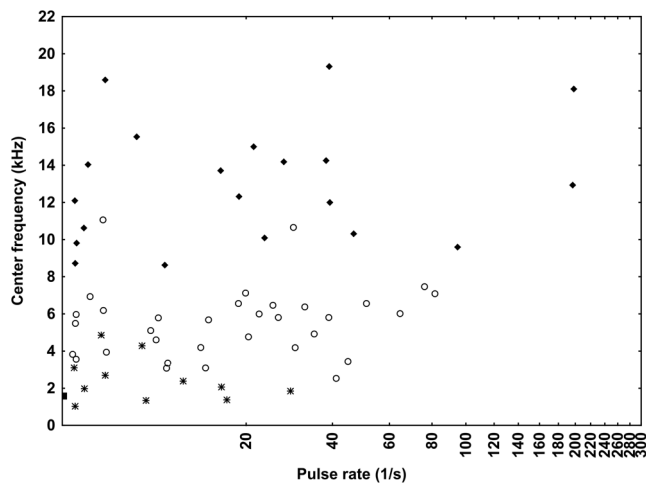
Figure 3. Distribution of acoustic species based on pulse rate and frequency at the center of the pulse (diamond, katydid; open circle, cricket; asterisk, frog; square, bird).
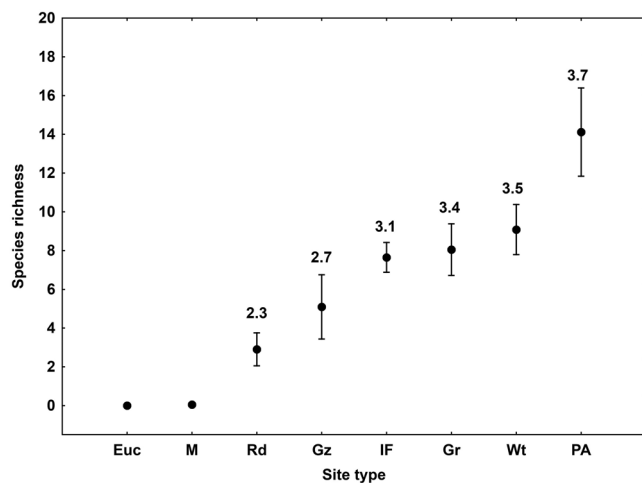

Figure 5. Difference in mean species richness among size categories of protected-area grassland patches (Wald $\chi^2 = 74.39$, P = 0.001) (bars, SD; values above bars, Shannon–Wiener index [H'] diversity scores; size categories: 1, <1 ha; 2, 1–3 ha; 3, 3–10 ha; 4, 10–150 ha; 5, >150 ha).

Figure 4. Difference in mean species richness among site categories (Wald $\chi^2 = 179.5$, P = 0.001) (bars, 95% confidence interval; values above bars, Shannon–Wiener index [H'] diversity scores; PA, protected area; Wt, wetland sites; Gr, grassland sites; IF, indigenous forest sites; Gz, grazed sites; M, managed sites; Euc, Eucalyptus timber stands).

(Gryllidae sp. 13) but no other acoustic species. The grassy road margins had low mean species richness (2.9, 13 species in total). However, *Ruspolia* sp. 5 was only in this site category, and *Ruspolia* sp. 4 was largely at this site type. Grazed grassland sites had higher mean species richness (5.1, 19 species in total) than road sites but contained no unique species and significantly lower mean species richness than in indigenous forest (7.6, 33 species in total), grassland (8.0, 45 species in total), wet-
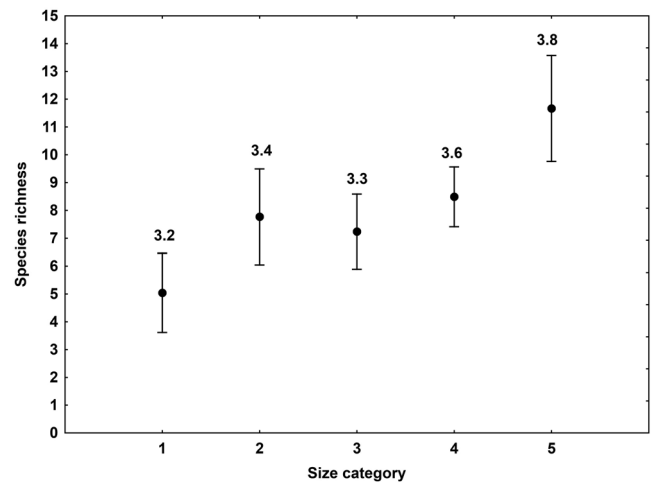
land (9.1, 50 species in total), or PA sites (14.1, 49 species in total).

There was no difference in mean species richness between indigenous forest, grassland, and wetland patches ($\chi^2 = 5.57$, P = 0.06), but there were differences in species composition (Table 1). Indigenous forests had 9 unique species, 2 crickets and 7 katydids (Gryllidae spp. 28 and 29, Tettigoniidae spp. 1–6, and *Conchotopoda belcki*), and wetlands had 4 unique species, 3 frogs and one cricket (*Hemisus guttatus, Hyperolius marmoratus, Ptychadena mossambica*, and Gryllidae sp. 25). Although grassland sites had comparable mean species richness to wetlands and indigenous forest, they contained no unique species. Generally, species within grasslands were detected more frequently in other cover types. Protected-area sites (large areas of natural grassland > 150 ha) had the highest mean species richness and contained 3 unique species, 2 crickets and one katydid (Gryllidae sp. 18 and 20 and *Conocephalus caudalis*).

Patch size had a significant effect on species richness ($\chi^2 = 74.39$, P = 0.001) (Fig. 5). Sites <1 ha had low mean species richness and Shannon–Wiener index scores (Fig. 5). Sites >1 and <150 ha supported similar mean species richness ($\chi^2 = 2.14$, P = 0.34), whereas those >150 ha supported the greatest mean number of species and had the highest Shannon–Wiener index scores. The shape of natural, unmanaged areas (indigenous forest, grassland patches, wetlands, and PA) seemingly had no effect on species richness ($\chi^2 = 0.68$, P = 0.41). Species richness increased as biotope heterogeneity increased ($\chi^2 = 140.81$, P = 0.001).

Across all sites (210), 46 sites (22%) contained some level of invasive crow's foot grass (range 3–85% cover; mean of 30.1% [SD 19.9]). Crow's foot grass was not detected in timber stands, road margins, or indigenous forest, likely due to high levels of shade from the tree canopies. The grass also did not occur in managed areas, likely due to biweekly mowing. Although it occurred to a lesser extent in grazed areas relative to grassland patches, wetlands, and PA ($\chi^2 = 8.58$, $P = 0.035$), there was no difference in percent cover among grassland patches, wetlands, and PA ($\chi^2 = 4.62$, $P = 0.099$). Percent cover of crow's foot grass also increased with smaller patch sizes ($\chi^2 = 10.65$, $P = 0.03$) but was not affected by patch shape ($\chi^2 = 3.17$, $P = 0.07$). Increasing cover of crow's foot grass at sites resulted in decreasing acoustic species richness ($\chi^2 = 27.45$, $P = 0.001$) and in decreasing Shannon–Wiener index values ($\chi^2 = 49.59$, $P = 0.001$). Furthermore, sites with >70% cover of this grass contained no acoustic species.

Discussion

Biodiversity assessments at the landscape scale are essential for accurately assessing and comparing species richness across diverse environmental gradients and over time. A considerable number of animal species indicate their presence by acoustic signals that can be easily detected, recorded, saved, and analyzed. Species-specific acoustic parameters such as frequency and amplitude modulation provide an excellent means for measuring acoustic species diversity and allowing for the definition of recognizable taxonomic units where taxonomic challenges exist (Riede 1993, 1998; Tishechkin 2014). Assessing biodiversity at the species level is important from a conservation perspective because species composition is often as important as species richness. This was especially the case here because the study area is in a global biodiversity hotspot. Detection of these acoustic signals across the landscape (ecoacoustics) therefore provides a noninvasive, effective method for investigating diversity patterns of acoustic taxa across ecological gradients, and ecoacoustics can be used in conservation decision making (Riede 1998; Pijanowski et al. 2011; Tucker et al 2014). Furthermore, use of ecoacoustics over time is a way of assessing the effectiveness of conservation strategies. It can also be used to monitor levels of restoration or deterioration of ecological integrity, based on acoustic diversity patterns (Riede 1993, 1998; Sueur et al. 2008; Pijanowski et al. 2011).

The mosaic of biotope types had a diverse range of acoustic signatures, which were readily detectable and identifiable. Our results were consistent with the fact that nonnative vegetation negatively affects species diversity (Samways et al. 1996; Litt & Steidl 2010; Simao et al. 2010). Nonnative vegetation had a distinct detrimental impact on acoustic signalers; timber stands supported no acoustics species. Increasing cover of invasive, nonnative crow's foot grass decreased species richness; there were no acoustic species in sites with >70% cover of crow's foot grass. Nonnative vegetation can often outcompete native vegetation and thus alter plant communities and negatively affect opportunities for indigenous insects. Here, these major deleterious changes were reflected in the acoustic profile and indicated that removal of crow's foot grass would contribute to restoring biodiversity within this plantation matrix. Success of restoration could be tracked using ecoacoustic assessments over time.

Managed sites within plantations such as powerline servitudes and firebreaks are an integral part of the production landscape and require biweekly mowing. These areas were acoustically similar to sites with alien vegetation and contained low acoustic species diversity; they had high levels of disturbance and short grasses that did not provide habitat for most species. Katydids were strongly affected by mowing because they are generally cryptic insects requiring late succession bushes and trees for camouflage; their establishment was prevented by mowing. Overall, the managed areas simulated early-succession stages after fire or heavy grazing, which benefits early-succession insects such as grasshoppers (Picaud & Petit 2007), at our sites (Bazelet & Samways 2010).

Grassy road margins did not support high species diversity, yet the tall grass within these sites was suitable for certain katydid species, which contributed marginally to overall biodiversity of the plantation matrix. At a landscape scale, this emphasizes the importance of particular natural landscape features containing special niches to maximize overall biodiversity (Crous et al. 2013). The grazed sites had higher acoustic diversity than either the managed sites or grassy margins. Here, grazing was the conflated effects from both natural megaherbivores and domestic livestock (cows).

Although grassland, wetland, and indigenous-forest patches had similar species richness, there were differences in acoustic species composition among them. Indigenous forest patches in particular contained the most unique acoustic species, as well as certain mute taxa (Pryke & Samways 2011). This emphasizes that ecoacoustics can be used to improve ENs in the context of this timber-plantation mosaic, including finding ways to improve functional connectivity between the threatened indigenous forest patches (Eeley et al. 2001).

Wetlands had the highest total number of acoustic species, unique species, and species strongly associated with the plantation landscape. This emphasizes the major contribution wetlands make to overall biodiversity in these systems. The extensive PA grassland sites had the highest mean species richness of all sites and supported some unique species. Although isolated grassland patches in the timber matrix had lower species richness, they

nevertheless had similar but poorer species composition relative to the PA. This shows that isolated grassland patches surrounded by a timber matrix function similarly to PA grassland sites and contribute to maintaining biodiversity across the mosaic. However, this may not be the case in perpetuity; ecological relaxation may take place over time. Future comparative ecoacoustics could test this.

There is generally positive association between increased patch size and improved reproductive success or survival (Fahrig 2003; Hokit & Branch 2003). Organisms in smaller patches can be more susceptible to edge effects, although some species respond positively to edges (van Halder et al. 2011), including some at our sites (Pryke & Samways 2012). For overall biodiversity, the interior zone is the most important to conserve, yet this can be difficult to do because it requires enough space to proportionately reduce the edge zone (Pryke & Samways 2012); species richness and assemblage composition is negatively affected within <32 m of plantation edges in this area (Pryke & Samways 2011). This indicates that remnant patches within this timber matrix must be >1 ha to avoid immediate adverse edge effects and to maintain resistance to invasive nonnative vegetation.

Another factor that influences level of biodiversity within patches is spatial habitat heterogeneity, and the greater it is the more niches are available (Rocchini et al. 2010; Schmidt et al. 2012). Although habitat heterogeneity on its own does not ensure conservation of rare or endemic species, it does contribute to overall biodiversity across the landscape (Grant & Samways 2011). On a landscape scale, this has important implications for the design of ENs, as we found here. Selecting habitats with special landscape features and containing many special and even unique niches maximizes the level of biodiversity conserved within plantation matrices (Crous et al. 2013). Conserving the natural structural aspects of a landscape therefore contributes to conserving biodiversity and thus improves the persistence of populations within transformed landscapes (Samways et al. 2009). Ecoacoustics can improve understanding of these issues because these measures are sensitive not only to changes in species and assemblages over space and time and also to the density of individuals.

To mitigate effect of landscape transformation, ENs with patches of indigenous forest, wetland, and grassland have been incorporated into the design of the timber plantation mosaic (Samways & Pryke 2016). Good EN design protects important local biodiversity, whereas afforestation can be done in less critical areas. To achieve this, biodiversity patterns across patches of indigenous vegetation must be identified accurately, and ecoacoustics is a valuable tool for doing so.

Detection of sound patterns across the landscape provided an accurate biodiversity assessment to guide conservation relative to, in particular, complementarity of the different ecosystems, from wetland to indigenous forest; importance of indigenous remnant patch size; risk from nonnative plants relative to their density; the relationship between megaherbivores and other biodiversity; and effects of human activities. Putting in place more effective management of the mosaic over time could also be addressed using ecoacoustics, with particular emphasis on restoration and testing improvements to EN design. In sum, ecoacoustics holds great promise not just for assessing biodiversity but also for monitoring change. Its value goes well beyond our plantation-PA mosaic and could be used in any production system with various agroecological approaches where the intention is to harmonize production, conservation, and sustainability well into the future.

Acknowledgment

Literature Cited

Bazelet CS, Samways MJ. 2010. Relative importance of management vs. design for implementation of large-scale ecological networks. Landscape Ecology **26:**341–353.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution **24:**127–135.

Clavero M, García-Berthou E. 2005. Invasive species are a leading cause of animal extinctions. Trends in Ecological & Evolution **20:**110.

Costanza JK, Moody A, Peet RK. 2011. Multi-scale environmental heterogeneity as a predictor of plant species richness. Landscape Ecology **26:**851–864.

Crous C, Samways MJ, Pryke JS. 2013. Exploring the mesofilter as a novel operational scale in conservation planning. Journal of Applied Ecology **50:**205–214.

Dell Statistica. 2015. Statistica. Version 12. Dell Statistica, Tulsa, Oklahoma.

Diwakar S, Balakrishnan R. 2007. The assemblage of acoustically communicating crickets of a tropical evergreen forest in southern India: call diversity and diel calling patterns. Bioacoustics **16:**113–135.

du Preez L, Carruthers V. 2009. A complete guide to the frogs of southern Africa. Struik Nature, Cape Town.

Eeley HAC, Lawes MJ, Reyers B. 2001. Priority areas for the conservation of subtropical indigenous forest in southern Africa: a case study from KwaZulu-Natal. Biodiversity and Conservation **10:**1221–1246.

Fahrig L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics **34:**487–515.

Farina A, Lattanzi E, Malavasi R, Pieretti N, Piccioli L. 2011. Avian soundscapes and cognitive landscapes: theory, application and ecological perspectives. Landscape Ecology **26:**1257–1267.

Grant PBC, Samways MJ. 2011. Micro-hotspot determination and buffer zone value for Odonata in a globally significant biosphere reserve. Biological Conservation **144:**772–781.

Greenfield MD. 2002. Signalers and receivers. Mechanisms and evolution of arthropod communication. Oxford University Press, Oxford.

Guisan A, Edwards TC, Hastie T. 2002. Generalized linear and generalized additive models in studies of species distributions: setting the scene. Ecological Modelling **157:**89–100.

Hokit DG, Branch LC. 2003. Associations between patch area and vital rates: consequences for local and regional populations. Ecological Applications **13:**1060–1068.

Laiolo P. 2010. The emerging significance of bioacoustics in animal species conservation. Biological Conservation **7:**1635–1645.

Lindenmayer DB, Fischer J. 2006. Habitat fragmentation and landscape change: an ecological and conservation synthesis. Island Press, Washington D.C.

Litt AR, Steidl RJ. 2010. Insect assemblages change along a gradient of invasion by a nonnative grass. Biological Invasions **12:**3449–3463.

MacSwiney G, Cristina M, Clarke FM, Racey PA. 2008. What you see is not what you get: the role of ultrasonic detectors in increasing inventory completeness in Neotropical bat assemblages. Journal of Applied Ecology **45:**1364–1371.

Malkmus R, Riede K. 1996. Frogs from Kinabalu. Borneo Sauria **18:**37–37.

Mittermeier RA, Gil PR, Hoffman M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, da Fonsecs GAB. 2005. Hotspots revisited. Cemex, Mexico City.

Montealegre-Z F, Morris GK, Mason AC. 2006. Generation of extreme ultrasonics in rainforest katydids. Journal of Experimental Biology **209:**4923–4937.

Nagendra H. 2002. Opposite trends in response for the Shannon and Simpson indices of landscape diversity. Applied Geography **22:**175–186.

Olenin S, Ducrotoy JP. 2006. The concept of biotope in marine ecology and coastal management. Marine Pollution Bulletin **53:**20–29.

Penone C, Le Viol I, Pellissier V, Julien J-F, Bas Y, Kerbiriou C. 2013. Use of large-scale acoustic monitoring to assess anthropogenic pressures on Orthoptera communities. Conservation Biology **27:**979–987.

Picaud F, Petit DP. 2007. Primary succession of Acrididae (Orthoptera): differences in displacement capacity in early and late colonizers of new habitats. Acta Oecologica **32:**59–66.

Pijanowski BC, Farina A, Gage SH, Dumyahn SL, Krause BL. 2011. What is soundscape ecology? An introduction and overview of an emerging new science. Landscape Ecology **26:**1213–1232.

Pryke JS, Samways MJ. 2011. Conservation management of complex natural forest and plantation edge effects. Landscape Ecology **27:**73–85.

Pryke JS, Samways MJ. 2012. Ecological networks act as extensions of protected areas for arthropod biodiversity conservation. Journal of Applied Ecology **49:**591–600.

Riede K. 1993. Monitoring biodiversity: analysis of Amazonian rainforest sounds. Ambio **22:**546–548.

Riede K. 1998. Acoustic monitoring of Orthoptera and its potential for conservation. Journal of Insect Conservation **2:**217–223.

Rocchini D, Balkenhol N, Carter G, Foody GM, Gillespie TW, He KS, Neteler M. 2010. Remotely sensed spectral heterogeneity as a proxy of species diversity: recent advances and open challenges. Ecological Informatics **5:**318–329.

Samways MJ, Bazelet CS, Pryke JS. 2009. Provision of ecosystem services by large scale corridors and ecological networks. Biodiversity and Conservation **19:**2949–2962.

Samways MJ, Caldwell PM, Osborn R. 1996. Ground-living invertebrate assemblages in native, planted, and invasive vegetation in South Africa. Agriculture, Ecosystems & Environment **59:**19–32.

Samways MJ, Moore SD. 1991. Influence of exotic conifer patches on grasshopper (Orthoptera) assemblages in a grassland matrix at a recreational resort, Natal, South-Africa. Biological Conservation **57:**117–137.

Samways MJ, Pryke JS. 2016. Large-scale ecological networks do work in an ecologically complex biodiversity hotspot. Ambio **45:**161–172.

Schmidt AKD, Römer H, Riede K. 2012. Spectral niche segregation and community organization in a tropical cricket assemblage. Behavioral Ecology **24:**470–480.

Sedláček O, Vokurková J, Ferenc M, Djomo EN, Albrecht T, Hořák D. 2015. A comparison of point counts with a new acoustic sampling method: a case study of a bird community from the montane forests of Mount Cameroon. Ostrich **2015:**1–8.

Simao MC, Flory SL, Rudgers JA. 2010. Experimental plant invasion reduces arthropod abundance and richness across multiple trophic levels. Oikos **119:**1553–1562.

Sloan S, Jenkins CN, Joppa LN, Gaveau DLA, Laurance WF. 2014. Remaining natural vegetation in the global biodiversity hotspots. Biological Conservation **177:**12–24.

Standish RJ. 2004. Impact of an invasive clonal herb on epigaeic invertebrates in forest remnants in New Zealand. Biological Conservation **116:**49–58.

Sueur J, Pavoine S, Hamerlynck O, Duvail S. 2008. Rapid acoustic survey for biodiversity appraisal. PLoS ONE **3:**e4065 DOI: 10.1371/journal.pone.0004065.

Sueur J, Farina A. 2015. Ecoacoustics: the ecological investigation and interpretation of environmental sound. Biosemiotics **8:**493–502. DOI: 10.1007/s12304-015-9248-x.

Tishechkin DY. 2014. The use of bioacoustics characters for distinguishing between cryptic species in insects: potentials, restrictions and prospects. Entomological Review **94:**289–309.

Tucker D, Gage SH, Williamson I. 2014. Linking ecological condition and the soundscape in fragmented Australian forests. Landscape Ecology **29:**745–758.

van Halder I, Barbaro L, Jactel H. 2011. Conserving butterflies in fragmented plantation forests: Are edge and interior habitats equally important? Journal of Insect Conservation **15:**591–601.

Villanueva-Rivera LJ. 2007. Digital recorders increase detection of *Eleutherodactylus* frogs. Herpetological Review **38:**59–63.