

Ecological indexes of arthropods on *Sapindus saponaria* plants with dehydrated sewage sludge as fertilizer

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Abstract

Plant species, monitoring measures, and alternative fertilization with sewage sludge accelerate recovering of degraded areas. The objective of this study was to evaluate the numbers of leaves/branch and branches/plant of *Sapindus saponaria* L., the ground cover by this plant and the ecological indexes of abundance, diversity, and species richness of insects and spiders, and of their interactions, during 24 months on trees grown in a degraded area with or without dehydrated sewage sludge as fertilizer. The metrics of plant, soil cover, and arthropods were higher on the *S. saponaria* plants fertilized with dehydrated sewage sludge. The abundance of chewing insects, tending ants, and spiders was greater on larger *S. saponaria* tree crown. The abundance of these last groups of organisms associated, negatively, with that of Coleoptera, but those of ants reduced that of the predators. The ecological indexes of arthropods on *S. saponaria* plants shows the suitability of this plant for recovering programs of degraded areas and the use of dehydrated sewage sludge as fertilizer increased these indices and the possibility of using insects and spiders on the crown of this plant as bioindicators.

Introduction

Deforestation and fire negatively affect biotic and abiotic factors and, consequently, the ecosystem heterogeneity [1] what increases the abandonment of cultivatable land [2]. Conservation techniques and approaches, including planting trees to fix atmospheric nitrogen through symbiotic bacteria [1] for timber production, erosion prevention, and carbon sequestration help to recover these areas [3]. Nutrient application (e.g., animal slaughterhouse waste, swine and poultry farm residues, and sewage sludge) can increase plant growth and development [4, 5, 6].

The restoration of geochemical and hydrological factors, evaluated by monitoring programs, defines the success of recovering programs in biological systems [7]. Traditional restoration methods rely on soil recovery and plant growth [7], but monitoring the arthropod community provides information on the quality of the area [4]. Many arthropods (Arthropoda) are environmental bioindicators in recovery processes and their ecological relationships with the vegetation should be studied [4, 5, 6]. Abundance, richness, and diversity of soil fauna increased and the abundance of the predator/saprophagous group, mainly Formicidae (Hymenoptera) was higher in areas in more advanced natural regeneration in the Seasonal Semi-deciduous Forest in Pinheiral, Rio de Janeiro State, Brazil [8]. These organisms and detritivores, herbivores, hyperparasitoids, parasitoids, and predators were important for nutrient cycling in the recovering of degraded grassland fields in Dublin, Ireland [9]. Changes in the diversity of insects, important as food for different groups of animals such as birds [10], can be used to make decisions in choosing the most suitable plant species per biome and local fauna [5].

Wing leaf soapberry, *Sapindus saponaria* L. (Sapindales: Sapindaceae), native to the Americas [11], is a late secondary forest species growing up to 8 m high [12] and reported from north (e.g., Pará state) to south (e.g., Rio Grande do Sul state) Brazil [13]. Carbon storage, survival (95%) and annual increment of *S. saponaria* plants were high in the Atlantic Forest [14]. This plant adds economic value to recovered

lands, especially those from deactivated mines in Brazil [15], Colombia [16], and the United States of America (USA) [17]. Besides, Sapindaceae species are used to treat dermatitis, stomachache, and as anthelmintics, expectorants, diuretics, sedatives, and stimulants [18]. Fruits of this tree are used to wash clothes and, in the cutlery, due to its chemical compound saponin and amphipathic glycoside. *Sapindus saponaria* seeds are dark round and used to make buttons and necklaces, and its wood splits easily to make baskets [12]. Symbiosis of *S. saponaria* with atmospheric nitrogen-fixing bacteria is low and the application of nutrient-rich organic materials increased the growth of this plant [19].

The residue sewage sludge from municipal or industrial wastewater processing is a semi-solid material rich in organic matter and chemical elements with potential as fertilizer for plant seedlings and accelerating land recovery process [20]. Plants absorb nutrients from the sludge, including toxic ones (e.g., heavy metals), reducing the environmental contamination in forest plantations and helping to recover degraded lands [21]. Sewage sludge use can reduce synthetic fertilizer application, production costs, and environmental problems in agriculture and forestry [21]. The application of dehydrated sewage sludge as fertilizer did not increase the soil pathogens and heavy metal content in maize, *Zea mays* L. (Poales: Poaceae), and cowpea, *Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae) grains.

The importance of organisms as bioindicators needs to be studied per group [22], including their establishment, spatio-temporal relationships, and ecological parameters [23] to understand the role of each group in land recovering programs [24]. The reestablishment of the ecological functions of organisms is the main parameter to evaluate programs of environment recovering focusing mainly on the plant structure, but it does not guarantee its effectiveness [25]. Arthropod community metrics vary with grass cover, tree density, and pesticides, as found for ants (Hymenoptera) and dung beetles (Coleoptera) with higher numbers of individuals in areas with low management levels in the savannah at Uberlândia, Minas Gerais state, Brazil [22]. The arthropod numbers and diversity varied with season, fire occurrence, and host plant species [26], plant nutrition, and cover by litter and weeds [27, 28], besides land management history [22]. The application of sewage sludge increased plant growth and development [29], nutritional indexes, chemical defences, fertilization [27], macro (e.g., Nitrogen and Phosphorus) and micronutrients (e.g., Copper and Zinc). These factors and the nutritional quality of flora affected diversity of herbivorous insects and of their natural enemies, including spiders [30] with, generally, larger values on bigger trees [31, 32]. The odds of extinction of rarer arthropod species is lower on these plants because they function as island biogeography with large resource availability and/or habitat from their great leaf area as explained in the Theory of Island Biogeography (TIB) [33, 32].

Sapindus saponaria is widely used to recover degraded areas and saponins are obtained from this plant. The objective of this work is to evaluate the numbers of leaves/branch and branches/plant of *S. saponaria*, the ground cover by this plant and the ecological indexes of abundance, diversity, and species richness of insects and spiders, and their interactions on the *S. saponaria* plants with the application or not of sewage sludge. The hypotheses tested are: i) crown of fertilized plants is larger, forming more litter, helping to recover degraded land [34, 28]; ii) fertilized plants are larger (greater TIB) with a greater ground cover and higher abundance of chewing insects [31, 32], and predator numbers varying with those of their

prey [35]; (iii) abundance of chewing insects, phytophagous Coleoptera and Diptera, tending ants, and spiders increases with the *S. saponaria* leaf mass; (iv) the abundance of tending ants and/or spiders is negatively correlated with chewing insects; and (v) chewing insects and spiders.

Materials And Methods

Experimental site

This experiment was carried out in the municipality of Montes Claros, Minas Gerais State, Brazil from March 2012 to February 2014 (insect collecting period). The collection site was a degraded area of the *Instituto de Ciências Agrárias (ICA)* of the *Universidade Federal de Minas Gerais (UFMG)* (latitude of 16° 51' S, longitude of 44° 55' W and altitude of 943 m). The area is degraded due to intense grazing activity without proper management resulting in soil losses and changes in its chemistry and hydrology [36]. The climate is Aw, according to the Köppen classification: tropical savannah, with dry winter and rainy summer [37]. The soil type is litolic neosoil [38], and its chemical and physical characteristics have been described [28].

Experimental design

Tree seeds from the institution were used to produce *S. saponaria* seedlings, which were planted in plastic polybags (8 cm diameter × 12 cm high) in a nursery, with a substrate composed of 10% reactive natural phosphate, 30% sand, 30% clay soil and 30% organic fertilizer (160 g/seedling) in March 2011. The organic fertilizer is constituted by one part of tanned Nelore manure, *Bos taurus indicus* L., 1758 (Artiodactyla: Bovidae), and two parts of garden pruning debris (plant fragments ≤ 5 cm). Seedlings were watered twice a day.

Six-month-old seedlings (around 30-cm high) of *S. saponaria* were planted in manually dug holes (40 × 40 × 40 cm) in a degraded area. The soil pH in the holes was corrected with dolomitic limestone (90% total relative neutralization power) (187 g/pit), increasing base saturation to 50% [39]. Natural phosphate (80 g/pit), fritted trace elements (10 g/pit), and marble rock dust (1 Kg/pit) were added according to soil analysis. One *S. saponaria* seedling was planted per hole with eight per row spaced two meters between plants, in six parallel rows. These seedlings were spaced two meters between rows, with four plants with dehydrated sewage sludge and four without it per line in September 2011. The plants were irrigated with water from a nearby river twice a week. The irrigation period started at the beginning of the rainy season (early November). The cultural treatments were carried out with properly sanitized blades, cutting the branches exceeding the limit of 5 cm long and eliminating those with up to one-third of the crown height. The residues were incorporated into the soil of their respective planting lines. The experimental design was completely randomized in blocks (planting rows), with two treatments (20 L of dehydrated sewage sludge/hole in a single dose during planting as fertilizer or without it with six replications) and four plants each with a total of 48 plants. *Sapindus saponaria* is a species that can be easily identified and does not require an exsiccate to be deposited in an herbarium. The necessary licenses for its cultivation were obtained through the Ministério da Agricultura, Pecuária e Abastecimento (MAPA).

Dehydrated sewage sludge

Dehydrated sewage sludge (5% moisture) was acquired at the sewage treatment plant (STP) in the municipality of Juramento, north of Minas Gerais state, Brazil at 40 Km from the *S. saponaria* experimental site. The Minas Gerais Sanitation Company S.A. manages this STP producing 217 m³ of sewage sludge/day. The system extracts more than 93% of the organic matter from the sewage.

The sewage sludge was exposed to sunlight in impermeabilized tanks with coarse sand for three months at the STP. This reduces the thermotolerant coliform bacteria according to the criteria established by the National Environmental Council (Resolution N° 375) of the Brazilian Ministry of the Environment for agricultural use, which is $< 10^3$ of the most predictable and acceptable number/g of total solids. The basic chemical and biological attributes of this dehydrated sewage sludge have been characterized [29].

Plant mass production and soil coverage

The number of leaves/branch and branches/plant and the percentage of soil covered by litter and grasses and herbaceous vegetation was quantified monthly by visual observations per plot (1 m²) in the projected area of the canopy of the 48 *S. saponaria* plants.

Ecological arthropod indexes

Arthropods were counted visually, every fifteen days, on the adaxial and abaxial *S. saponaria* leaf surfaces from 7:00 to 11:00 A.M., randomly at the basal, middle, and apical (0 to 33%, 33 to 66%, and 66 to 100% of canopy sapling height) in the eastern, northern, southern and western sides of each tree (1 leaf per side; 4 leaves/canopy height; 12 leaves/plant), respectively, per evaluation over 24 months. Adults, larvae and nymphs of arthropod were counted in 27,648 leaves covering the entire plant (vertical and horizontal axes), including the most uncommon ones on the 48 *S. saponaria* plants.

The evaluator approached, carefully, assessing the adaxial leaf surface and in the case of not visualizing the abaxial one, lifted the leaf with a delicate and slow movement to visualize it. Insects with greater mobility (e.g., Orthoptera), that flew, on approach, were counted if they were recognized (e.g., Order). The arthropods (insects and spiders) were not removed from the saplings during the evaluation.

A few arthropod specimens (up to three individuals) per species were collected using an aspirator (two hours per week), at the beginning of the study (between transplantation and first evaluation, six months after), stored in flasks with 70% alcohol, separated into morphospecies, and sent to specialists for identification (see acknowledgments). Any visible arthropod species not previously observed, was collected, coded, and sent to a taxonomist of its group. The arthropods identified were deposited in the laboratory collection of the UFMG. Permission to collect at these sites/activities was granted by the landowner (UFMG). The arthropods collected are not threatened with extinction and do not represent protected species.

The Arthropod individuals observed on 12 leaves (three heights and four sides of each sapling) composed each replication. Ecological indexes (abundance, diversity, and species richness) were calculated per group of species identified per treatment (with or without dehydrated sewage sludge as fertilizer) and tree using the BioDiversity Pro 2.0 software [40]. Abundance and species richness were based on the total number of individuals and species per tree, respectively [41]. Diversity was calculated using Hill's formula $N1 = \exp(H')$, where H' is the Shannon–Weaver diversity index using the actual number of individuals per species [42].

Statistics

Data on leaves/branch, branches/plant, percentages of ground cover by litter, grasses, and herbaceous vegetation, and the ecological indexes of abundance, diversity, and richness of chewers (Coleoptera + Lepidoptera + Orthoptera), coleopterans, dipterans, orthopterans, pollinators (Hymenoptera), spiders, and of total predators (Arachnida + Insecta), besides the association between groups (chewing insects, phytophagous Coleoptera and Diptera, tending ants and natural enemy abundance) with *S. saponaria* leaf mass were submitted to analysis of variance (one way ANOVA) ($P < 0.05$). The data were transformed to $\sqrt{x} + 0.5$ (when necessary) after testing with Liliefors (normal distribution) and Cochran and Bartlett (homogeneity of variances). The means were compared using the Tukey range test [43] ($P < 0.05$) with the Statistical Analysis System and Genetics, version 9.1 [44] (Supplier: Federal University of Viçosa).

The association between groups of the chewing insects, phytophagous Coleoptera and Diptera, tending ants and natural enemy abundance with *S. saponaria* leaf mass were tested with simple regression analyses, and their parameters performed using the statistical software SAEG [44]. The selection of simple equations (linear or quadratic regression) was based on the criteria: i) distribution of the data in the figures (linear or quadratic response), ii) the most significant parameters used in these regressions ($P < 0.05$), iii) $P < 0.05 < 0.05$ and F of the Analysis of Variance of these regressions, and iv) the determination coefficient of these equations (R^2). The independent and dependent variables for the regressions (e.g., $y = b_0 + b_1 * x$) were defined, as example, chewing insects/tree = $b_0 + b_1 * \text{leaves/tree}$.

Results

Plant mass production and soil coverage

The numbers of leaves/branch ($F = 11.66$), branches/plant ($F = 105.63$) and the percentage of ground cover (litter, grasses, and herbaceous vegetation) ($F = 84.02$) were higher ($P < 0.05$; $Df = 1$) for *S. saponaria* plants with dehydrated sewage sludge (Table 1).

Table 1

Numbers of leaves/branch and branches/plant and percentages of soil cover by *Sapindus saponaria* (Sapindales: Sapindaceae)/plant (mean $\pm$ SE of the mean) with or without dehydrated sewage sludge as fertilizer

	Dehydrated sewage sludge		ANOVA	
	With	Without	<i>F</i>	<i>P</i>
Branches/plant	19.51 $\pm$ 2.30a	7.22 $\pm$ 0.73b	11.66	0.00
Leaves/branch	16.55 $\pm$ 0.83a	6.88 $\pm$ 0.39b	105.63	0.00
Soil cover (%)	24.69 $\pm$ 1.05a	5.96 $\pm$ 0.41b	84.02	0.00
Averages followed by the same lower case letter per row do not differ by the Tukey test ($P < 0.05$). Degrees of freedom: treatment = 1, block = 5, and residual = 41. SE = standard error.				

Ecological arthropod indexes

Abundance ($F = 11.29$), diversity ($F = 12.37$), and species richness ($F = 64.06$) of total predators (including spiders) and abundance and species richness of chewing ($F = 5.70$, $F = 9.18$), dipterans ($F = 24.54$, $F = 41.87$), and phytophagous coleopterans ($F = 2.80$, $F = 1.94$) were higher ($P < 0.05$, $Df = 1$) on *S. saponaria* plants with dehydrated sewage sludge (Table 2). The abundance (≈ 0.15 /tree), diversity (0.00/tree), and species richness (≈ 0.04 /tree) of pollinators (Hymenoptera) were similar ($P > 0.05$) on trees with or without treatment.

Table 2

Abundance, diversity, and species richness of coleopteran phytophagous, dipteran phytophagous, orthopteran phytophagous, spiders, total chewing, and predators on *Sapindus saponaria* (Sapindales: Sapindaceae)/plant (mean $\pm$ SE of the mean) with or without dehydrated sewage sludge as fertilizer

Ecological indexes	Dehydrated sewage sludge		ANOVA	
	With	Without	<i>F</i>	<i>P</i>
Abundance of Chewing Insects	1.17 $\pm$ 0.29a	0.33 $\pm$ 0.17b	5.70	0.02
Diversity of Chewing Insects	0.52 $\pm$ 0.33a	0.26 $\pm$ 0.15a	0.68	n.s.
Species richness of Chewing Insects	1.04 $\pm$ 0.23a	0.25 $\pm$ 0.10b	9.18	0.00
Abundance of Coleoptera	1.79 $\pm$ 0.30a	1.13 $\pm$ 0.25a	2.80	0.10
Diversity of Coleoptera	0.78 $\pm$ 0.22a	0.99 $\pm$ 0.28a	0.30	n.s.
Species Richness of Coleoptera	1.04 $\pm$ 0.14a	0.75 $\pm$ 0.15a	1.94	0.17
Abundance of Diptera	31.96 $\pm$ 5.73a	4.13 $\pm$ 1.84b	24.54	0.00
Diversity of Diptera	2.90 $\pm$ 0.40a	2.02 $\pm$ 0.38a	2.33	0.14
Species Richness of of Diptera	3.25 $\pm$ 0.24a	1.25 $\pm$ 0.19b	41.87	0.00
Abundance of Orthoptera	20.38 $\pm$ 3.55a	0.88 $\pm$ 0.45b	34.23	0.00
Diversity of Orthoptera	0.22 $\pm$ 0.17a	0.12 $\pm$ 0.08a	0.40	n.s.
Species Richness of Orthoptera	0.75 $\pm$ 0.12a	0.08 $\pm$ 0.05 b	36.96	0.00
Abundance of Spiders	3.08 $\pm$ 0.52a	0.71 $\pm$ 0.22b	18.21	0.00
Diversity of Spiders	1.37 $\pm$ 0.53a	1.44 $\pm$ 0.51a	0.01	n.s
Species Richness of Spiders	1.92 $\pm$ 0.23a	0.71 $\pm$ 0.22b	14.70	0.00
Abundance of Predators	28.00 $\pm$ 6.09a	6.79 $\pm$ 2.26b	11.29	0.00
Diversity of Predators	7.52 $\pm$ 1.08a	3.42 $\pm$ 0.75b	12.37	0.00
Species Richness of Predators	5.58 $\pm$ 0.37a	1.83 $\pm$ 0.33b	64.06	0.00
Averages followed by the same lowercase letter per row do not differ by Tukey test ($P < 0.05$). n.s. highly not significant by ANOVA ($P > 0.05$). Degrees of freedom: treatment = 1, block = 5, and residual = 41. SE = standard error.				

Interactions between groups and leaf mass production by *Sapindus saponaria* plants

The interaction between the abundance of chewing insects ($F = 16.74$, $R^2 = 0.27$) (Fig. 1A), phytophagous Coleoptera ($F = 7.95$, $R^2 = 0.15$) (Fig. 1B), Diptera ($F = 20.52$, $R^2 = 0.31$) (Fig. 1C), tending ants ($F = 9.08$, $R^2 = 0.17$) (Fig. 1D), and spiders ($F = 6.78$, $R^2 = 0.13$) (Fig. 1E) with the number of leaves/*S. saponaria* tree was positive.

The interaction between the abundance of phytophagous Coleoptera ($P < 0.05$) and spiders ($F = 3.3$, $R^2 = 0.13$) was negative (Fig. 2A) and that of spiders positive with chewing insects ($F = 8.6$, $R^2 = 0.16$) (Fig. 2B), phytophagous Coleoptera negative with tending ants ($F = 3.32$, $R^2 = 0.12$) (Fig. 2C), and spiders negative with these ants ($F = 3.9$, $R^2 = 0.15$) ($P < 0.05$) (Fig. 2D). The numbers of tending ants ($P < 0.05$) were negatively correlated with the abundance of chewing insects ($F = 10.5$, $R^2 = 0.32$) (Fig. 2E) and phytophagous Diptera ($F = 7.1$, $R^2 = 0.24$) (Fig. 2F) on *S. saponaria* leaves.

Discussion

The higher number of leaves/tree of *S. saponaria* plants with dehydrated sewage sludge is likely due to the organic matter and element richness, especially the nitrogen, in this material [29], increasing litter production as soil cover. Sewage sludge also benefits plants by boosting colonization of endophytic and soil microbes such as decomposers, increasing nutrient availability [45]. In addition, these plant crowns were larger (greater TIB) with more food resources and greater ecological indexes (e.g., abundance) of the arthropod fauna [31, 32].

The higher number of leaves/branch and branches/plant of *S. saponaria* with dehydrated sewage sludge increased ground cover with litter and confirms the first hypothesis (fertilized plants accelerate the recovery process of degraded areas), important to reduce erosion and increasing soil fertility [46]. The development of *S. saponaria* with 10 leaves/branch, 13 branches/plant, and 19% of soil cover was similar to that of *Acacia auriculiformis* A. Cunn. ex Beth [5] and *Acacia mangium* Willd. [28] (Fabales: Fabaceae) with, respectively, an average of seven and 11 leaves/branch, 26 and 24 branches/plant, and 23% and 23% soil cover in the same edaphoclimatic experimental conditions. Adequate growth of *S. saponaria* plants with dehydrated sewage sludge increases the importance of this plant to recover degraded land and, in addition, reduces the kinetic energy of the rainfall and, consequently, the erosion [47]. The increase in vegetation cover by plants fertilized with sewage sludge is important for the recovery process affecting different trophic levels, including herbivores and predators in the herbaceous layer with a more favourable microclimate and floristic composition [23] such as greater weed diversity [48]. The better plant growth with sewage sludge increases food quality and quantity besides more hiding and reproduction places for the predators [49]. Herbivorous insects are attracted to the tree canopies, searching for food, and thus their enemies follow them [49]. Insect colonization indicates that the recovery process is being effective to re-establish the ecosystem dynamics [28] with denser vegetation reducing the antagonistic effect between predators on herbivores [23]. The nutrients of the sewage sludge increased *S. saponaria* tree growth as found for *A. auriculiformis* and *A. mangium* and, consequently, the importance of evaluating each plant species in programs of recovering degraded areas. These results are important for decision management according to the expected attribute (e.g., greater production of branches, or vegetation cover) and of their ecological and social importance.

The greater abundance, diversity, and species richness of total predators (including spiders) and abundance and species richness of chewing, phytophagous coleopterans, and dipterans on *S. saponaria*

plants with dehydrated sewage sludge confirm the second hypothesis: a greater TIB leads to a higher number of chewing and predator insects. The application of sewage sludge increased plant growth and, consequently, the abundance, diversity, and richness of total predators, but with lower values on *S. saponaria* than on *Terminalia argentea* Mart. (Combretaceae) and *A. mangium* plants [4, 28]. This may be due to the response of each plant species to the sewage sludge but with greatest ecological indices on those with better growth [4, 28]. The lower abundance and species richness of chewing insects on *S. saponaria* were similar to that reported for *A. mangium* [28]. Besides, the abundance, diversity, and species richness of phytophagous coleopterans on *S. saponaria* were lower than that on *T. argentea* [4]. The greater number of phytophagous coleopterans on this last plant may be due to its origin, the Brazilian savannah [4]. The Diptera abundance and species richness on *S. saponaria* was higher than that on *T. argentea*, *A. mangium*, and *A. auriculiformis* without a single individual on these last two plants [4, 5, 28]. Compounds in the *S. saponaria* leaves may explain the lower ecological indexes on this plant with sewage sludge compared to those on *T. argentea*, *A. mangium* and *A. auriculiformis*. This increases the importance of developing studies correlating leaf compounds and the ecological indexes of arthropods on different tree species.

The increased in soil cover by *T. argentea* and the abundance of chewing, predator and sucking insects on this plant were similar to that reported for *A. auriculiformis*, *A. mangium* with sewage sludge [4, 5, 28, 6]. Variation in the abundance of arthropod fauna (e.g., coleopterans and spiders) in different environments, ecological successions, temporal distribution, seasons [8], temperature, and gradient increase [50] makes them monitoring tools for environment quality [22]. The increase in the vegetable matter produced and arthropod community with sewage sludge application allow evaluating the invertebrate community and their relationships in the recovering area [51]. The abundance of coleopterans may be due to the increase in the number of leaves (feeding) and plant complexity (habitat) [51]. The greater plant production and increase soil cover may favour the abundance of dipterans and weeds as shelter against predators [48]. This contributes to understanding the relationships between the organisms and the sludge application similar to that of the tree density directly affecting species richness of these insects [52, 53] and of ants and dung beetle species abundance and composition [22]. The vineyard canopy also correlated with ground cover after application of the sewage sludge providing greater shelter and prey availability, increasing spider survival and reproduction [48]. The diversity of foraging sites and better plant production and soil cover with the application of sludge and increasing numbers of sites for the ants to build their nests in a Neotropical rain forest in the USA helped to explain the higher abundance of arboreal ants [54]. This condition increases alternative food sources favouring prey survival and abundance of spiders in vineyards in Croatia [48] and in wheat fields, *Triticum aestivum* L. (Poales: Poaceae), in the North China Plain, China [55]. The competition between spiders and ground beetles (Carabidae) affected the arthropod diversity [55]. Tree canopies with fruits, flowers, and leaves attracted herbivorous insects (e.g., coleopterans, formicids, homopterans, and termites) in the Australian tropical rainforest [49]. The orthopterans success was attribute to their generalist diet feeding on crops, grasses, and weeds [56] and a more developed canopy protect them from excessive sunlight and to

predators such as birds, coleopterans, dipterans, and heteropterans [49]. Besides the vegetation structure influences on microclimate conditions affect the development and reproduction of these insects [56].

The higher abundance of chewing insects, phytophagous Coleoptera and Diptera, tending ants and spiders on *S. saponaria* trees with greater leaf mass confirm our third hypothesis. The arthropod abundance is related to the leaf mass of this plant (> BGI) as found in North American grasslands [57]. The better quality and higher quantity of nutrients in plants with larger crowns may explain the greater numbers of arthropod individuals and the effect on the subsequent trophic levels [57] as herbivorous insects [30]. The greater abundance of tending ants with the *S. saponaria* leaf mass increase was similar to that reported for ant colonies on larger *Hirtella myrmecophila* trees in the Amazon [58]. The larger number of spiders on *S. saponaria* plants fertilized with sewage sludge is similar to that of these organisms in areas with greater heterogeneity in terrestrial environments [59] with the vegetation structure increasing prey populations [59].

The negative correlation between the abundance of phytophagous Coleoptera with spiders on *S. saponaria* plants confirms the fourth hypothesis: a greater number of ants and/or spiders reduce that of chewing insects [51]. Natural enemies protect trees from pests (Coleoptera and Lepidoptera) [31], increasing their importance in the agrosystem balance [61]. Ants, generally, contribute to the plant leaf area increase [62] by predating any arthropods threatening their food sources [63, 58]. Spiders are important predators, and their presence even with their mouthpiece partially bonded reduced the herbivore abundance [62]. However, the combined action of ants and spiders can be synergistic or antagonistic, the first when both act on herbivores and antagonistic when ants reduce spider populations [62].

The negative correlation between the abundance of phytophagous Coleoptera and spiders with that of total tending ants on *S. saponaria* trees confirms the fifth hypothesis: ants reduced predator populations. Spiders and ants are key groups in terrestrial ecosystems affecting directly or indirectly each other distribution [64]. The ant behavioural and structural defences [64] are advantages when interacting with spiders in common habitats [65] and these last organisms tend to avoid ants through escaping mechanisms and abandoning the plant [65]. This explains the reduction in the spider abundance as the ant population increase was similar to the 90% of spiders displaced by ants on *Psychotria carthagenensis* inflorescences in the south of the Brazilian wetland [59]. This behaviour increases the importance of studies on post-dispersal mechanisms because this competition can be ecologically positive, by forcing the spiders to colonize other areas.

The higher complexity of the canopy with the application of sewage sludge attracted more herbivorous insects and, consequently, of their natural enemies defending the plant [49]. The application of sewage sludge increased plant mass and spider populations, which reduced damage by herbivores. This is a low-cost alternative to improve plant development and ecological relationships between pests and natural enemies.

The higher number of leaves/branch and branches/plant and the percentage of ground cover by *S. saponaria* plants with dehydrated sewage sludge increased the ecosystem recovering. The application of

sewage sludge may also increase *S. saponaria* seed production, important for the pharmaceutical industry due to the compounds of economic interest in them. The higher abundance, diversity, and species richness of total predators (including spiders) and abundance and species richness of chewers, phytophagous coleopterans, and dipterans on *S. saponaria* plants with dehydrated sewage sludge is a promising strategy to recover degraded areas by increasing plant growth with the positive correlation between the ecological indexes of chewing insects, Coleoptera and Diptera phytophagous, tending ants, and spiders. The abundance of phytophagous Coleoptera was negatively and positively correlated with that of spiders and chewing insects, respectively. The abundance of phytophagous and spiders and coleopterans was negatively correlated with that of total tending ants, and this last group was also negatively correlated with the abundance of chewing insects and phytophagous dipterans. Such relationships can be used to assess the effectiveness of recovering programs and the adequacy of the plant species for the ecological functions of the environment. The relationships between insects and their ecological indexes are important for monitoring programs and to establishing them as bioindicators.

Declarations

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Author Contributions

G.L.D.L: conceptualization, data curation, methodology, sampling. D.L.T., G.L.D.L analysis, writing-original draft. R.A.S., W.S.T., T.M.M.F., J.E.S., R.Z., R.S.C., J.C.Z.: writing, reviewing, and editing. J.C.Z: supervision.

Competing Interests

The authors declare that they have no conflicts of interest to this work.

Data availability

The datasets used during the current study available from the corresponding author on reasonable request.

Ethics declarations

The authors declare that the work complies with the requested ethical standards.

Study complies

All study procedures on plants in the present study complies with relevant institutional, national, and international guidelines and legislation.

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Figures

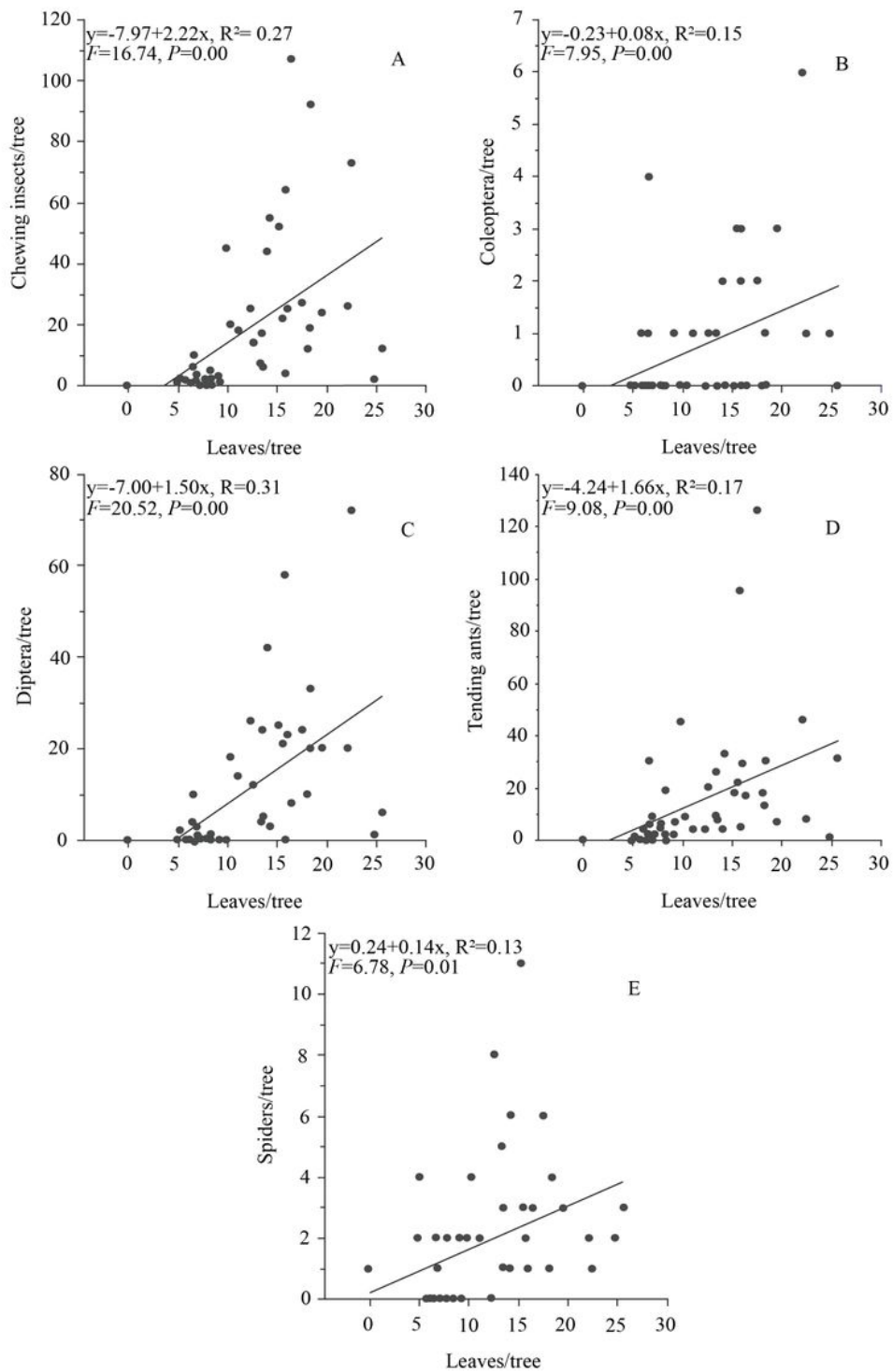


Figure 1

Chewing insects, phytophagous Coleoptera, Diptera, tending ants and spider abundance as function of the leaf mass/ *Sapindus saponaria* tree. n= 48.

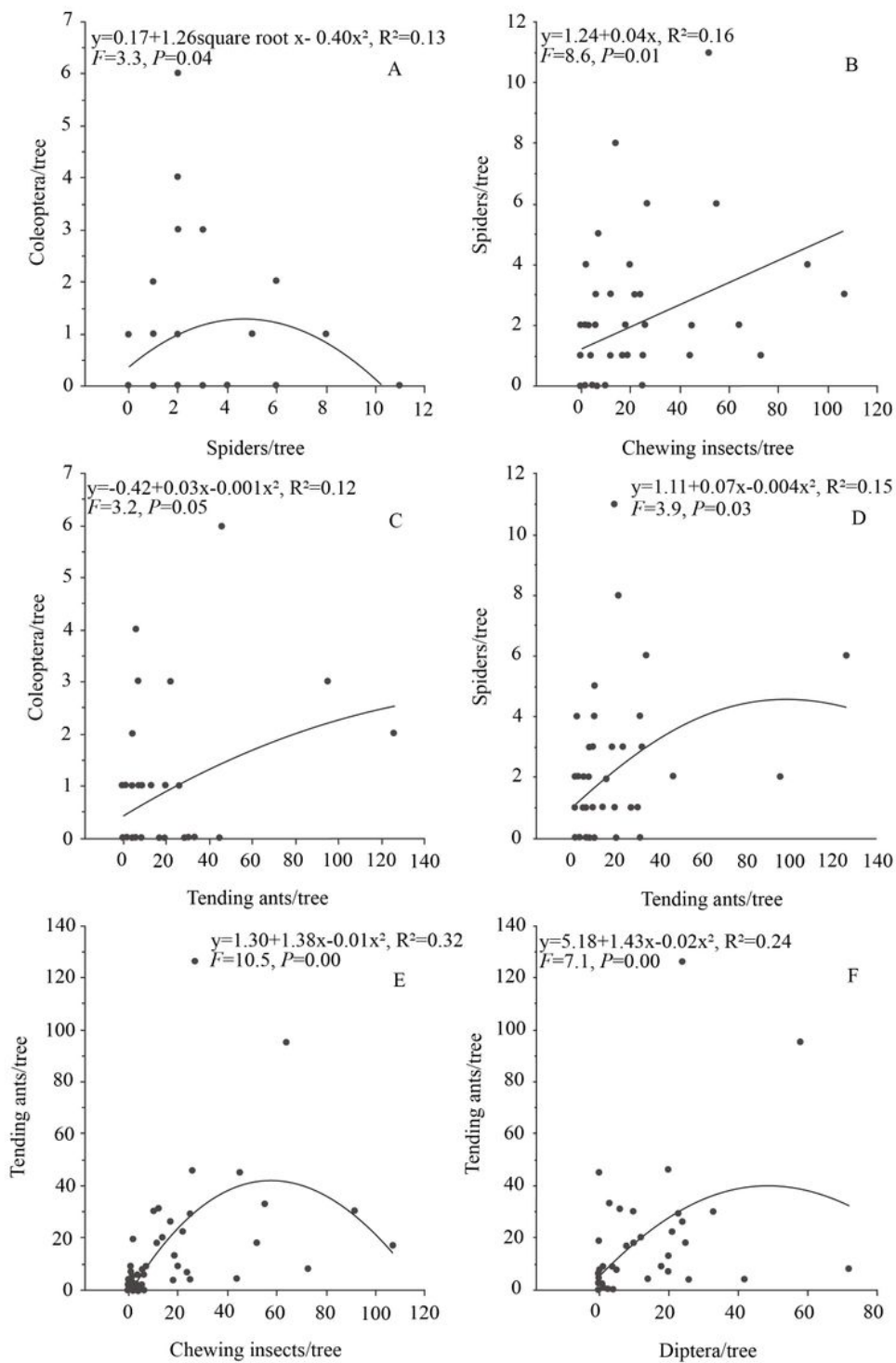


Figure 2

Relationships between Coleoptera, Diptera, chewing insects, spiders and tending ant abundance on *Sapindus saponaria*/tree. $n = 48$.