

Collembola as bioindicators of restoration in mined sand dunes of Northeastern Brazil

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Received: 29 March 2008 / Accepted: 8 October 2008 / Published online: 21 October 2008
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Abstract Opencast mining causes severe environmental impacts by removing the vegetation cover and depleting the fauna. Reforestation methods using native species and diverse pre- and post-disturbance approaches aim to recover the original richness and diversity of species found before the impact. Bioindicators are powerful tools to evaluate the restoration of the original environmental conditions in disturbed areas. We used species richness, endemism and diversity measurements of Collembola to compare successional stages in reforested sites of different ages compared with a control undisturbed area. Richness and abundance of Collembola were subjected to correlation analysis with age of plots and vegetational variables. Areas that were reforested for up to 16 years supported a much lower Collembola species richness than undisturbed areas. Both the age of reforestation plots and vegetation variables (number of trees, diameter of crowns, depth of leaf litter and tree species richness) were positively and significantly correlated to collembolan abundance and richness. The results showed that the diversity of the 16-year-old plot was significantly higher than that of younger areas, but significantly less diverse than that of the control area. Endemic species were more sensitive to disturbance than non-endemics. Thus, species richness and diversity of soil Collembola can be only partially restored with appropriate reforestation methods, and although it takes many years, to some extent even endemic species can be gradually restored. Nevertheless, the maintenance of undisturbed

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Federal Inscription number 05.117.699.000.198, Cabedelo, PB, Brazil

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diversity reservoirs linked by ecological corridors to reforested plots is imperative, as only undisturbed areas can support most of the endemic species able to re-colonize reforested sites.

Keywords Collembola · Bioindicators · Diversity recovery · Mined sand dunes · Reforestation

Introduction

The restoration of environmental conditions and species diversity in opencast mined areas is demanded by environmental authorities in Brazil. However, reforestation methods are poorly studied and reported in South America. Restoration should involve a combination of treatments: (a) in situ improvement of physical and chemical substrates; (b) top-soil spreading and later improvement of the recovered soil; (c) soil stabilization and protection with herbaceous species and (d) planting of shrubs and trees (Greenslade and Majer 1993; Kumssa et al. 2004; Andrés and Mateos 2006).

The evaluation of the success of restoration demands measurable criteria to define the degree of recovery of a given site. Surrounding undisturbed areas can give an indication of the natural conditions for comparison (Andrés and Mateos 2006). Physical and chemical indicators do not necessarily reflect the capability of the soil to maintain the ecological functions necessary to recover soil faunal diversity (van Bruggen and Semenov 2000; Cassagne et al. 2003). Soil mesofauna taxa are useful bioindicators for assessing the success of biodiversity recovery, as they are sensitive to human disturbance and are involved in diverse ecological processes in the formation of soil (Rusek 1998; Kumssa et al. 2004).

Collembola are good representatives of soil faunal diversity (Cassagne et al. 2003) and play a major role in nutrient cycling, decomposition of organic matter and soil formation; all basic features in the functioning of forest ecosystems (Faber 1992; Bardgett et al. 1998; Cassagne et al. 2003; Kumssa et al. 2004). The diversity of collembolan species and the density of populations are influenced by many aspects of the soil such as pH, aeration, organic matter composition, nutrient availability, humus type, vegetation cover and soil physical structure (Oliveira 1993; Salomon et al. 2004; Cole et al. 2005). The richness of endemic species is particularly sensitive to environmental disturbance and forest replacement, more so than that of non-endemic species (Deharveng 1996). These features emphasize the importance of Collembola as bioindicators of environmental quality (Huhta et al. 1967; Hole 1981; Faber 1992; Oliveira 1993; Detsis et al. 2000; Cassagne et al. 2003).

The main objective of this study was to determine whether strategies to improve the restoration program applied, in the area studied, by the mining company, are able to achieve recovery of the levels of diversity found in undisturbed adjacent areas, as the removal of vegetation previous to mining operations and the extraction of minerals of commercial interest completely eradicate the fauna leaving behind a sterile newly formed and bare sand dune. This was considered by comparing the diversity of Collembola in plots at different regeneration ages with diversity in a control (undisturbed) area. The species richness of endemics was used as an indicator of the restoration success. As a consequence of this study it was possible, for the first time, to obtain a Collembola diversity dataset for South America.

Materials and methods

Study site

We conducted the study in a set of reforested plots of known ages and included an undisturbed (control) area adjacent to the study site. The reforestation is part of a restoration plan of a heavy metal (mainly rutile, zircon and ilmenite) mining company. The study site is located on the north coast of Paraíba state, in northeastern Brazil, between the estuary of the river Guaju ($6^{\circ}29'27''\text{S}$, $34^{\circ}58'03''\text{W}$) and the limits of the mining company property about 5 km to the south ($6^{\circ}32'40''\text{S}$, $34^{\circ}57'58''\text{W}$). The climate following Köppen's system (Koppen and Geiger 1936; Shear 1966) is Am, the mean annual rainfall in 2006 was 1,246 mm, 177.4 in each of the six winter months (wet season) and 30.2 in each of the six summer months (dry season). The mean annual temperature is 26, 23.7°C during the winter and 27.2°C in summer (Rosado 2001). The whole area was originally covered with sand dune forest typical of the region, known as "Restinga". This vegetation is still present in undisturbed adjacent areas which will be mined in the near future. The mining process involves the removal of all vegetation and the processing of the sand to extract minerals of interest. The post-mining restoration plan started in 1988 is composed of five stages: (a) reestablishment of dune topography, (b) top soil reposition (20–30 cm), (c) fertilization, (d) planting and grass sowing, and (e) control of pests and exotic species. More than 30 species are planted, most of which are native to the original local flora. The areas are planted until they reach a density of 2,500 seedlings/ha. Previous studies recorded 337 plant species, from grasses to trees, in undisturbed sites prior to mining activities and adjacent areas, waiting to be mined (Oliveira-Filho and Carvalho 1993; Rosado 2001), the average density of trees in control areas being 3,316 trees/ha.

Sampling and identification of soil Collembola

This work was part of a multi-taxonomic study conducted in 2005–2006, to analyze the ecological succession on mined sand dunes. Collembola were collected in Malaise traps, used in the general entomological survey, even though Malaise traps are aimed at collecting Pterygota. Collembola were fixed in 70% ethanol in the collection tube of the trap. They were manually extracted from the alcohol using small brushes and dissecting needles with flattened tips, sorted under a stereomicroscope, counted, and mounted in Hoyer's media for identification to species level. Taxonomic studies of Collembola pose some problems, especially in Brazil where only 210 species have been described, a small fraction of the actual estimated diversity (about 5,000 species). We used all available literature including electronic identification keys (Bellinger et al. 2007). However, on occasion, it was likely that different but very similar species that we could not distinguish were identified as the same species. This kind of uncertainty would have resulted in an underestimation of the actual number of species in the study area.

We sampled four plots of 2, 4, 8, and 16 years since reforestation and a control (undisturbed) area in which no logging, hunting or other exploitation had been allowed in the last 30 years. Each plot was divided into three replicates and a trap was set at random in each one resulting in 15 samples for the whole area. Sampling periods lasted 21 days in the dry season (November 8–29 2005, total rainfall during sampling 0.50 mm) and 21 days in the wet season (April 4–25 2006, rainfall 281.9 mm), each trap was left open for 7 days on each occasion, resulting in a total of 30 samples. The alcohol in the collection tubes of traps was replenished daily to avoid drying out.

Given the constraints imposed on the study by the mining and reforestation program undertaken by the mining company, there was only a single restoration plot for each of the different ages and a single control plot. While three samples, at least 100 m apart from each other, were taken from each plot, these do not constitute true plot replication and the three samples from each plot were combined for the purposes of the analyses. Nevertheless, the study documents a real world restoration process from the perspective of an often ignored group of animals.

Endemic species

To ascertain the status of endemism of a species partly depends on the availability of information about distributional patterns of the fauna; however, biogeographic studies of many of the soil fauna, particularly in Brazil, are rare or nonexistent. A complete survey of the local Collembola fauna, including descriptions of new species and biogeographic studies is in progress, but will take years to complete. Therefore, we assumed for practical purposes that the species that did not have a known distribution beyond the study area were the local endemics. This was based on bibliographic records and our collections of the wider regional fauna.

Data analysis

Sampling site means, variances and diversity indices for all vegetation measurements are listed by Costa et al. (2006). The vegetation variables used for statistical correlation were: (a) number of trees, (b) number of species of trees, (c) maximum diameter of the crown, and (d) depth of leaf litter.

The completeness of Collembola sampling was checked using a cumulative curve of species richness (total number of species) estimated with the software Estimates 7.51 (Colwell 2005) and the jackknife estimator of the total theoretical richness was performed. Graphics were plotted with the software package Statistica (Statsoft 2001). Relative abundances, means and variances were calculated for each plot. The number of individuals and the number of species of Collembola were correlated (Spearman correlation r_s) with vegetation variables and with the regeneration age, using Statistica (Statsoft 2001). Species richness was used as the universal estimate of diversity, in addition to Shannon, Brillouin and Simpson diversity indices, Camargo evenness, Morisita–Horn similarity indices and percentages of similarity. These indices were calculated using Ecological Methodology 5.2 (Krebs 1999; Krebs and Kenney 2000). Similarity analysis was performed using the softwares SPADE (Chao and Shen 2003–2005) and Estimates 7.51 (Colwell 2005). A dendrogram was obtained using Morisita–Horn values and cluster analysis was performed using Statistica (Statsoft 2001). The similarity index was calculated with the square root of the absolute abundance of each species.

Results

Species abundance and richness

We collected a total of 12,183 individual collembolans, distributed in 24 species, 13 genera and 10 families. Three known species [*Entomobrya* cf. *nivalis* (Linnaeus 1758),

E. griseoolivata (Packard 1873) and *Sphyrotheca mucroserrata* Snider 1978] and three genera (*Sphyrotheca* Börner 1906, *Prorastriopes* Delamare Deboutteville 1947 and *Rastriopes* Börner 1906) were new records for Brazil (Culik and Zeppelini 2003; Bellini and Zeppelini 2004). Thirteen species were newly identified and are still undescribed, one species could not be positively identified to the species level, 14 species are endemic to the coast of Paraíba. At least 10 species are known to be distributed beyond the study area, either in other parts of Brazil [*Arlesia albipes* (Folsom 1927), *Seira brasiliana* (Arlé 1939), *S. mirianae* Arlé and Guimarães 1981, *S. prodiga* (Arlé 1959), *S. xinguensis* (Arlé 1959) and *Hemisotoma thermophilus* (Axelson 1900)] or elsewhere in the world [*E. cf. nivalis*, *E. griseoolivata*, *Brachystomella cf. agrosa* (Schäffer, 1896) and *S. mucroserrata*]. Table 1 presents the total number of individuals, their distribution in the sampling sites and the community indices. The cumulative curve of overall species richness indicates that the actual diversity of the area was still not fully assessed. Jackknife estimates of a total of 30 species present in this area (Fig. 1) confirmed that not all species were recorded during the study.

Comparison of confidence limits estimated for species richness among sites shows that the number of observed species in the 16-year-old reforested area was significantly higher than that in the 2-, 4- and 8-year-old areas, yet species richness in this plot was still significantly lower than that of the control undisturbed area (Fig. 2). Indeed species richness in the control area is higher (19 species) than the combined values of all reforested areas (15 species).

Effects of vegetation and age of plots on Collembola abundance and richness

The abundance and species richness of Collembola were strongly and positively correlated to all of the vegetation variables and to the age of reforestation plots. Number of trees and diameter of tree crowns are both directly related to shading of the soil and affected both number of Collembola individuals ($r_s = 0.519$ $P = 0.047$; $r_s = 0.663$ $P = 0.007$, respectively) and species richness of Collembola ($r_s = 0.711$ $P = 0.003$; $r_s = 0.653$ $P = 0.008$, respectively). The depth of leaf litter was significantly related to the number of individuals of Collembola ($r_s = 0.573$ $P = 0.025$) and species richness ($r_s = 0.742$ $P = 0.002$). The species richness of trees was positively correlated with the number of collembolan individuals ($r_s = 0.574$ $P = 0.025$), and was the variable most highly correlated with Collembola species richness ($r_s = 0.846$, $P < 0.001$). The age of restoration plots, which determined the successional stage of vegetation, was strongly correlated with the number of individuals ($r_s = 0.644$, $P = 0.010$) and the species richness of Collembola ($r_s = 0.913$, $P < 0.001$).

Analysis of similarity

Similarity dendrograms, obtained with the similarity index of Morisita–Horn, indicated that there were two groups of similarity, one formed by the 2-, 8- and 4-year-old plots and another group formed by the 16-year-old and control plots. The 2-year dry plot did not group with either one, because no specimens were collected in this area. The 16-year-old and control areas were more similar to each other during wet season than they were to themselves in the dry season (Fig. 3), stressing the influence of the climate on Collembola diversity, mainly as an effect of variation in the abundance of the rare species.

Table 1 Numbers of individuals of collembolan species, with abundance, species richness and diversity indices

Species	2 years	4 years	8 years	16 years	Control	Total/sp.
<i>Seira</i> sp. n. 1	0	440 ^a	9 ^a	0	0	449
<i>Seira brasiliiana</i>	0	0	35	0	0	35
<i>Seira miriana</i>	0	0	0	0	363	363
<i>Seira prodiga</i>	0	0	4	5	9	18
<i>Seira xinguensis</i>	0	12	69	1,464	2,751	4,296
<i>Seira</i> sp. n. 2	108 ^a	190 ^a	2,532 ^a	288 ^a	534 ^a	3,652
<i>Seira</i> sp. n. 3	0	0	0	383 ^b	0	383
<i>Seira</i> sp. n. 4	0	0	0	0	167 ^b	167
<i>Entomobrya</i> cf. <i>nivalis</i>	0	0	0	3	0	3
<i>Entomobrya grisoolivata</i>	0	0	0	100	0	100
<i>Lepidocyrtus</i> sp. n. 1	0	0	0	6 ^a	17 ^a	23
<i>Lepidocyrtus</i> sp. 2	0	0	0	0	8 ^b	8
<i>Hemisotoma thermophilus</i>	0	0	0	0	84	84
<i>Arlesia albipes</i>	0	0	0	0	6	6
<i>Brachystomella</i> cf. <i>agrosa</i>	0	0	0	281	19	300
<i>Xenylla</i> sp. n.	0	0	0	0	27 ^b	27
<i>Sphaeridia</i> sp.	0	0	0	138 ^a	34 ^a	172
<i>Sphyrotheca mucroserrata</i>	0	0	0	0	6	6
<i>Prorastriopes</i> sp. n.	0	2 ^a	0	0	58 ^a	60
<i>Rastriopes</i> sp. n.	0	3 ^a	270 ^a	124 ^a	39 ^a	436
<i>Sminthurinus</i> sp. n. 1	0	0	18 ^a	0	86 ^a	104
<i>Sminthurinus</i> sp. n. 2	0	0	0	0	12 ^b	12
<i>Dicyrtoma</i> sp. n. 1	0	0	0	1,115 ^a	351 ^a	1,466
<i>Dicyrtoma</i> sp. n. 2	0	0	0	0	13 ^b	13
Total of specimens	108	647	2,937	3,907	4,584	12,183
Rel. Abd. Ind. (%)	0.89	5.31	24.1	32.06	37.62	
Rel. Abd. Sp. (%)	4.16	20.83	29.16	45.83	79.16	
Total of endemic species	1 (7.1)	4 (28.5)	4 (28.5)	6 (42.8)	12 (85.7)	14 (58.0)
Total of common species	0	1 (10.0)	3 (30.0)	5 (50.0)	7 (70.0)	10 (41.0)
Richness per area dry season	0	2 (33)	3 (50)	4 (66)	4 (66)	6
Richness per area wet season	1 (4.1)	5 (20.8)	7 (29.1)	11 (45.8)	19 (79.1)	24
Total richness per area	1	5	7	11	19	24
Simpson	nd	0.147	0.203	0.545	0.581	
Shannon	nd	0.436	0.658	1.441	1.755	
Brillouin	nd	0.386	0.641	1.365	1.726	
Camargo	nd	0.461	0.295	0.492	0.283	

^a Species with known distribution restricted to the study area but recorded in more than one plot

^b Species with distribution known only from a single plot

Values in parentheses are percentages of the endemic species, common species and overall number of species in each plot and total

Rel. Abd. Ind., relative abundance of individuals; Rel. Abd. Sp., relative abundance of species; nd, no data

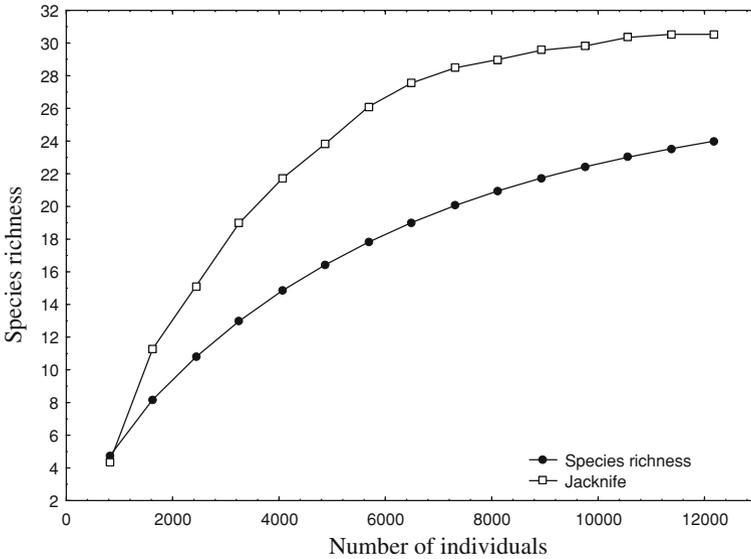


Fig. 1 Cumulative curve of species richness observed with increasing numbers of individuals collected, and Jackknife estimation of theoretical richness (100 replicates)

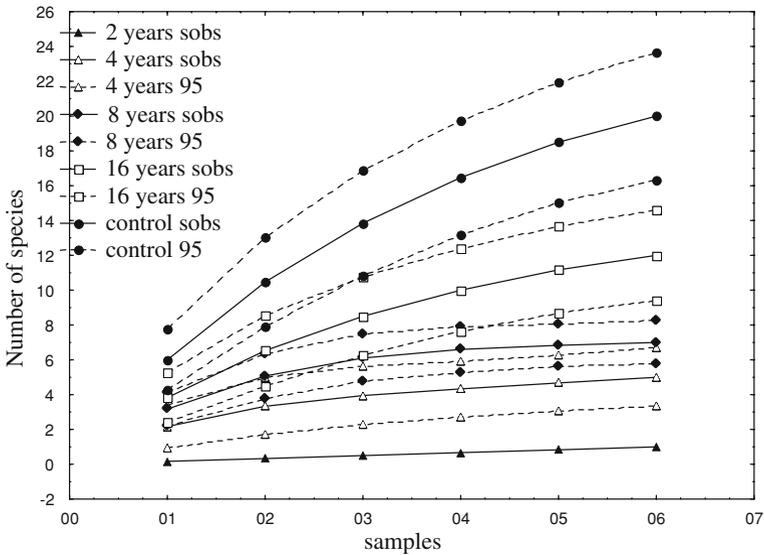


Fig. 2 Distribution of observed richness. *Solid line*, number of species observed (sobs); *dotted line*, 95% confidence intervals

Analysis of endemism

Ten species have a broad distribution, either in Brazil or elsewhere. Fourteen species (superscript ‘a’ in Table 1; 58% of the total number of species) are new to science and presumed endemic to the region; six of these were found in a single sample (superscript ‘b’

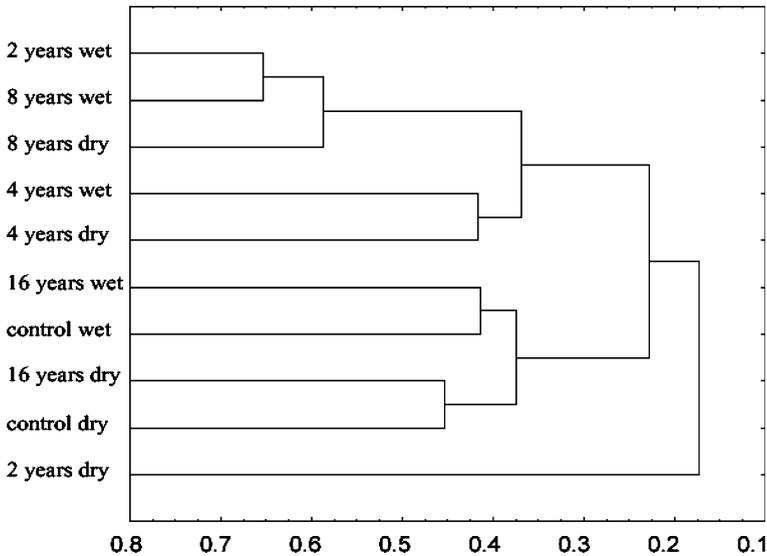


Fig. 3 Dendrogram of Morisita–Horn similarity for all plots with dry and wet season data separate

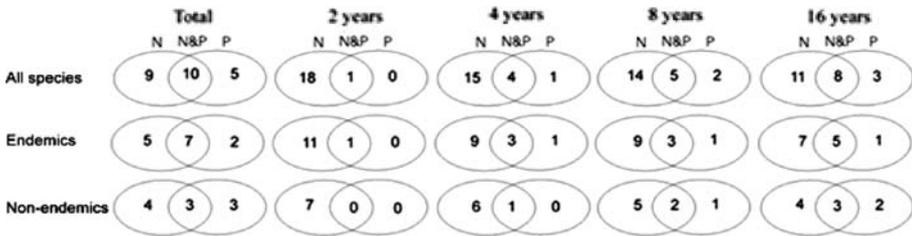


Fig. 4 Number of endemic and non-endemic species in 2-, 4-, 8- and 16-year-old reforested plots and the control plot. N, native control area; P, planted (reforested) area

in Table 1). Presumed endemic species were unevenly distributed among plots. Nine of the endemics (64% of the endemics, 37.5% of the total) were found in the combined reforested plots and 12 endemics (86% of the endemics, 50% of the total) were found in the control plot. Comparisons of the numbers of species in reforested and control plots showed that all reforested plots had fewer species (Fig. 4).

Discussion

The data clearly indicate that reforested areas support a much lower richness of both endemic and non-endemic Collembola species than the undisturbed area, which is confirmed in studies conducted elsewhere (Greenslade and Majer 1993; Deharveng 1996; Kumssa et al. 2004; Andrés and Mateos 2006). The vegetation variables (number of trees, diameter of crowns, depth of leaf litter), all factors that affect shading of the soil and availability of organic matter, were positively correlated with the number of individuals and species richness of Collembola. Tree species richness seems to be the most important

vegetational variable. Similar results were found by Greenslade and Majer (1993) in bauxite mines in Western Australia. This is in contrast to findings by Salomon et al. (2004) that richness of plants and functional groups of plants were not significantly linked to collembolan richness in an experimental grassland ecosystem.

Post-disturbance recovery of invertebrate communities is slow and not shorter than 15 years (Neumann 1991; Greenslade and Majer 1993; Webb 1994), with an estimated 80–102 years for cold temperate forest collembolans (Addison et al. 2003). This is confirmed herein. Similarity and diversity analyses showed that the 16-year-old plot was the most diverse among reforestation areas and the most similar to the control plot. Nevertheless, its species richness is significantly lower than that of the undisturbed plot. The reforestation method applied in the mined sand dunes studied is, therefore, able to partially recover collembolan diversity, but this will take much longer than 16 years, and there is no guarantee that all the original diversity will be restored, especially the endemic species which are more sensitive to disturbance. A similar analysis of endemism in the Pyrenees (France) indicated that areas artificially reforested with conifers did not recover the richness of endemic *Collembola* species 20–25 years after reforestation (Deharveng 1996). It is important to note that canopy collembolan species were not collected in this study and are likely to take a longer time to recover as they may depend on trees maturing.

The colonization of newly restored soils depends on the active and passive movement of animals from undisturbed areas (St. John et al. 2002). In the present study we strongly reinforce the importance of maintaining ecological corridors linking disturbed areas with native undisturbed sites. This approach combined with locally appropriate reforestation techniques (Rosado 2001; Andrés and Mateos 2006) will optimize efforts for restoration and shorten the time span to recover, as much as possible, the original diversity and richness. It is imperative to preserve undisturbed areas as reservoirs of biodiversity, as reforestation cannot restore endemic species diversity if the plots are isolated from natural reservoirs of diversity, especially for non-flying insects. Finally, soil *Collembola* confirm their importance as bioindicators for disturbed areas, as they proved to be highly sensitive to different stages of ecological succession, have a short life cycle, and are species rich in both endemics and non-endemics.

Acknowledgments This research project was funded by CNPq project # 507127/2004-8, with a grant to the senior author under the CNPq/DTI program, M.I.M. Hernández had a CAPES/PRODOC grant. Claudéci S. Silva provided data concerning planting on restored areas, Rodrigo A. Costa, Valderêz H. Costa, Rembrandt R.A.D. Rothéa helped in field work, Helder F. Araújo assisted with statistical analyses, Peter Iverson revised the English and L. Deharveng provided important comments and suggestions. Two anonymous referees greatly improved the manuscript.

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