

Variation in dung removal by dung beetles in subtropical Atlantic Rainforests

Valentina Amore¹ , Pedro Giovâni da Silva^{1,2} , Maristela Carpintero Hensen¹ ,
Malva Isabel Medina Hernández¹  & Jorge M. Lobo^{3*} 

¹Depto. Ecologia e Zoologia, Universidade Federal de Santa Catarina, CEP 88040-900, Florianópolis, Santa Catarina, Brazil, ²Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Universidade Federal de Minas Gerais, CEP 31270-901, Belo Horizonte, Minas Gerais, Brazil, and ³Depto. de Biogeografía y Cambio Global, Museo Nacional de Ciencias Naturales, C.S.I.C., Madrid, Spain

Accepted: 15 June 2018

Key words: Scarabaeinae, tropical rainforest, small droppings, variance partitioning, air temperature, biomass, Coleoptera, Scarabaeidae

Abstract

Dung consumption and removal is a fundamental ecological process carried out by dung beetles that drive soil nutrient cycling and associated ecosystem services. In this study, the removal rate of small droppings by dung beetles was estimated in natural subtropical rainforests of southern Brazil located along an elevational gradient, in order to understand the factors influencing variation in dung removal. To do that, the comparative explanatory capacity of three main types of variables was quantified: assemblage characteristics, local climatic and habitat conditions, and seasonal variation. The complete disappearance of dung within 48 h after deposition occurs in 73% of occasions. The highest explanatory capacity correspond to the combined effects of the three types of variables; however, average air temperature during the sampling period, total volume of dung beetles collected in the traps in which dung removal was measured, and the seasonal transition from summer to winter were the most important and representative predictors of dung removal. Thus, the dung nutrient incorporation into the soil will most likely be greater during spring-summer conditions, when the air temperature at the time of dung deposition is high and the biomass of the dung beetle assemblage is bigger.

Introduction

The role of biodiversity in ecosystem functioning has been investigated intensively in the last decades (Naeem et al., 2012). Despite the many advances that have been made linking biodiversity and ecosystem functioning, many uncertainties remain (Balvanera et al., 2014). For example, the simultaneous effects have been reported of various components of biodiversity (richness, abundance, composition) and environmental factors influencing the functional role of a species assemblage, thus hiding clear relationships between biodiversity and ecosystem functioning (Balvanera et al., 2014). Therefore, there is a need to investigate which biodiversity components and environmental factors interact the most in providing specific ecosystem functions.

Dung beetles (Coleoptera: Scarabaeidae, Scarabaeinae) are a species-rich group involved in several ecological processes that provide relevant ecological functions (Sakai & Tamiji, 1999; Nichols et al., 2007). Coprophagous beetles are known around the world for their removal and burial of faeces in the soil (Nichols et al., 2008; Beynon et al., 2012). In fact, the majority of dung beetle species feed on the microorganism-rich liquid component of mammalian dung and, on some occasions, on the dung of other vertebrate groups, rotting fruits, fungus, or carrion, the latter mainly in the neotropics (Halffter & Matthews, 1966; Halffter & Edmonds, 1982; Simmons & Ridsdill-Smith, 2011). The consumption and relocation of dung by beetles drives a series of ecological processes, such as soil nutrient cycling, aeration and bioturbation, secondary seed dispersal and burial, and nematode and fly control (Nichols et al., 2007). These are key processes in human modified ecosystems (such as cattle farming or agriculture), but also in natural biomes like tropical forests.

Most literature about dung beetle removal is focused on experimental or mesocosmos designs. Several studies have

*Correspondence: Jorge M. Lobo, Depto. de Biogeografía y Cambio Global, Museo Nacional de Ciencias Naturales, C.S.I.C. c/ José Gutiérrez Abascal 2, 28006 Madrid, Spain.
E-mail: jorge.lobo@mncn.csic.es

estimated dung removal efficiency and other ecological functions according to the (low) number, identity, and functional characteristics of selected species (Horgan, 2001; Finn & Giller, 2002; Slade et al., 2007; Rosenlew & Roslin, 2008; Nervo et al., 2014, 2017; Tixier et al., 2015; Yoshihara & Sato, 2015; Manning et al., 2017). However, aside the importance of dung removal to understand nutrient cycling and ecosystem functioning, not many studies have directly estimated dung removal in field samples (Holter, 1979; Lee & Wall, 2006), and only few have done this under neotropical forest conditions (Herrick & Lal, 1996; Andresen, 2003; Amézquita & Favila, 2010; Braga et al., 2013; Batilani-Filho & Hernández, 2017).

Assemblage characteristics – such as species richness, abundance, biomass, or composition – have been shown to be associated experimentally with the rate of dung removal in dung beetles (Beynon et al., 2012; Nervo et al., 2014; Tixier et al., 2015). Similarly, local habitat and climatic variables have also been linked to the activity of dung beetle species and their seasonal and spatial distribution (Numa et al., 2012; Kaspari et al., 2015; da Silva et al., 2018), as all organisms have a thermo-comfort zone and limits of environmental conditions that cannot be surpassed (Angilletta, 2009). Seasonal variation can be another important variable in dung removal by dung beetles, not only because of the associated change in climatic conditions, but also as a consequence of the species specific time required to complete a specific instar (Bull, 1987; Kivelä et al., 2016).

In this study, we estimate the rate of dung removal by dung beetles at six sites representing natural subtropical rainforests located along an elevational gradient in southern Brazil. A former study carried out on these sampling sites indicated the existence of two contrasting assemblages above and below 800 m above sea level, as well as a mid-elevation peak in species richness, coinciding with the lowest species richness values at the highest elevations (da Silva et al., 2018). Taking into account the likely influence of assemblage characteristics and local climatic or habitat conditions on dung removal rates, these rates are probably associated with faunistic and environmental variations. We therefore compared the capability of three types of predictors – assemblage characteristics, local climatic and habitat conditions, and seasonal variation – to determine whether disappearance of dung varies environmentally or whether such removal can be considered a widespread process in these subtropical rainforests.

Materials and methods

Study area

The study was performed in the Atlantic Forest in the Serra Catarinense, state of Santa Catarina, southern Brazil, and

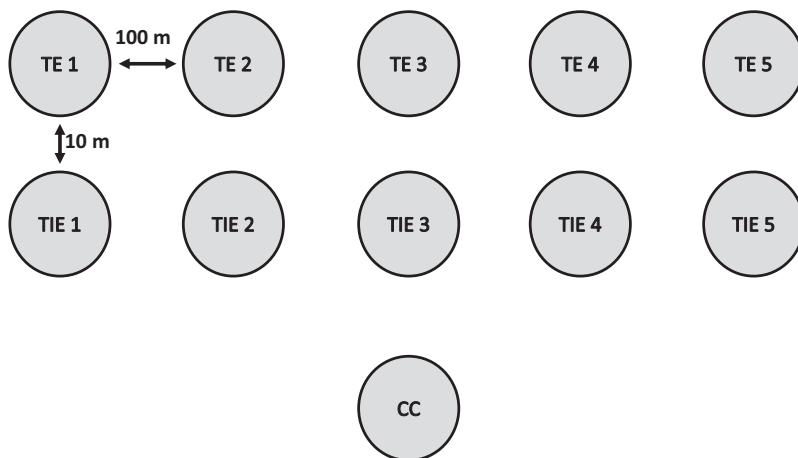
was comprised of an elevational transect of 93.7 km long extending northeast-southwest from 27.73 to 28.15°S and from 48.80 to 49.61°W. This is a highland region with subtropical climate and mountains that surpass 1 800 m of elevation. Regular rainfall and relatively well-defined seasons are characteristic of this region: winter is usually cold, with frequent frosts due to the influence of elevation. Temperatures vary from a minimum close to zero in the winter to a maximum of 35 °C during summer (IBGE – Instituto Brasileiro de Geografia e Estatística, 1992), with an appreciable daily temperature range (from our data: 35 °C at noon to 9 °C at night in January and 30 to 4 °C in August). The two Atlantic Forest ecosystems found in the state of Santa Catarina within the elevational gradient are the Dense Ombrophilous Forests (below 800 m elevation) and Mixed Ombrophilous Forests (higher regions). Dense Ombrophilous Forests are mainly comprised of evergreen plant species with low constancy and restricted distribution (Scudeller et al., 2001). The canopy can reach a height of 30 m. The understory is dense shrub vegetation mainly consisting of ferns, bromeliads, and palms. Mixed Ombrophilous Forests (Araucaria Forest) occur at high elevations and are among the most endangered and fragmented types of forest due to historical anthropic activities (Ribeiro et al., 2009). *Araucaria angustifolia* is the most characteristic species of this forest, which is accompanied by a rich diversity of trees and dense shrub vegetation.

Sampling method

Dung beetle assemblages were surveyed monthly from August 2015 to June 2016 (11 sampling periods) using baited traps. Six forest sampling sites were chosen at 250, 430, 840, 1 050, 1 350, and 1 625 m above sea level, approximately (Table 1). The two sites at lower elevation were located within a Dense Ombrophilous Forest, the remaining sites were found within Mixed Ombrophilous Forests (Table 1). All the surveyed sites can be considered primary forests slightly altered by human actions; sampling sites at lower elevations (250 and 430 m) are at the edge of the Tabuleiro State Park, whereas the remaining sites are in the São Joaquim National Park or in private reserves (Leão da Montanha Natural Heritage). At each sampling site, two types of traps were placed: baited pitfall traps that allow both immigration and emigration (TIE), and traps that attract dung beetles but prevent emigration (TE). The collected specimens in both types of traps were used to estimate the complete dung beetle fauna present in each survey locality, whereas only TIE traps are used to estimate removal rates. These two types of traps were placed in the forest soil in pairs (one TE and one TIE, 8–10 m apart) to diminish possible environmental differences among them. Both types of traps were placed in five locations separated

Table 1 Basic characteristics of the sampling sites. Latitude and longitude are in decimal degrees, elevation (m above sea level), mean air temperature (°C), and mean precipitation (mm) of all sampled days across the complete studied period (mean \pm SD)

Sampling site	Longitude (°W)	Latitude (°S)	Elevation	Temperature	Precipitation	Forest type
Santo Amaro da Imperatriz (1)	-48.809	-27.735	254 \pm 10.6	18.6 \pm 4.2	333 \pm 205	Dense Ombrophilous Forest
Santo Amaro da Imperatriz (2)	-48.812	-27.742	434 \pm 12.8	18.2 \pm 3.7	434 \pm 319	Dense Ombrophilous Forest
Rancho Queimado	-49.015	-27.690	839 \pm 15.2	16.5 \pm 3.7	231 \pm 143	Mixed Ombrophilous Forest
Urubici (1)	-49.375	-28.008	1060 \pm 12.4	14.0 \pm 4.1	362 \pm 284	Mixed Ombrophilous Forest
Urubici (2)	-49.631	-28.139	1357 \pm 7.2	13.5 \pm 4.3	206 \pm 146	Mixed Ombrophilous Forest
Urubici (3)	-49.630	-28.159	1632 \pm 14.1	12.7 \pm 3.9	155 \pm 101	Mixed Ombrophilous Forest

**Figure 1** Schematic representation of the sampling design carried out at each of the six sites. TE, trap that prevents emigration (replicates 1–5); TIE, trap that allows both immigration and emigration; CC, control container.

by 100 m to ensure the independence of collected specimens (Larsen & Forsyth, 2005; da Silva & Hernández, 2015) (Figure 1).

TIEs consist of plastic containers (15 cm diameter, 20 cm deep) perforated with approximately 50 small holes of about 1 mm diameter at the bottom to avoid excessive retention of rain water. They were buried in the soil to the rim and filled with local soil up to 5 cm from the rim. A dung portion of approximately 2 cm in diameter of human excrement (10.85 ± 0.9 g) was placed at the center of each TIE as bait. Human excrement was used because it is the most attractive for Neotropical native dung beetles (Whipple & Hoback, 2012). This amount of bait was chosen for logistical reasons, being enough to measure the burial activity of dung beetles under tropical conditions (Andresen, 2001). Considering the width and weight of the faeces of the mammal species present in the study region (Table S1), this amount of bait would reflect the attractiveness toward the droppings of most mammal species with a small and medium body size (Bogoni et al., 2016). Previously, this excrement was mixed with a little dose of blue food coloring to facilitate the visualization and quantification of the remaining faeces. The addition

of this coloring has proven to be safe, and does not affect the natural behavior of the Scarabaeinae (Batilani-Filho & Hernández, 2016). Lastly, a plastic cover was placed approximately 10 cm above the trap in order to protect it against the rain.

After sieving all the soil within each container, all the beetles buried or within the dung were separated. TE traps are similar to TIE, but in this case 300 ml of water was added to each container to catch the attracted insects. A few drops of detergent were added to the water in order to break the surface tension of the liquid thus avoiding their escape. Five TE traps were also placed at each site (8–10 m distant) baited with the same quantity of human dung (ca. 10 g). Dung bait was wrapped in a thin cloth and tied to the central part of the plastic cover that protected the trap from the rain. At each sampling site and sampling period, a control container (CC) similar to TIE traps was also placed; however, the access of dung beetles was avoided by means of thin nylon gauze fixed with a rubber band around the edge of the plastic container. The mass of the bait in the CC served to correct the loss or gain of bait mass in TIEs due to drying or hydrating. A total of 330 TIE, 330 TE (11 sampling periods \times 6 sampling sites \times 5

traps \times 2 trap types) and 66 CC were placed in the field (11 sampling periods \times 6 sampling sites). All the traps remained in the field for 48 h, and after this period the remaining dung bait was weighed using a field digital balance with a precision of ± 0.01 g. We were not able to sample a particular site $2\times$ due to road obstruction (flooding): five traps in September 2015 and five traps in March 2016 for the site at 1 000 m, and another five traps were damaged during various periods and locations. Thus, from the 330 TIE traps 315 were used in the statistical analyses (sampling units). The same number of TE traps was also used to estimate the complete potential dung beetle fauna present and flying in the area (TIE+TE traps). All beetles were identified to species level and preserved in 90% alcohol. A complete list of the collected species in each sampling site is in Table S2. Voucher specimens (dried and mounted on entomological pins) were deposited in institutional collections (Universidade Federal de Santa Catarina and Universidade Federal de Mato Grosso, Brazil). All the collections were made with the required permissions issued by Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/MMA, permit no. 49486-1), the Brazilian institution that regulates biodiversity.

Two waterproof data loggers (HOBO 64K Pendant; Onset Computer Corporation, Bourne, MA, USA) were placed at each sampled site, one at 1 m above the ground in order to take both temperature and luminosity measurements and the other buried in the soil at an approximate depth of 5 cm. Data loggers were installed in the middle of each transect and programmed to take measurements every 15 min throughout the complete sampling period. Additionally, a pluviometer was installed at each sampling site taking measurements of the quantity of rain during the 48 h of the sampling period.

Data analysis

The dung buried or removed by dung beetles in each TIE was calculated as the difference between the initial and the remaining mass of dung after 48 h, considering the loss or gain of mass observed in dung CC due to drying or hydrating. As moisture loss or gain can vary among sampling periods and sites, the so generated values are expressed in relative frequencies calculated as: final mass of dung/(starting mass of dung—loss or gain of dung mass in the CC). The calculated dung removal frequencies were submitted to a logit transformation to fulfil linear modeling assumptions (Warton & Hui, 2011).

We used General Linear Models (GLMs) to relate the removal activity of dung beetles during 48 h (response variable) against three types of explanatory variables that reflected: dung beetle assemblage characteristics (A), local habitat and climatic conditions of the environment (E),

and seasonal periods (S). Type A variables aim to estimate the influence of the number, diversity, and descriptive features of the assemblages of dung beetles on the disappearance of dung. On the other hand, E variables were included with the purpose of examining the influence of environmental conditions on the rate of dung loss. Finally, S variables were included to assess the influence of the time of year on dung removal as consequence of phenological and developmental constrains (different seasons of the year may have similar climatic conditions but representing different stages of development).

The features of the dung beetle assemblages are species richness (S), number of individuals (N), and volume (V), the last two expressed as natural logarithms. These three variables were calculated both considering TIE and TIE + TE (= TOT, total) traps (S_{TIE} , N_{TIE} , V_{TIE} , S_{TOT} , N_{TOT} , and V_{TOT} , respectively) in order to represent both the characteristics of the observed dung beetles directly interacting with the dung (TIE traps) and those of the complete potential fauna present and flying in the area (TIE + TE traps). The average volume (V) of each specimen was calculated measuring thorax length (l), width (w), and height (h) of 10 randomly selected individuals with a manual millimetre gauge. After approximating the volume of a beetle to an ellipsoid and converting the linear dimensions to an approximate radius, the volume was calculated as $4/3 \times (l \times w \times h)$. Due to the lack of fresh body mass, the measurement volume was used as a surrogate for biomass, taking into account the recognized high and linear relationship between the volume of insects and their body mass (Kühnel et al., 2017), including Scarabaeidae beetles (Rios & Hernández, 1993). All the available individuals of the uncommon species were measured (number of individuals <10). The average volume of each species was used to estimate total volume and biomass of all the dung beetle specimens collected at each sampling trap.

Local habitat and climatic conditions include: elevation, average air temperature during the 48 h of sampling (T_{AIR}), temperature range experienced in 48 h ($T_{RANGE\ AIR}$, the difference between the maximum and the minimum temperature), average soil temperature (T_{SOIL}) and its corresponding thermal range ($T_{RANGE\ SOIL}$), the amount of rain (R_{ain}) during the sampling period, and luminosity (L). Seasonal variables are represented as circular variables so that the date of the spring equinox in the southern hemisphere (22 September) is represented as zero (or 360) and the day before this equinox by 359. After converting the figures for each of the sampling periods into radians, the calculated values were transformed into their cosine and sine values. Thus, the spring-autumn transition is a variable represented by the cosine of the date

and oscillates from 1 to -1 , whereas the summer-winter oscillation between solstices is represented by the sine of the date and is also on the scale 1 to -1 .

Firstly, three GLMs were carried out with each type of predictors. Type III sum of squares are used to estimate the partial effects of each variable while controlling for the effects of the remaining variables (pure variability). Only statistical relationships significant at $P < 0.01$ are retained and considered. After estimating the partial explanatory capacity of these variables, we created saturated models including all the variables at the same time in order to estimate the full explanatory capacity of each type of predictors, as well as the complete variability accounted for by the three types of predictors. The comparative influence of A, E, and S variables in the variation of dung removal was estimated by a variation partitioning method or partial regression analysis (Legendre & Legendre, 1998). In this method the complete explained variability is divided into eight pure and combined components: three pure effects for each type of predictor, three combined variations between pairs of predictors, one variation due to the combined effect of the three types of predictors, and lastly, the variation not explained by the predictor variables included in the analysis. This analysis aims to discriminate the genuine relevance of the three types of predictors, as well as their combined explanatory capacity as a consequence of the correlation between them (variability that can be indifferently attributed to one or another set of predictors). All analyses were performed using StatSoft's STATISTICA v.12.0 (StatSoft, Tulsa, OK, USA).

Results

Dung removal in all considered sampling units was $80.6 \pm 3.8\%$ (mean \pm 95% confidence interval). The dung was completely removed in 230 of the 315 studied sampling units (73%), and in 79% of sampling units at least half of the dung was removed.

The characteristics of the dung beetle assemblages (A) explain 38% of variability in dung removal ($R^2 = 38.21\%$; $F_{6,308} = 31.75$, $P < 0.001$). V_{TIE} accounts for 5% of total variability (pure variability; $F_{1,308} = 25.10$, $P < 0.001$). S_{TOT} ($F_{1,308} = 6.92$, $P = 0.009$) and N_{TOT} ($F_{1,308} = 8.37$, $P = 0.004$) are also significant variables, whose pure explanatory capacity is only 1.4 and 1.7%, respectively. Thus, when the total volume of dung beetles increase, the disappearance of dung is more probable (Figure 2), a positive relationship similar to that of total richness or total abundance to dung removal rate (not shown). All other beetle assemblage predictors have an almost negligible influence (4.3% in total); most of the variability accounted

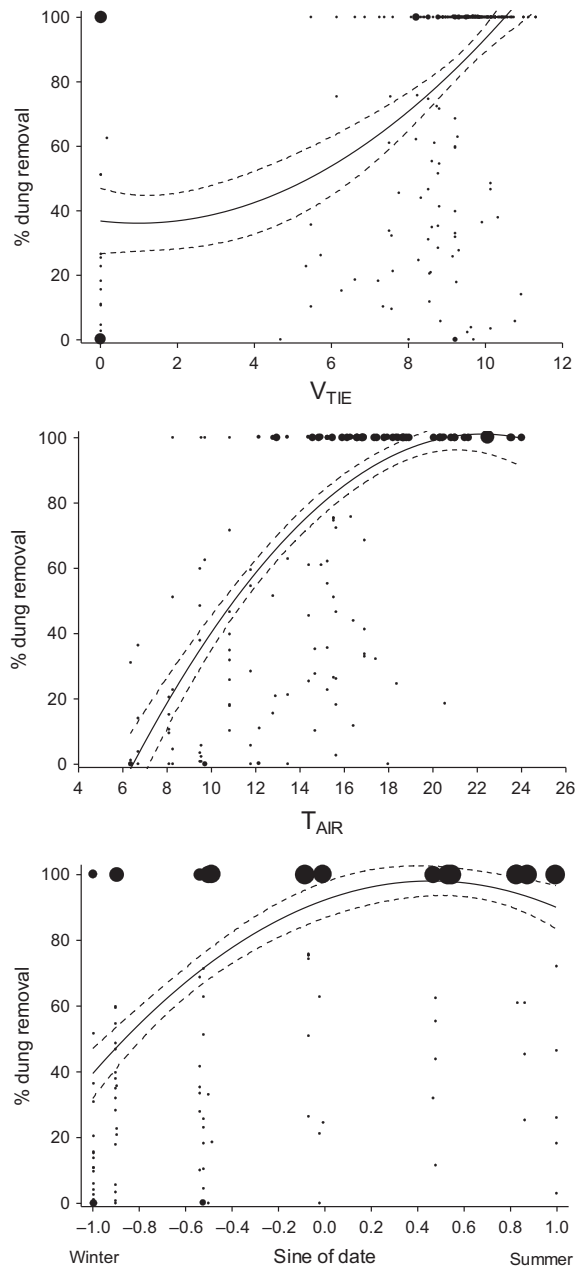


Figure 2 Relationships between dung removal (%) and total volume of dung beetles collected in traps in which dung removal was measured (V_{TIE}), average air temperature during the 48 h of sampling (T_{AIR}), and the sine of date reflecting the summer-winter oscillation between solstices. The solid lines indicate a quadratic polynomial adjusted to the data (\pm 95% confidence intervals, broken lines). The size of the dots reflects the frequency of sampling units with the same values.

for by these predictors is due to their joint or shared influence. When V_{TIE} is considered as the unique predictor, it explains 26.2% of the total variability (compared to 68%

of the variability accounted for by all assemblage-related predictors together).

Seasonal predictors (S) accounted for 26% of total variability of dung removal ($R^2 = 25.95\%$; $F_{2,312} = 54.66$, $P < 0.001$). Both the sine ($F_{1,312} = 104.18$, $P < 0.001$) and cosine of the date ($F_{1,312} = 9.09$, $P = 0.002$) are significant, although the sine, representing the solstice variation (summer-winter), has a much higher pure explanatory variability (24.7%) than the cosine (2.2%), representing the equinox variation. Thus, the higher activity of dung removal seems to occur at the end of spring (December) (Figure 2).

Habitat and climate predictors (E) explain 45% of variability in dung removal ($R^2 = 44.99\%$; $F_{7,307} = 35.87$, $P < 0.001$). Only one explanatory variable was significant (T_{AIR} ; $F_{1,307} = 15.32$, $P < 0.001$), whose pure explanatory capacity is 2.7%. In general, dung removal is higher at higher air temperatures; when $T_{AIR} > 21$ °C all dung is removed from every trap (Figure 2). Variability is accounted for most by the shared influence of habitat and climate variables. When T_{AIR} is considered alone, it explains 44.1% of the total variability, that is, almost all of the variability accounted for by all these predictors combined (45%).

A saturated model including all three types of predictors together explains 56.3% of the total variability. The total pure variability accounted for by the three types of predictors is 20.3%, whereas the sum of the combined effects is 36.0%. The pure variability accounted for by A and E variables is 10.1 and 9.7%, respectively, whereas the pure variability explained by S predictors is negligible (0.5%; Figure 3). Among the combined effects, the variability that can be attributed indifferently to the three types of predictors stands out (17%; Figure 3). The three main predictors previously selected from each type of variable (V_{TIE} , T_{AIR} , and sine of the date) represent almost all the complete variability in removal rates accounted for by the complete set of predictors (viz. 56.3%) – these three predictors account for 54.2% of total variability ($F_{3,311} = 122.80$, $P < 0.001$). In this case, the pure effects are 8.6, 13.0, and 1.5%, for V_{TIE} , T_{AIR} , and sine of date, respectively. The highest combined contributions are those of T_{AIR} and sine of date (13.5%), V_{TIE} and T_{AIR} (7.9%), and the variability that can be indifferently attributed to the three predictors (9.7%).

Discussion

Our study quantifies the contribution of several types of factors in explaining the variation in dung removal rates by dung beetles across the surveyed localities. One of the most interesting results is the moderate level of overall

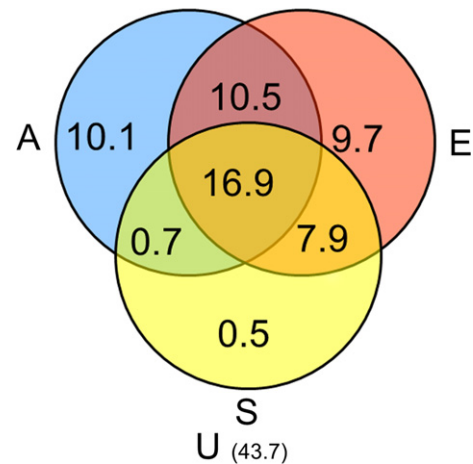


Figure 3 Variance partitioning (%) of dung removal considering the influence of assemblage characteristics (A), climatic conditions of the environment (E), and seasonal period (S). U, unexplained variance. [Colour figure can be viewed at wileyonlinelibrary.com]

explanatory capacity in accounting for dung removal despite the many and diverse predictors: ca. 44% of total variability in dung removal remains unexplained. Considering the mass of the bait used (ca. 10 g) and the high percentage of sampling units where the dung was completely removed (73%), our results indicate that the complete disappearance of dung within 48 h after deposition is the rule in these forests. Of course, this result can be due to the amount of excrement used as bait, reflecting, for example, the dung pellets of small and medium-sized mammal species. However, similar results were found in nearby forests of southern Brazil when 50 gram of domestic dog dung was used: 80% of the dung portions were completely removed after 48 h (Batilani-Filho & Hernández, 2017). These results are in agreement with other studies carried out under tropical and sub-tropical conditions in which dung beetles are able to transfer all deposited mammal faeces into the soil within hours after deposition (Slade et al., 2007). According to our results, small pieces of dung will disappear completely and quickly under a high variety of situations. The complete removal of dung can happen during all seasons, under very different types of dung beetle assemblages and environmental conditions: from sites located at 200–1 600 m, from mean air temperatures of 8–24 °C, and from a situation in which no dung beetles were observed inside the dung or in the soil beneath it (i.e., the dung is buried but the dung beetles emigrate) to a situation in which eight species and 115 specimens were collected in TIE traps.

Some important factors influencing the rate of dung removal could be recognized, although the highest

explanatory capacities corresponded to the combined effects of several types of variables, where the three types of variables exercise a joint and indivisible effect on dung removal (fastest under summer conditions with high temperatures and assemblages with a high biomass). In experimental as well as field studies, dung beetle diversity is identified as a key factor in explaining ecological functions, such as dung removal or soil perturbation (Rosenlew & Roslin, 2008; Beynon et al., 2012; Braga et al., 2013; Manning et al., 2017). In our study, the main predictor was the total volume, represented by dung beetles collected in the traps in which dung removal was measured (TIE traps). Considering that volume is tightly linked to biomass, our results corroborate the relevance of this variable in the rate of dung removal estimated by other studies conducted under neotropical conditions (Tixier et al., 2015; Ortega-Martínez et al., 2016; Batilani-Filho & Hernández, 2017).

When habitat and climatic variables are considered, average air temperature during the sampling period best predicts the role played by all other environmental variables. Thus, the rate of dung removal is positively related to an increase in air temperature, which is associated with the arrival of summer conditions and the location of lowland sites across the studied gradient. The approach of summer conditions linked with an increase in temperature and precipitation is the best seasonal predictor, although its pure explanatory capacity can be considered negligible due to the correlation between this seasonal predictor and those reflecting environmental conditions. This could mean that developmental constraints linked to seasonal changes (Kivelä et al., 2016) would have a minor influence in dung removal rates in tropical forests, and that the seasonal variation in these rates (Amézquita & Favila, 2010) is mainly related to the associated environmental and temperature changes (Hernández & Vaz-de-Mello, 2009; da Silva et al., 2013).

In summary, we showed that assemblage metrics, seasonal variation, and local climatic and habitat conditions jointly affect dung removal. The results indicate that the removal of small pieces of dung in Atlantic subtropical forests is quick and pervasive, and that the lack of nutrient incorporation into the soil from dung of small mammals more likely occurs when the air temperature at which this dung is deposited is low, during winter conditions, and when the biomass of the dung beetle assemblage is limited.

Acknowledgements

We would like to thank Diogo Roesner, Eloisa Alves, Victor Michelon, and Renata Campos from Laboratório de Ecologia Terrestre Animal (UFSC) who helped us during

fieldwork. Thanks to Fernando Brüggemann and Pedro Castilho for permission to collect on their properties, and to Plaza Caldas da Imperatriz Resort & Spa for logistical support and permission to access the Parque Estadual da Serra do Tabuleiro. We would like to thank the Instituto Chico Mendes de Conservação da Biodiversidade for permission to collect beetles and for logistical support at the São Joaquim National Park. Furthermore, we would like to thank Ciências Sem Fronteiras program of the Ministério da Educação via Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES and CNPq (process 88881.068089/2014-01) for their financial support. PGdS and VA thank CAPES for a post-doctoral grant (process 88887.094506/2015-00 and 88887.100542/2015-00) and MIMH thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the Productivity grant (process 309030/2013-7).

References

- Amézquita S & Favila ME (2010) Removal rates of native and exotic dung by dung beetles (Scarabaeidae: Scarabaeinae) in a fragmented tropical rain forest. *Environmental Entomology* 39: 328–336.
- Andresen E (2001) Effects of dung presence, dung amount and secondary dispersal by dung beetles on the fate of *Micropholis guyanensis* (Sapotaceae) seeds in Central Amazonia. *Journal of Tropical Ecology* 17: 61–78.
- Andresen E (2003) Effect of forest fragmentation on dung beetle communities and functional consequences for plant regeneration. *Ecography* 26: 87–97.
- Angilletta MJ (2009) *Thermal Adaptation: A Theoretical and Empirical Analysis*. Oxford University Press, New York, NY, USA.
- Balvanera P, Siddique I, Dee L, Paquette A, Isbell F et al. (2014) Linking biodiversity and ecosystem services: current uncertainties and the necessary next steps. *BioScience* 64: 49–57.
- Batilani-Filho M & Hernández MIM (2016) Staining method for assessing the ecological function of excrement removal by dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae). *The Coleopterists Bulletin* 70: 880–884.
- Batilani-Filho M & Hernández MIM (2017) Decline of ecological functions performed by dung beetles in areas of Atlantic Forest and contribution of rollers and tunnelers in organic matter removal. *Environmental Entomology* 46: 784–793.
- Beynon SA, Mann DJ, Slade EM & Lewis OT (2012) Species-rich dung beetle communities buffer ecosystem services in perturbed agro-ecosystems. *Journal of Applied Ecology* 49: 1365–1372.
- Bogoni JA, Graipel ME, de Castilho PV, Fantacini FM, Kuhnen VV et al. (2016) Contributions of the mammal community, habitat structure, and spatial distance to dung beetle community structure. *Biodiversity and Conservation* 25: 1661–1675.
- Braga RF, Korasaki V, Andresen E & Louzada J (2013) Dung beetle community and functions along a habitat-disturbance

- gradient in the Amazon: a rapid assessment of ecological functions associated to biodiversity. *PLoS ONE* 8: e57786.
- Bull JJ (1987) Evolution of phenotypic variance. *Evolution* 41: 303–315.
- Finn JA & Giller PS (2002) Experimental investigations of colonisation by north temperate dung beetles of different types of domestic herbivore dung. *Applied Soil Ecology* 20: 1–13.
- Halffter G & Edmonds WD (1982) The Nesting Behavior of Dung Beetles (Scarabaeinae). An Ecological and Evolutionary Approach. Man and Biosphere Program UNESCO, Mexico City, Mexico.
- Halffter G & Matthews EG (1966) The natural history of dung beetles of the subfamily Scarabaeinae (Coleoptera, Scarabaeidae). *Folia Entomologica Mexicana* 12(14): 1–312.
- Hernández MIM & Vaz-de-Mello FZ (2009) Seasonal and spatial species richness variation of dung beetle (Coleoptera, Scarabaeidae s. str.) in the Atlantic Forest of southeastern Brazil. *Revista Brasileira de Entomologia* 53: 607–613.
- Herrick JE & Lal R (1996) Dung decomposition and pedoturbation in a seasonally dry tropical pasture. *Biology and Fertility of Soils* 23: 177–181.
- Holter P (1979) Effect of dung-beetles (*Aphodius* spp.) and earthworms on the disappearance of cattle dung. *Oikos* 32: 393–402.
- Horgan FG (2001) Burial of bovine dung by coprophagous beetles (Coleoptera: Scarabaeidae) from horse and cow grazing sites in El Salvador. *European Journal of Soil Biology* 37: 103–111.
- IBGE – Instituto Brasileiro de Geografia e Estatística (1992) Manual Técnico da Vegetação Brasileira. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro, Brazil.
- Kaspari M, Clay NA, Lucas J, Yanoviak SP & Kay A (2015) Thermal adaptation generates a diversity of thermal limits in a rainforest ant community. *Global Change Biology* 21: 1092–1102.
- Kivelä SM, Välimäki P & Gotthard K (2016) Evolution of alternative insect life histories in stochastic seasonal environments. *Ecology and Evolution* 6: 5596–5613.
- Kühnel S, Brückner A, Schmelzle S, Heethoff M & Blüthgen N (2017) Surface area-volume ratios in insects. *Insect Science* 24: 829–841.
- Larsen TH & Forsyth A (2005) Trap spacing and transect design for dung beetle biodiversity studies. *Biotropica* 37: 322–325.
- Lee C & Wall R (2006) Cow-dung colonization and decomposition following insect exclusion. *Bulletin of Entomological Research* 96: 315–322.
- Legendre P & Legendre L (1998) *Numerical Ecology*. Elsevier, Amsterdam, The Netherlands.
- Manning P, Slade EM, Beynon SA & Lewis OT (2017) Effect of dung beetle species richness and chemical perturbation on multiple ecosystem functions. *Ecological Entomology* 42: 577–586.
- Naem S, Duffy JE & Zavaleta E (2012) The functions of biological diversity in an Age of Extinction. *Science* 336: 1401–1406.
- Nervo B, Tocco C, Caprio E, Palestini C & Rolando A (2014) The effects of body mass on dung removal efficiency in dung beetles. *PLoS ONE* 9: e107699.
- Nervo B, Caprio E, Celi L, Lonati M, Lombardi G et al. (2017) Ecological functions provided by dung beetles are interlinked across space and time: evidence from ¹⁵N isotope tracing. *Ecology* 98: 433–446.
- Nichols E, Larsen T, Spector S, Davis AL, Escobar F et al. (2007) Global dung beetle response to tropical forest modification and fragmentation: a quantitative literature review and meta-analysis. *Biological Conservation* 137: 1–19.
- Nichols E, Spector S, Louzada J, Larsen T, Amézquita S & Favila ME (2008) Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological Conservation* 141: 1461–1474.
- Numa C, Lobo JM & Verdú JR (2012) Scaling local abundance determinants in Mediterranean dung beetles. *Insect Conservation and Diversity* 5: 106–117.
- Ortega-Martínez IJ, Moreno CE & Escobar F (2016) A dirty job: manure removal by dung beetles in both a cattle ranch and laboratory setting. *Entomologia Experimentalis et Applicata* 161: 70–78.
- Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ & Hirota MM (2009) The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation* 142: 1141–1153.
- Rios RI & Hernández MIM (1993) Avaliação do tamanho do corpo de insetos em três famílias de Coleoptera: Scarabaeidae, Elateridae e Hydrophilidae. *Revista Brasileira de Biologia* 53: 37–41.
- Rosenlew H & Roslin T (2008) Habitat fragmentation and the functional efficiency of temperate dung beetles. *Oikos* 117: 1659–1666.
- Sakai S & Tamiji I (1999) A new pollination system: dung-beetle pollination discovered in *Orchidantha inouei* (Labiaceae, Zingiberales) in Sarawak, Malaysia. *American Journal of Botany* 86: 56–61.
- Scudeller V, Martins F & Shepherd G (2001) Distribution and abundance of arboreal species in the Atlantic ombrophilous dense forest in Southeastern Brazil. *Plant Ecology* 152: 185–199.
- da Silva PG & Hernández MIM (2015) Spatial patterns of movement of dung beetle species in a tropical forest suggest a new trap spacing for dung beetle biodiversity studies. *PLoS ONE* 10: e0126112.
- da Silva PG, Vaz-de-Mello FZ & Di Mare RA (2013) Diversity and seasonality of Scarabaeinae (Coleoptera: Scarabaeidae) in forest fragments in Santa Maria, Rio Grande do Sul, Brazil. *Anais da Academia Brasileira de Ciências* 85: 679–697.
- da Silva PG, Lobo JM, Hensen MC, Vaz-de-Mello FZ & Hernández MIM (2018) Turnover and nestedness in subtropical dung beetle assemblages along an elevational gradient. *Diversity and Distributions* 24: 1277–1290.
- Simmons LW & Ridsdill-Smith TJ (2011) *Ecology and Evolution of Dung Beetles*. John Wiley & Sons, Oxford, UK.
- Slade EM, Mann DJ, Villanueva JF & Lewis OT (2007) Experimental evidence for the effects of dung beetle functional group

- richness and composition on ecosystem function in a tropical forest. *Journal of Animal Ecology* 76: 1094–1104.
- Tixier T, Bloor JM & Lumaret JP (2015) Species-specific effects of dung beetle abundance on dung removal and leaf litter decomposition. *Acta Oecologica* 69: 31–34.
- Warton DI & Hui FK (2011) The arcsine is asinine: the analysis of proportions in ecology. *Ecology* 92: 3–10.
- Whipple SD & Hoback WW (2012) A comparison of dung beetle (Coleoptera: Scarabaeidae) attraction to native and exotic mammal dung. *Environmental Entomology* 41: 238–244.
- Yoshihara Y & Sato S (2015) The relationship between dung beetle species richness and ecosystem functioning. *Applied Soil Ecology* 88: 21–25.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Mammal species that appear in the survey sites according to Bogoni et al. (2016), their diet and body mass (B, kg) according to Paglia et al. (2012), and the width (W, cm), length (L, cm), and weight (We, g) of their faecal pellets according to various literature sources

Table S2. Dung beetles collected in each sampling site

1 **Supporting Information**

2

3 **Table S1** Mammal species that appear in the survey sites according to Bogoni et al. (2016), their diet and body mass (B, kg) according to Paglia
4 et al. (2012), and the width (W, cm), length (L, cm), and weight (We, g) of their faecal pellets according to various literature sources

Species	Diet	B (kg)	W (cm)	L (cm)	We (g)	References
<i>Cerdocyon thous</i> (L.)	Insectivore/omnivore	6.5	1.7-2.3	1.5-10.8		Chame (2003)
<i>Lycalopex gymnocercus</i> (G. Fischer)	Carnivore/omnivore	4.4			7.3	Birochio (2008)
<i>Hydrochoerus hydrochaeris</i> (L.)	Herbivore	50.0	1.2			Eberhardt (2014)
<i>Mazama gouazoubira</i> (G. Fischer)	Frugivore/herbivore	21.0	0.5-2.8	0.9-5.1		Chame (2003)
<i>Mazama nana</i> (Hensel)	Frugivore/herbivore	17.5	0.5	1.0		
<i>Cuniculus paca</i> (L.)	Frugivore/herbivore	9.3	1.5	3.0		Chame (2003)
<i>Cabassous tatouay</i> (Desmarest)	Myrmecophage	5.4	≈1.5	≈2.5	≈2.8	da Silveira Anacleto (2007)
<i>Dasybus novemcinctus</i> L.	Insectivore/omnivore	3.6	1.1-2.8	1.1-3.7		Chame (2003)
<i>Dasyprocta azarae</i> Lichtenstein	Frugivore/granivore	3.0	1.5	4.0		Chame (2003)
<i>Didelphis aurita</i> Wied-Neuwied	Frugivore/omnivore	1.2	≈1.9	≈5.0	≈0.2-0.3	Fitzgerald (1977)
<i>Didelphis albiventris</i> Lund	Frugivore/omnivore	1.6	≈1.9	≈5.0	≈0.2-0.3	Fitzgerald (1977)
<i>Coendou spinosus</i> (Cuvier)	Frugivore/folivore	1.8	≈1.0	≈2.0		Aranda Sanchez (2012)
<i>Leopardus guttulus</i> (Hensel)	Carnivore	2.2				
<i>Leopardus wiedii</i> (Schinz)	Carnivore	6.0	1.5-2.5	10.0-15.0		Aranda Sanchez (2012)
<i>Leopardus pardalis</i> (L.)	Carnivore	9.5	1.6	12.7		Chame (2003)
<i>Herpailurus yagouaroundi</i> (Geoffroy)	Carnivore	4.5	1.0-2.0	5.0-10.0		Aranda Sanchez (2012)
<i>Puma concolor</i> (L.)	Carnivore	45.0	2.2-3.2	3.7-6.1		Chame (2003)
<i>Lepus europaeus</i> (Pallas)	Herbivore	3.8	1.1	1.7		Chame (2003)
<i>Eira barbara</i> (L.)	Frugivore/omnivore	7.0	1.0-2.0	5.0-10.0		Aranda Sanchez (2012)
<i>Galictis cuja</i> (Molina)	Carnivore	2.0	1.0-2.0	5.0-10.0		Aranda Sanchez (2012)
<i>Lontra longicaudis</i> (Olfers)	Piscivore	6.0	1.5-2.5			Aranda Sanchez (2012)
<i>Tamandua tetradactyla</i> (L.)	Myrmecophage	5.2	≈1.5-2.5	≈1.8-8.1	10.9	Chame (2003), Cazón & Juárez (2014)
<i>Nasua nasua</i> (L.)	Frugivore/omnivore	5.7	1.0-2.0	5.0-10.0		Aranda Sanchez (2012)
<i>Procyon cancrivorus</i> (Cuvier)	Frugivore/omnivore	4.9	≈1.0-2.0	≈5.0-10.0		Aranda Sanchez (2012)
<i>Guerlinguetus ingrami</i> (Thomas)	Frugivore/granivore	0.2	≈0.5	≈1.0		Aranda Sanchez (2012)
<i>Tapirus terrestris</i> (L.)	Herbivore/frugivore	260.0	5.8	10.9	38.1	Chame (2003), Cazón & Juárez (2014)
<i>Pecari tajacu</i> (L.)	Frugivore/herbivore	25.0	1.2-2.2	0.9-2.4	9.3	Chame (2003), Cazón & Juárez (2014)

5 Bogoni JA, Graipel ME, de Castilho PV, Fantacini FM, Kuhnen VV et al. (2016) Contributions of the mammal community, habitat structure,
6 and spatial distance to dung beetle community structure. *Biodiversity and Conservation* 25: 1661-1675.

- 1 Paglia AP, da Fonseca GAB, Rylands AB, Herrmann G, Aguiar LMS et al. (2012) Lista Anotada dos Mamíferos do Brasil [Annotated Checklist
2 of Brazilian Mammals], 2nd edn. Occasional Papers in Conservation Biology, Occasional Paper No. 6. Conservation International, Arlington,
3 VA, USA.
- 4 Cazón AV & Juárez VD (2014) Identificación de mamíferos silvestres y confirmación de su presencia por cromatografía en cada fina (TCL) de
5 sus ácidos biliares fecales. Memorias X Congreso Internacional de Fauna Silvestre de America Latina, Salta, Argentina, 2012, pp. 1-7.
- 6 Chame M (2003) Terrestrial mammal feces: a morphometric summary and description. Memorias del Instituto Oswaldo Cruz 98: 71-94.
- 7 da Silveira Anacleto TC (2007) Food habits of four armadillo species in the Cerrado area, Mato Grosso, Brazil. Zoological Studies 46: 529-537.
- 8 Fitzgerald AE (1977) Number and weight of faecal pellets produced by opossums. Proceedings of the New Zealand Ecological Society 24: 76-
9 78.
- 10 Eberhardt MAT (2014) Evaluación de la Dinámica de Salud en Poblaciones de *Hydrochoerus hydrochaeris* L. 1766: Intensidad del Parasitismo
11 Gastrointestinal. PhD Dissertation, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.
- 12 Birochio D (2008) Ecología Trófica de *Lycalopex gymnocercus* en la Región Pampeana: Un Acercamiento Inferencial al Uso de los Recursos.
13 PhD Dissertation, Universidad Nacional del Sur, Bahía Blanca, Argentina.
- 14 Aranda Sanchez JM (2012) Manual Para el Rastreo de Mamíferos Silvestres de México. Conabio, Mexico City, Mexico.

1 **Table S2** Dung beetles collected in each sampling site

Species	Sites					
	200	500	800	1000	1300	1600
<i>Canthidium</i> aff. <i>dispar</i>	1	10	1			
<i>Canthidium</i> aff. <i>lucidum</i>			1	196	15	62
<i>Canthidium</i> aff. <i>trinodosum</i>	36	278	200	142	1	
<i>Canthidium</i> <i>bovinum</i>			1	2		
<i>Canthon</i> aff. <i>luctuosus</i>		2	3	4	5	
<i>Canthon</i> aff. <i>oliverioi</i>		1				
<i>Canthon</i> <i>angularis obenbergeri</i>			61	336	416	32
<i>Canthon</i> <i>lividus seminitens</i>			1		1	
<i>Canthon</i> <i>luctuosus</i>				39		
<i>Canthon</i> <i>rutilans cyanescens</i>	582	37	17			
<i>Canthon</i> <i>rutilans rutilans</i>					7	
<i>Canthonella</i> aff. <i>instriata</i>			10			
<i>Coprophanaeus</i> <i>cerberus</i>		11				
<i>Coprophanaeus</i> <i>saphirinus</i>	82	44	72	25	2	
<i>Deltochilum</i> <i>brasiliense</i>	13	48	17	58	17	10
<i>Deltochilum</i> <i>dentipes</i>			1			
<i>Deltochilum</i> <i>furcatum</i>	2	71				
<i>Deltochilum</i> <i>morbillosum</i>	12	115	85			
<i>Deltochilum</i> <i>multicolor</i>	37	5	13			
<i>Dichotomius</i> aff. <i>acuticornis</i>			64	13	12	4
<i>Dichotomius</i> <i>ascanius</i>	62	4	3			
<i>Dichotomius</i> <i>assifer</i>		16	49			
<i>Dichotomius</i> <i>fissus</i>			48			
<i>Dichotomius</i> <i>mormon</i>	2	4	6			
<i>Dichotomius</i> <i>opalescens</i>					8	
<i>Dichotomius</i> <i>quadrinodosus</i>	5	34				
<i>Dichotomius</i> <i>sericeus</i>	143	219	105			
<i>Eurysternus</i> <i>cyanescens</i>	12	21	29			
<i>Eurysternus</i> <i>inflexus</i>	5	32	15			
<i>Eurysternus</i> <i>parallelus</i>			2			
<i>Homocopris</i> sp.			10	22	111	85
<i>Ontherus</i> <i>azteca</i>			1			
<i>Onthophagus</i> <i>catharinensis</i>	3	5	11			
<i>Onthophagus</i> <i>tristis</i>			1	14	101	59
<i>Paracanthon</i> sp.				6		
<i>Phanaeus</i> <i>splendidulus</i>	12	96	47			
<i>Uroxys</i> <i>dilaticollis</i>			1	2		1
<i>Uroxys</i> sp. 1	9	4		1842		
<i>Uroxys</i> sp. 2		1	68			
<i>Uroxys</i> sp. 3				1	2	
<i>Uroxys</i> sp. 4					1	

2

3