WHEN TO SAMPLE GROUND BEETLES (COLEOPTERA: CARABIDAE) IN THE NEOTROPICS FOR BIODIVERSITY ASSESSMENTS? A CASE STUDY IN THE BRAZILIAN AMAZON

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Abstract. Temporal and spatial variation in Ground beetles diversity was described for neotropical ecosystems. The present study evaluated the seasonal patterns of Ground beetles assemblages within habitats in the Brazilian Amazon, to determine the most appropriate time-window to undertake significant ecological sampling. The study area comprised the most representative habitats such as primary and secondary forests, agriculture and pastures for livestock. Pronounced differences in richness and abundance of Ground beetles assemblages between seasons and habitats were noticed. The abundance and richness were significantly higher in the rainy season, and this pattern was related for all habitats. Our results show that when logistical constrains prevent multiple sampling, the most appropriate season to monitor Ground beetles communities in the scope of ecological status assessments is during the rainy season. On the other hand, we also highlight the importance of sampling different seasons if the study objective is a full community characterization.
Keywords: *Amazonia, biodiversity, ground beetles, seasonal variability, neotropical forest*

Introduction

Neotropical forests are considered biodiversity hotspots. Human activities, however, have been causing considerable deforestation and diversity erosion (Pan et al., 2011; Lewis, 2009; Kim et al., 2015). Diversity erosion is mostly linked with modifications in ecosystem processes and the emergence of new combinations of species that are ultimately linked with land use/cover change (LUCC), such as habitat conversion and fragmentation (Marques et al., 2002). Moreover these changes threaten the functionality of neotropical ecosystems, with major implications for the socio-ecological services associated (Gibson et al., 2011; Kim et al., 2015). Also, the recognition that neotropical forests are essential for the earth's functioning and ultimately for the survival of humankind (Cajaiba et al., 2017a) has increase the need for swift assessments and likeminded conservation strategies.

Invertebrates, especially insects, play a crucial role in most ecological processes and are considered key components of functional ecosystems (Bicknell et al., 2014; Viegas et al., 2014; Campos and Hernández, 2015; Cajaiba et al., 2017a, b). Insect abundance and richness are related not only with other taxa diversity, but also with specific

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environmental characteristics. Hence, insects represent potential indicators of environmental change (e.g. Nichols et al., 2008) and could support decision-making and robust management/recovery approaches for endangered ecosystems in the scope of need for rapid, standardized and cost-efficient assessment methodologies (Godfray et al., 1999).

Ground beetles (Coleoptera: Carabidae) (GB) have been the focus of numerous ecological studies (Eyre et al., 2005; Maveety et al., 2014). GB are considered relevant ecological indicators, namely due to their sensitivity to temperature, moisture and shade variation (Thiele, 1977), food quality and abundance (Bilde et al., 2000; Bohan et al., 2011), habitat structure (Brose, 2003; Taboada et al., 2008), and substrate concentration of salts, sugars and acidity (Merivee et al., 2006; Milius et al., 2006) (see complete review in Koivula, 2011). In the neotropics and especially in the Amazon region, integrity studies using GB are scarce and were performed in specific locations near the most important cities/universities (but see Cajaiba et al., 2018a). Additionally, for estimating the possible changes in the ecological status, it is fundamental to obtain the reference condition, supported on estimates of overall GB diversity (Coddington et al., 1991; Maveety et al., 2014). These estimates are supported by intense sampling and/or trapping duration for encompassing GB spatial and temporal patterns (Erwin and Scott, 1980; Wang et al., 2014). Anyhow logistic constrains (e.g. lack of manpower, reduced survey times and funding) and the vastness of the Amazon region impose cost-effective sampling methods, namely choosing the most informative time of year (Wang et al., 2014).

In fact the choice of the time-window is fundamental to determine optimal sampling periods for relating GB diversity and ecological status of habitats. Additionally as GB are often used for biological control, seasonal information is needed to understand their co-occurrence and interrelations with pest species (Suenaga and Hamamura, 2001; Werner and Raffa, 2003). Seasonal data can also be used to evaluate the potential effects of non-native fauna on native species (Niemelä et al., 1997; Werner and Raffa, 2003): such displacements may be less likely to occur when temporal separation exists (Werner and Raffa, 2003; Wang et al., 2014). Finally, knowing GB seasonal patterns is fundamental to support decision-making and reliable management/ recovery approaches for endangered ecosystems. This study evaluates seasonal patterns of GB within a disturbance gradient in the Amazon in the scope of ecological status assessments (Cajaiba et al., 2018b). The gradient encompasses habitats such as primary forest, secondary forests, cocoa plantations and pastures. More specifically, we aimed to answer the following questions: Which is the best period for sampling the highest diversity of GB in the Amazonian biome? Are the GB seasonal trends comparable for all habitats?

Materials and methods

Study area

The study area was located in the municipality of Uruará, state of Pará, northern Brazil (*Fig. 1*). Extensive livestock production and the exploitation of timber (mostly illegal) are currently considered the most serious environmental pressures (Cajaiba et al., 2016). The studied areas contain the most representative habitats of the region, in terms of biophysical and ecological characteristics, for understanding the response of GB communities, such as Native Vegetation (NV), Early Secondary succession (ES -

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secondary vegetation with 5 years of regeneration), Mature Secondary succession (MS - secondary vegetation with 15 years of regeneration), Agriculture (Ag - cocoa plantations, *Theobroma cacao* L.) and Pasture for extensive livestock (Pa). The climate is characterized as hot-humid (Köppen's classification), with annual average temperature and precipitation of 26 °C and 2000 mm respectively (Peel et al., 2007; Da Silva et al., 2018).

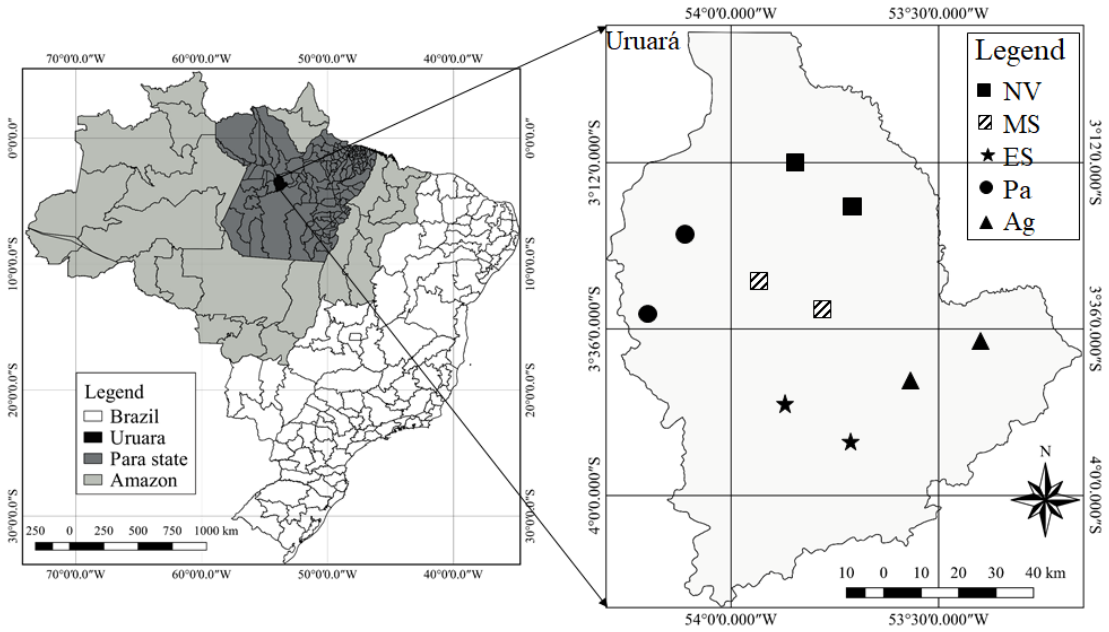

Figure 1. *Location of the study region in the municipality of Uruará, state of Pará, northern Brazil. Location of the sampling areas (sites) associated with each ecosystem: NV, Native vegetation; MS, Mature secondary succession; ES, Early secondary succession; Pa, Pasture; Ag, Agriculture*

Carabidae sampling

Fieldworks were carried out in 2015, during the rainy season (February-March), the intermediate season (June) and the dry season (September-October). The selected locations were positioned at a minimum distance of 100 m from ecotones, to guarantee that most GB captured were associated with the monitored habitat (Cajaiba et al., 2017a). For each habitat, 28 locations were selected, placed 100 m apart. Individuals were trapped using pitfall traps with 75 mm diameter and 110 mm depth, filled with preservative liquid consisting of formalin, alcohol, water and a few drops of detergent to break the surface tension. A roof was attached to each pitfall trap to prevent the damaging effect of direct rainwater for 48 h prior to collection. This protocol was applied to all areas and periods of collection, generating a total sampling effort of 840 traps (see *Table A1* in the *Appendix*).

The GB captured were preserved in 70% ethanol and taken to the laboratory for subsequent identification using specific taxonomic keys - Reichardt (1967, 1977). All specimens were deposited in the MCNU (Museu de Ciências Naturais da Univates – Univates Natural Sciences Museum, Brazil) and Coleção de Zoologia, Departmento de Biologia da Universidade Federal do Pará (Zoological collection of the Biology Department of the University of Pará, Brazil).

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Assemblage analysis

Rarefaction curves were used to assess whether the sampling effort was enough to identify most diversity by habitat per sampling period. Possible statistical differences in the seasonal patterns of GB abundance and richness were gauged using ANOVA, complemented by specific multiple comparisons tests (Tukey test). Prior to statistical analysis, the data was examined to ensure compliance with the underlying assumptions of parametric tests (e.g. normal distributions), using Shapiro-Wilk test. In order to homogenize the variances and normalize the residues, the GB abundance was logarithmized ($\log x + 1$).

The taxonomic composition of GB assemblages was examined across the seasons (dry, intermediary and rainy) within each habitat using Permutational Multivariate Analysis of Variance (PERMANOVA). Non-metric Multidimensional Scaling (NMDS) plots were used to help interpreting the results found with PERMANOVA. Similarity matrices were built using Bray-Curtis index.

To determine indicator species and possible influences of seasonal patterns, single value indicator (IndVal) developed by Dufrêne and Legendre (1997) was calculated, combining specificity (patterns of relative abundance) of a given species in a given habitat with its fidelity within that environment (patterns of incidence). Species with a high specificity and high fidelity within a habitat achieve the highest indicator value. Only taxa with IndVal > 25% were considered (Dufrêne and Legendre, 1997).

Results

A total of 2378 Ground bettles (GB) were captured, distributed within 32 species and morphospecies (hereinafter designated by species). Overall, 29 species were identified within 859 specimens captured in Native Vegetation (NV), 25 species were identified from the 414 specimens captured in the Mature Secondary (MS), 18 species were identified within 201 individuals captured in Early Secondary (ES), 25 species and 590 individuals were captured in Agriculture (Ag) and 13 species were identified within 314 individuals captured in Pasture (Pa) (*Table A2* in the *Appendix*). Rarefaction curves show that the sample effort was sufficient for estimating richness for most habitats within all sampling periods (*Fig. 2*).

Regarding the temporal variation, the analysis of ANOVA showed that overall richness and abundance were significantly higher in the rainy season than in the intermediary and dry season and richness and abundance were significantly higher in intermediary season than the in the dry season ($F = 15.43$, $p < 0.001$, $F = 13.85$, $p < 0.01$, respectively) (the associated differences are shown in *Fig. 3,* Tukey test) (*Table A2* in the *Appendix*).

The results of NMDS showed that GB assemblages of different sampling periods could not be easily separated from each other by ordination of species composition (*Fig. 4*). Anyway the Permutational Multivariate Analysis of Variance (PERMANOVA) showed that GB taxonomic composition varied significantly among the three periods studied ($F = 7.21$, $p < 0.0001$).

The inspection of the seasonal variation within each habitat showed similar trends to the overall data: richness and abundance were, with some exceptions, significantly higher in the rainy season. For richness, NV, MS and ES presented higher values in the rainy season, while Ag and Pa, higher in the intermediary period (ANOVA): NV ($F = 17.82$, $p < 0.01$), MS ($F = 82.14$, $p < 0.0001$), ES ($F = 23.12$, $p < 0.01$), Ag

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($F = 43.68$, $p < 0.01$) and Pa ($F = 14.29$, $p < 0.05$) (the associated differences are shown in *Fig. 5a*, Tukey test). In the case of abundance, significantly superior values were obtained in the rainy season for all habitats (ANOVA): NV ($F = 11.53$, $p < 0.001$), MS ($F = 50.52$, $p < 0.0001$), ES ($F = 19.62$, $p < 0.001$), Ag ($F = 25.80$, $p < 0.05$) and Pa ($F = 22.36$, $p < 0.01$) (the associated differences are shown in *Fig. 5b*, Tukey test).

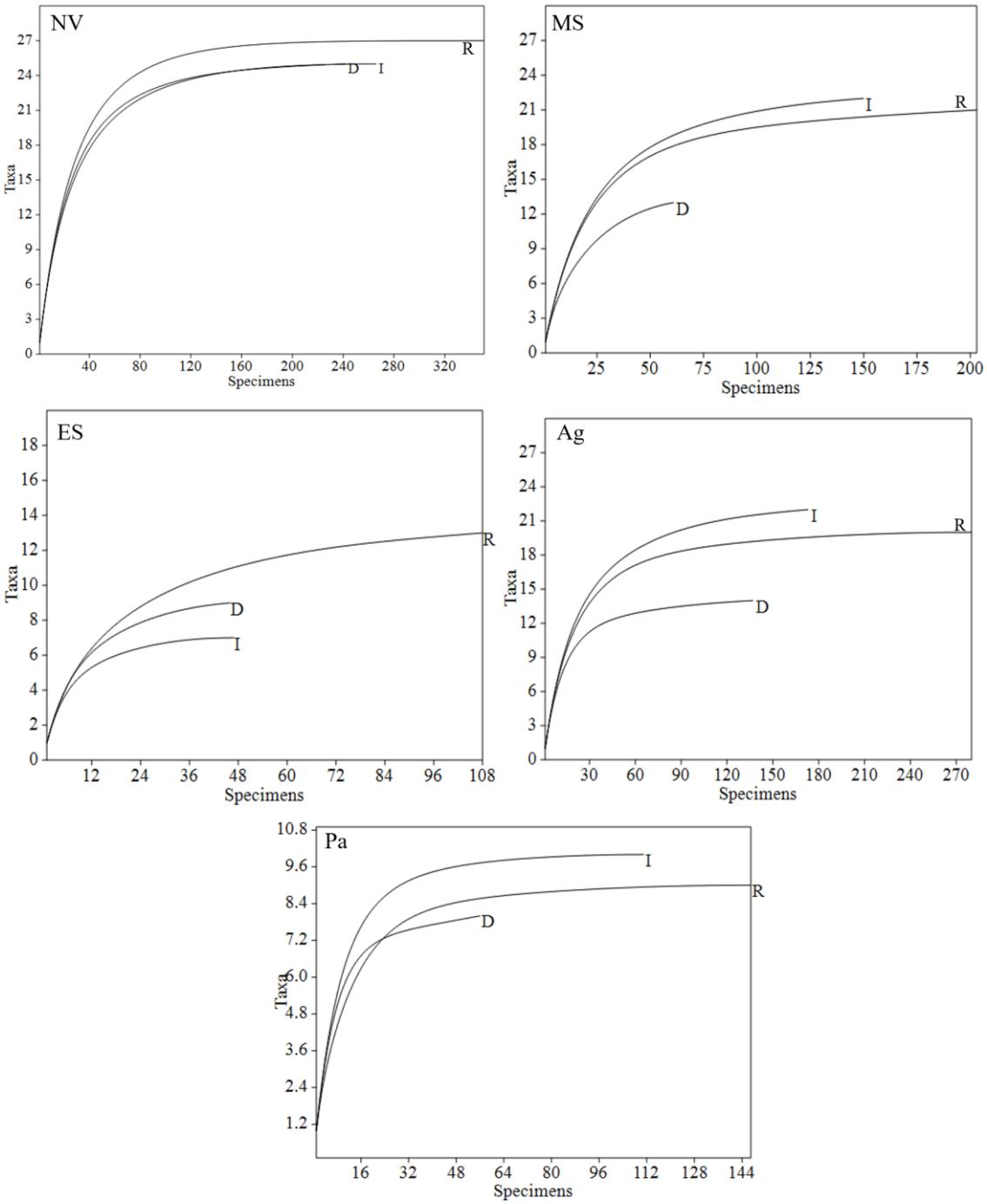

Figure 2. *Individual-based rarefaction curves for the studied sites. Abbreviations: NV, Vegetation native; MS, Maturing Secondary succession (vegetation with 15 years of regeneration); ES, Early Secondary succession (vegetation with 5 years of regeneration); Ag, Agriculture; Pa, Pasture. D, dry; I, intermediary; R, rainy*

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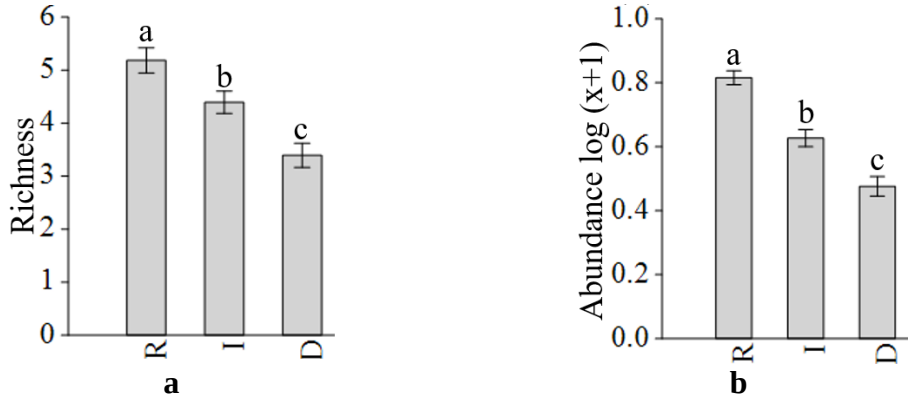

Figure 3. *Richness (number of species ± SE) (a) and abundance (number of individuals ± SE) (b) and of Carabidae in different periods of sampling in the Brazilian Amazon. The values labelled with the same letters are not significantly different according to the Tukey post-hoc test. Abbreviations: D, dry; I, intermediary; R, rainy*

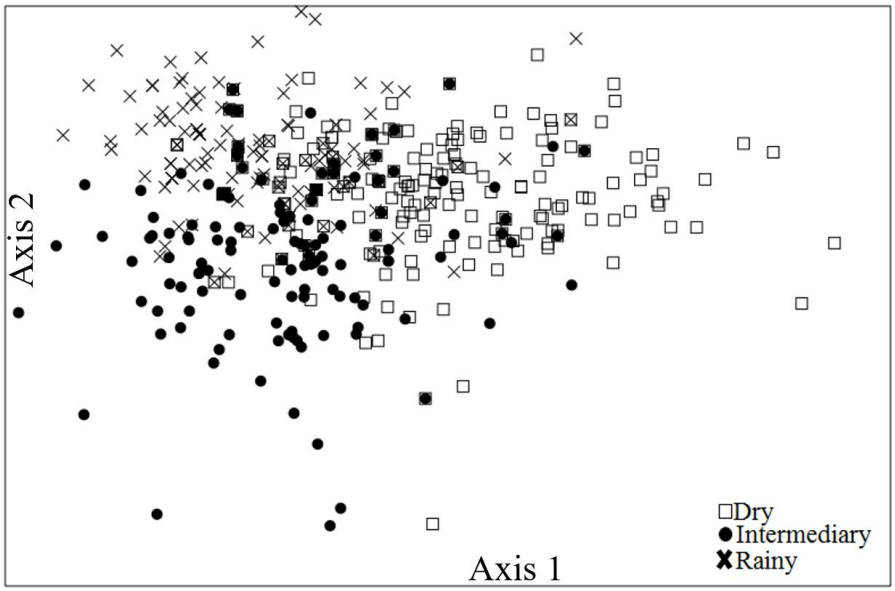

Figure 4. *Non-metric multidimensional scaling (NMDS) showing groups of Carabidae according to each periods studied (using Bray-Curtis similarity, stress 0.13)*

The taxonomic composition of the GB assemblage within each habitat varied significantly among the different seasons (PERMANOVA, $p < 0.05$). The NMDS results show an arch, indicating that the Carabidae assemblages changed gradually from the rainy towards the dry period (*Fig. A1* in the *Appendix*).

Twelve species out of 32 (ca. 37%) were considered habitat indicators. According to IndVal, two species of GB were significantly associated with NV, one with MS, six with Ag, and two with Pa. No species was indicative for ES (*Table 1*). Our analyses of GB patterns showed that of the 12 species identified by IndVal, five species present peaks of abundance in the rainy season; two species with peaks in abundance in the dry season and two species had peaks in abundance in the intermediary season (*Fig. A2* in the *Appendix*).

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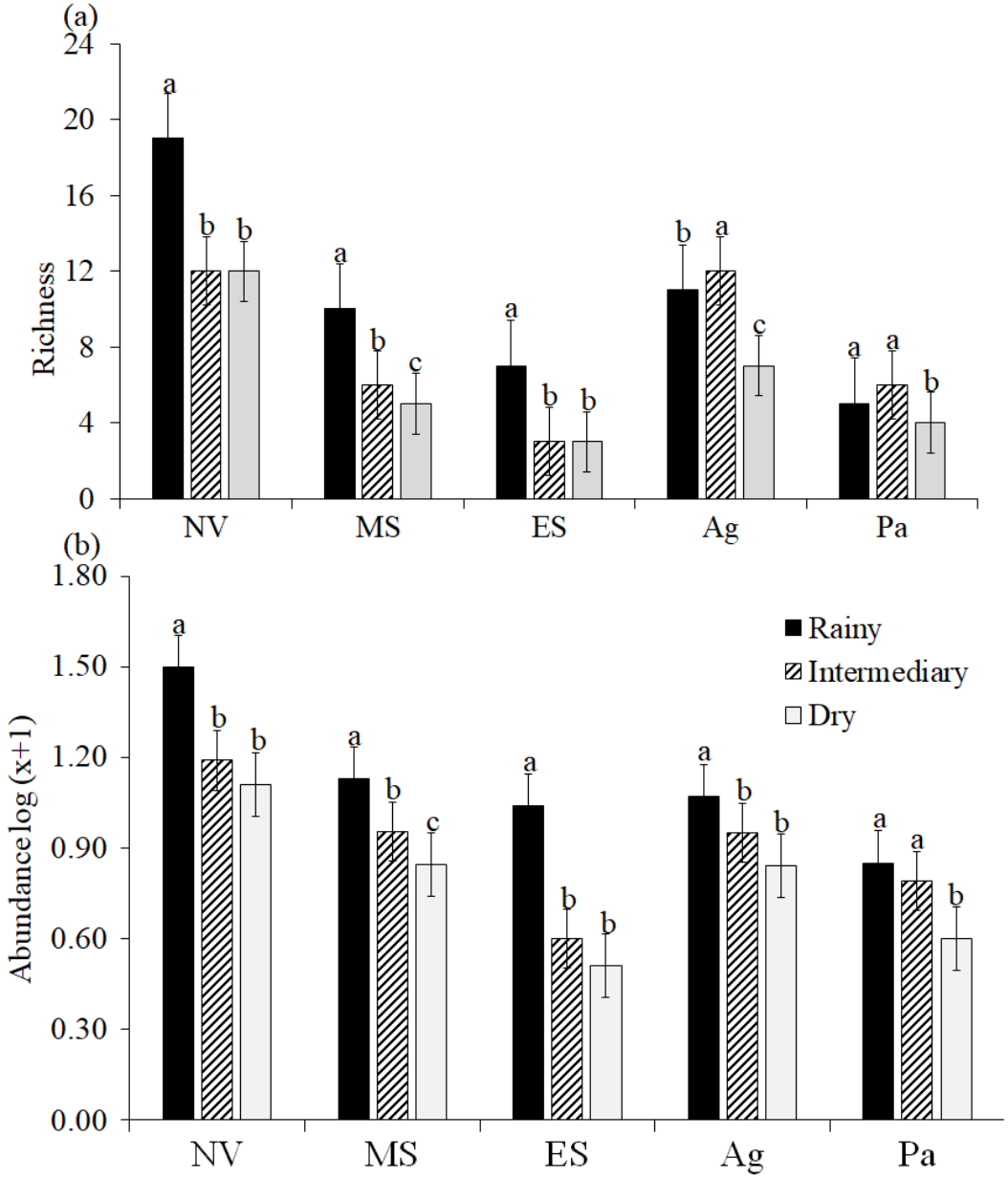

Figure 5. *Temporal variation of the richness (a) and abundance (b) of Carabidae by ecosystem. The values followed by the same letters are not significantly different according to the Tukey post-hoc test. Abbreviations: NV, Vegetation native; MS, Maturing Secondary succession (vegetation with 15 years of regeneration); ES, Early Secondary succession (vegetation with 5 years of regeneration); Ag, Agriculture; Pa, Pasture. D, dry; I, intermediary; R, rainy*

Discussion

Seasonality is an important component of ecosystems and should be taken into consideration in most biodiversity assessments. If the goal of a biodiversity study is to perform a complete inventory for a specific group, decisions concerning not only where (e.g. altitudinal gradient, location) and how (e.g. collection methods) to optimize the number of species sampled, but also when (e.g. time of year) collections should be performed are quite relevant (Maveety et al., 2014; Cajaiba et al., 2017c, d).

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Table 1. *Habitat indicators selected by the IndVal method. NV, Native vegetation; MS, Mature Secondary succession (vegetation with 15 years of regeneration); ES, Early Secondary succession (vegetation with five years of regeneration); Ag, Agriculture; Pa, Pasture (only taxa with IndVal > 25% were considered)*

Species	Indicator value	P value	Habitat
Calosoma sp1	0.88	0.005	NV
Brachininae sp	0.87	0.005	NV
Pelecium sp2	0.73	0.001	MS
Calosoma sp1	0.68	0.005	NV+MS
Loxandrus sp	0.79	0.005	Ag
Tetracha sp1	0.68	0.005	Ag
Athrostictus sp2	0.67	0.01	Ag
Pterostichini sp2	0.63	0.02	Ag
Selenophorus sp2	0.59	0.01	Ag
Pterostichinae sp2	0.53	0.005	Ag
Harpalinae sp2	0.76	0.005	Pa
Badistrinae sp	0.63	0.005	Pa

In our study, the richness and abundance of Ground Beetles (GB) collected at different seasons tended to decrease as aridity increased (Yu et al., 2006; Wang et al., 2014). The importance of the microclimate in defining the structure of GB assemblages has been emphasized in previous studies (Butterfield, 1996). Vegetation structure and its derived changes in microclimate (e.g. temperature and air moisture) are likely to be some of the most important factors controlling and structuring the distribution and diversity of GB (Magura et al., 2000). This outcome is probably associated with evolutionary strategies, allowing the organisms to optimize and synchronize their life cycle with favorable environmental conditions (Kotze et al., 2011; Wang et al., 2014). Higher richness and abundance in wetter periods can also be attributed to an increase in dispersal, responding to food dispersion/shortage (Moraes et al., 2013). In fact temperature and moisture are the major factors influencing the activity, flight, foraging behavior, and metabolism of GB (Saska et al., 2010). Wang et al. (2014) examined the effects of temperature fluctuations on GB activity-density within a year, and Silva et al. (2010) offered two hypotheses that may explain the lower diversity of adult GB in the dry season: (1) adults in open habitats are sensitive to the effects of drought and remain underground during this period; or (2) adults die in the dry season and only the immature GB survive in the nest, reaching the adult stage at the beginning of the rainy season. Additional studies are needed to understand the extent of these changes, which might be especially relevant in light of the potential effects of climate change (Maveety et al., 2014). Seasonal variations in diversity and composition emphasize the influence of phenology on survey timings in studying GB/habitat associations.

Although richness and abundance were greater in the rainy season, our results demonstrate the importance of GB inventory in different seasons to determine overall richness: Brachininae sp (not in D); *Tetracha* sp1 (only in R), Pterostichinae sp2, (only in D); Harpalinae sp2 (only in I) and *Calosoma* sp1 (not in D). Seasonal information of GB is also essential to understand the relevant ecological processes and associated functioning, such as pest predation (Paill, 2004) and seed consumption (Honek et al.,

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2003). GB phenology and asynchronous cycles may contribute to the reduction of interspecific competition among Carabidae, shaping their diversity (Werner and Raffa, 2003). Finally, the lack of information on seasonal activity patterns of GB is a major obstacle for GB conservation: detailed species information is fundamental for the several stages of resource use (Wang et al., 2014). Additionally, active species in times of intensive management practices (e.g. pesticide application and soil cultivation) could be also more sensitive to anthropogenic stressors (Wang et al., 2014).

Key findings

The results of this study demonstrate that surveying of Carabidae assemblages in different seasons provides the most accurate representation of the biodiversity of these beetles in the rainy season, however, each period of sampling may result in distinct species records. Determining which season(s) provides the most comprehensive representation of Carabidae biodiversity in tropical forests (Amazonia) can provide more accurate information for the development and implementation of conservation and management strategies of this biome and the communities they support. In fact, the present study should be complemented with studies linking carabid beetles with other taxa for understanding and assessing the state of conservation of the diverse ecosystems of the studied region.

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APPENDIX

Table A1. *Habitats surveyed, number of sampled areas, number of traps per sampled area, sampling repetitions and the total number of traps by habitat. NV = Native Vegetation; MS = Mature secondary succession (15 years of regeneration); ES = Early secondary succession (5 years of regeneration); Ag = Agriculture (Cocoa); Pa = Pasture*

Habitat sampled	Number of sampled areas	Number of pitfalls by sampled area	Repetitions (periods of the year)	Total traps/habitat
NV	2	28	3	168 (2 x 28 x 3)
MS	2	28	3	168 (2 x 28 x 3)
ES	2	28	3	168 (2 x 28 x 3)
Ag	2	28	3	168 (2 x 28 x 3)
Pa	2	28	3	168 (2 x 28 x 3)
Total number of pitfalls			840 (168 × 5)	

Table A2. *Abundance of the different species of Carabidae in the ecosystems monitored in the Brazilian Amazon. NV = Native Vegetation; MS = Mature secondary succession (15 years of regeneration); ES = Early secondary succession (5 years of regeneration); Ag = Agriculture (Cocoa); Pa = Pasture. R= Rainy season; I = Intermediate season; D = Dry season*

Species	NV			MS			ES			Ag			Pa			Total
	R	I	D	R	I	D	R	I	D	R	I	D	R	I	D	
Pelecium sp1	25	-	41	5	-	2	-	-	-	14	-	10	8	15	4	124
Pelecium sp2	3	1	1	12	12	6	-	-	-	-	-	-	-	-	-	35
Athrostictus sp1	29	5	-	5	2	-	-	14	10	-	-	-	32	15	13	125
Athrostictus sp2	2	5	-	-	-	-	-	-	-	4	6	26	-	-	-	43
Harpalinae sp1	34	4	-	4	-	-	14	-	-	-	-	-	12	-	-	68
Harpalinae sp2	-	-	-	-	-	-	-	-	-	-	4	-	-	22	-	26
Pterostichus sp1	26	38	-	-	30	-	-	-	-	14	11	-	10	9	5	143
Pterostichus sp2	18	8	2	7	3	1	3	-	-	6	3	3	8	7	4	73
Brachininae sp	33	9	-	4	1	-	-	-	-	-	-	-	-	-	-	47
Pterostichinae sp1	-	-	39	19	-	-	2	-	-	2	-	19	-	-	-	81
Pterostichinae sp2	-	-	4	-	-	-	-	-	6	-	-	23	-	-	-	33
Laemostenus sp1	4	-	-	7	5	-	-	-	-	8	7	-	-	-	-	31
Laemostenus sp2	8	4	4	14	4	2	1	2	-	3	-	2	-	-	-	44
Selenophorus sp1	26	10	8	10	6	4	4	8	-	-	9	-	-	-	-	85
Selenophorus sp2	4	-	-	-	-	-	-	-	-	7	16	8	-	-	-	35
Selenophorus sp3	14	10	8	8	6	6	7	6	-	8	3	7	-	-	-	83
Helluomorphoides squiresi	4	3	3	-	-	-	4	8	-	-	-	-	-	-	-	22
Lebiini sp1	7	5	5	7	-	-	-	-	-	10	-	-	-	-	-	34
Lebiini sp2	8	3	2	4	-	1	1	-	-	1	1	1	-	-	-	22
Pterostichini sp1	15	9	8	-	1	-	30	5	-	30	-	23	5	9	3	138
Pterostichini sp2	3	-	-	5	5	3	-	-	-	13	12	14	-	-	-	55

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Pterostichini sp3	14	6	4	12	8	2	4	-	-	11	10	9	-	-	-	80
Loxandrus sp	-	-	-	-	-	-	-	-	-	14	12	13	-	-	-	39
Galerita sp1	36	24	16	34	8	7	4	2	-	21	25	-	11	-	-	178
Badistrinae sp	-	-	-	-	-	-	-	-	-	-	-	-	22	16	9	47
Tetracha sp1	14	-	-	5	-	-	-	-	-	35	-	-	-	-	-	54
Tetracha sp2	5	2	2	4	3	1	-	1	2	1	2	-	-	-	-	23
Odontocheila sp	32	17	14	21	3	4	25	7	3	31	23	16	38	19	6	259
Calosoma sp1	32	24	-	13	-	-	-	-	-	-	-	-	-	-	-	72
Carabus sp1	18	15	11	16	11	-	10	-	-	5	3	-	-	-	-	89
Carabus sp2	-	5	2	15	8	1	-	9	4	10	8	2	4	4	-	69
Carabus sp3	32	19	13	15	9	3	5	-	-	7	-	4	1	3	-	111

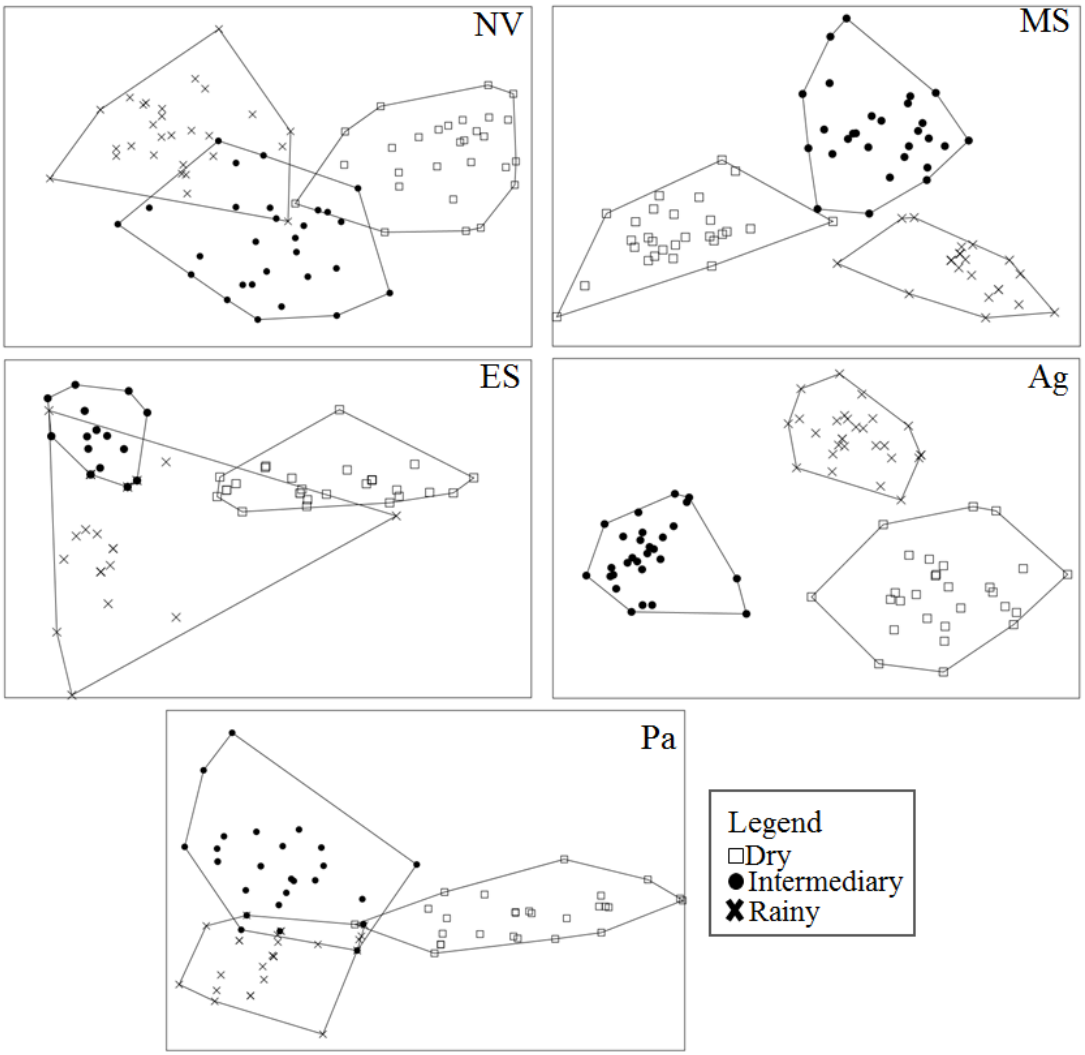

Figure A1. *Non-metric multidimensional scaling (NMDS) showing groups of Carabidae according to each periods studied by ecosystem (using Bray-Curtis similarity). Abbreviations: NV, Vegetation native; MS, Maturing Secondary succession (vegetation with 15 years of regeneration); ES, Early Secondary succession (vegetation with 5 years of regeneration); Ag, Agriculture; Pa, Pasture*

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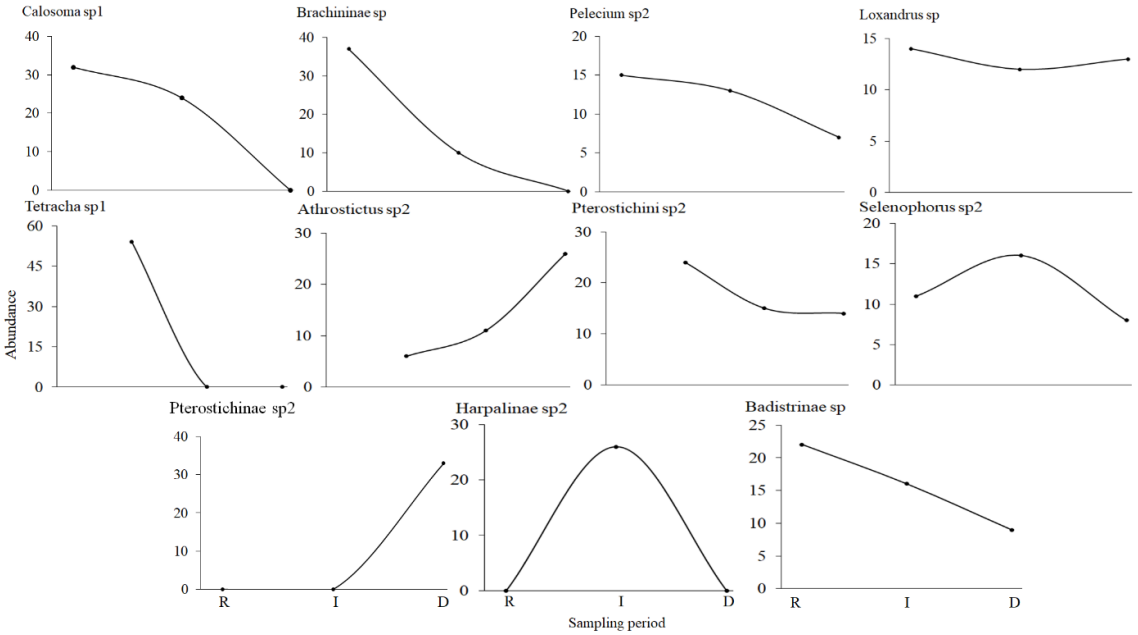

Figure A2. *Activity patterns of the 12 Carabidae species identified by the IndVal method. The indicator species of each environment are shown in Table 1. R= Rainy season; I = Intermediate season; D = Dry season*

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