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Sección Básica / Basic
Nota científica / Scientific note

Bottom-up effects on arthropod communities in *Platycyamus regnellii* (Fabaceae) fertilized with dehydrated sewage sludge

Efectos ascendentes en las comunidades de artrópodos de *Platycyamus regnellii* (Fabaceae) fertilizados con lodo de aguas residuales

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Abstract: Sewage sludge is a nitrogen-rich organic compound, which can be used to aid development in plants such as *Platycyamus regnellii* (Fabaceae), in the recovery of degraded areas. This study aimed to assess the bottom-up effects on leaf mass and percentage of ground cover (leaf litter, herbaceous plants, and grasses) in *P. regnellii* trees fertilized (or not) with dehydrated sewage sludge and arthropod communities over 24 months. *Platycyamus regnellii* trees fertilized with dehydrated sewage sludge presented significantly more leaves per branch, branches per tree, and a higher percentage of ground cover compared to unfertilized trees. *Phenacoccus* sp. (Pseudococcidae) was the most abundant phytophagous insect associated with *P. regnellii* trees. Fertilization did not affect the abundance, diversity, and species richness of Hemiptera phytophagous on *P. regnellii* trees. However, fertilized trees presented higher abundance and species richness of trophobiont-tending ants compared to unfertilized trees, with *Camponotus* sp. being the most abundant regardless of the treatment. Fertilized *P. regnellii* trees also presented higher species richness of natural enemies compared to unfertilized ones, with Aranae and Dolichopodidae being the most abundant. We concluded that fertilization with dehydrated sewage sludge improved *P. regnellii* trees leaf mass and ground cover and increased the diversity of trophobiont-tending ants and natural enemies. To our knowledge, this is the first study on the arthropods community associated with this tree species. This suggests that upon fertilization, *P. renellii* trees are useful for ecological restoration in severely disturbed areas.

Keywords: Arthropods, fertilization with sewage sludge, sucking insects, abundance of beneficial insects, trophobiont-tending ants, *Platycyamus regnellii.*

Resumen: El lodo de aguas residuales es un compuesto orgánico rico en nitrógeno que aumenta el desarrollo de plantas como *Platycyamus regnellii* (Fabaceae), que se usa comúnmente en la recuperación de áreas degradadas. Este estudio se centra en la evaluación de los efectos ascendentes en la producción en masa vegetal y la cobertura del suelo en plantas de *P. regnellii* fertilizados (o no) con lodo de aguas residuales y las comunidades de artrópodos durante 24 meses. Los árboles de *P. regnellii* fertilizados con lodo de aguas residuales presentaron significativamente más hojas por rama, ramas por árbol, y un mayor porcentaje de cobertura del suelo en comparación con los árboles no fertilizados. *Phenacoccus* sp. (Pseudococcidae) fue la especie fitófaga más abundante. La fertilización no afectó la abundancia, diversidad y riqueza de especies de Hemiptera fitófagos en árboles de *P. regnellii*. Sin embargo, los árboles fertilizados presentaron mayor abundancia y riqueza de especies de hormigas cuidadora de trofobiontes en comparación con los árboles no fertilizados. *Camponotus* sp. fue la specie más abundante independientemente del tratamiento. Los árboles de *P. regnellii* fertilizados también presentaron mayor riqueza de especies de enemigos naturales en comparación con los no fertilizados, siendo Aranae y Dolichopodidae los más abundantes. Se concluye que las coronas más grandes de *P. regnellii* fueron aquellas fertilizadas con lodo de aguas residuales deshidratadas, lo que aumenta la cobertura del suelo, la diversidad de hormigas cuidadoras de trofobiontes, y de enemigos naturales. Hasta donde se conoce, este es el primer estudio sobre la comunidad de artrópodos asociada a esta especie de árbol. Esto sugiere que las plantas de *P. renellii* fertilizadas con lodos de depurados son adecuadas para la recuperación de áreas degradadas.

Palabras clave: Artrópodos, fertilización con lodos depurados, insectos chupadores, abundancia de insectos benéficos, hormigas cuidadoras de trofobiontes, *Platycyamus regnellii.*

Introduction

Plants are primary producers that affect a wide range of different groups of organisms (e.g. phytophagous insects) in terrestrial food webs, providing them food and habitat. They are also present in resource-driven webs in which they provide profound bottom-up effects on higher trophic levels (Price 2002). Terrestrial food webs are composed of at least three interacting trophic levels: plants, herbivores, and natural enemies, and are controlled from the bottom-up via plant nutrition or from the top down via herbivory (Price *et al.* 1980; Rosemond *et al.* 1993). These hypotheses have been tested and empirically studied on two *Quercus* species, with fertilization used as the bottom-up effect, and the removal of natural enemies as top-down effects (Cornelissen and Stiling 2006). The authors found that fertilization affects herbivores density while the removal of natural enemies did not, suggesting that herbivores are more responsive to bottom-up compared to top-down effects.

Sewage sludge is a by-product from industrial or municipal waste treatment, rich in organic matter and, ideal for soil fertilization or substrate in seedling production (Antonkiewicz *et al.* 2019; Carvalho *et al.* 2020). This wasting product may be used in forest plantations and the recovery of degraded areas, ameliorating the risk of toxic elements entering the human food chain (Kimberley *et al.* 2004). Sewage sludge has the potential for usage as fertilizer in agriculture, and forests, reducing the production costs, the residual accumulation and environmental problems, and giving it an appropriate destination (Caldeira *et al.* 2014).

Platycyamus regnellii Benth (Fabaceae) is a native tree in Brazil, distributed in the states of Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, Paraná, São Paulo, and Goiás, especially in the semi-deciduous highland forests (Lorenzi 2008). This tree species is used in the reforestation of degraded areas and landscaping, ornamentation of parks and gardens (Brandão *et al.* 2002). *Platycyamus regnellii* has medicinal properties against fever, maldigestion, and inappetence, and is a proper wood for civil construction, carpentry, and manufacturing (Ferreira *et al.* 2015). Although there is a broad knowledge on its use as an ecological restorer, and other uses, little is known on the effects of sewage sludge on *P. regnellii* tree production and there are no studies on arthropods community associated with this tree species.

Sewage sludge increases humus content in the soil (Silva *et al.* 2011; Abreu *et al.* 2017) and is rich in phosphorus, nitrogen, macro (Ca, Mg), and micronutrients (Cu and Zn) (Nogueira *et al.* 2007), which favors the plant development and, consequently, increases ecological indices of phytophagous insects (> biogeographic island - BGI) and their natural enemies (Auslander *et al.* 2003). Changes in soil fertility may directly affect the nutrition and chemical defenses of the host plant, and this impacts the diversity of phytophagous insects and their natural enemies (Cornelissen and Stiling 2006; Staley *et al.* 2010; Graff *et al.* 2020). Insects are very susceptible to environmental changes, and such can be useful for monitoring the recovery of degraded areas (Burgio *et al.* 2015).

While interactions in terrestrial food webs depend on bottom-up effects on plant and herbivore communities, these interactions and forest productivity are usually structured by mutualism between ants and sap-sucking insects (Clark *et al.* 2019). Ants are an important group of insects that affect the interactions in host plant–herbivore systems in different ways, by attacking herbivores whilst defending the trophobionts; as seen in the positive relationship between ants and herbivores trophobiont richness on *Baccharis dracunculifolia* DC (Asteraceae) (Monteiro *et al.* 2020). Trophobiont-tending ants, hereafter, are those who defend hemipterans in exchange for food (honeydew) (Freitas and Rossi 2015).

The aim here was to assess leaf mass production and percentage of ground cover (leaf litter, herbaceous plants, and grasses) of *P. regnellii* trees fertilized and unfertilized with dehydrated sewage sludge and associated arthropods community on the trees over 24 months. The assumptions in the study were that i) trees fertilized with sewage sludge would have larger canopies, and therefore, an increased ground cover; ii) trees fertilized with sewage sludge would have larger canopies (> BGI) and thus an increased abundance of phytophagous insects and iii) larger trees would have more trophobiont-tending ants and natural enemies attracted by phytophagous insects.

Materials and methods

Experimental site. The study was carried out in a degraded area at the "Instituto de Ciências Agrárias (ICA)" of the "Universidade Federal de Minas Gerais (UFMG)", Montes Claros, Minas Gerais State, Brazil (16º51'38"S 44º55'00"W, 943 m) during 24 months (March 2015 to February 2017). The degraded area has severe loss and changes in soil chemistry and hydrology. According to Köppen's climate classification, it is a tropical dry climate area with annual rainfall between 1000-1300 mm, with dry winter and an annual mean temperature of ≥ 18 °C.

The soil is a litolic neosoil, loamy texture, total sand = 17 dag.Kg $^{-1}$, silt = 46.0 dag.Kg $^{-1}$, clay = 37.0 dag.Kg $^{-1}$, pH–H_2O = 4.3, organic matter = 0.73 dag.Kg $^{-1}$, organic carbon = 0.42 dag.Kg $^{-1}$, P = 0.35 mg.dm $^{-3}$, K = 41.0 mg.dm $^{-3}$, Ca = 1.6 $cmol_c$.dm $^{-3}$, Mg = 0.9 $cmol_c$.dm $^{-3}$, Al = 3.3 $cmol_c$.dm $^{-3}$, aluminum saturation in the capacity of cationic exchange = 55.1 %, sum of bases = 2.69 $cmol_c$ dm $^{-3}$, H + Al = 13.4 $cmol_c$.dm $^{-3}$, percentage of soil base saturation of the capacity of cationic exchange a pH 7.0 = 16.7, effective cation exchange capacity (CEC) = 5.9 $cmol_c$.dm $^{-3}$, and potential (pH 7.0) CEC = 16.1 $cmol_c$.dm $^{-3}$.

Experimental design. *Platycyamus regnellii* seedlings were produced from seeds of trees grown at the ICA/UFMG. Seedlings were planted in March 2014, in plastic bags (8 x 12 cm) in a nursery, with a substrate (160 g per seedling) containing 30 % of organic compost (two parts of debris gardening pruning and one-part bovine manure), 30 % of clay soil, 30 % of sand, and 10 % of reactive natural phosphate. The pH soil was corrected with dolomitic limestone (90 % relative total neutralization power) (187 g per pit), increasing base saturation to 50 %. Natural phosphate (80 g per pit), fritted trace elements (10 g per pit), and marble rock dust (1 Kg per pit) were added according to soil analysis. In September 2014, 48 *P. regnellii* seedlings (30 cm height) were planted in pits (40 × 40 × 40 cm) spaced two meters from each other, in six parallel lines (two meters between lines) with four seedlings per line. The seedlings were, fertilized with 20 L (in a single dose) of dehydrated sewage sludge, while a control group remained unfertilized. The seedlings were watered twice a week until the beginning of the rainy season and were pruned up to 1/3 of the canopy, eliminating 5 cm long branches. The experimental design was completely randomized with two factors (fertilized or unfertilized) of the treatment and 24 replicates.

Sewage sludge. Dehydrated sewage sludge with 5 % moisture content was collected at the sewage treatment plant - "Estação de Tratamento de Esgoto (ETE)" in Juramento, state of Minas Gerais, Brazil. The ETE treats 217 m^3 sewage per day with an efficiency of organic matter removal of ~ 90 %. The sewage sludge treatment occurs via solarization in coarse sand tanks for three months, and it aims to reduces the thermotolerant coliforms to acceptable levels (< 10^3 coliforms number/g of total solids) for use in agriculture (CONAMA, Resolution N375, Brazil). Chemical and biological characteristics of the dehydrated sewage sludge are pH - H_2O = 4.40, N = 10.4 mg.Kg $^{-1}$, P = 2.9 mg.Kg $^{-1}$, K = 5.8 mg.Kg $^{-1}$, Cd = 0.1 µg.g $^{-1}$, Pb = 56.9 µg.g $^{-1}$, Cr = 46.7 µg.g $^{-1}$, and fecal coliforms = 4.35 most likely number g $^{-1}$ (Nogueira *et al.* 2007).

Leaf mass production and ground cover. Leaves per branch, branches per tree numbers, and the percentage of ground cover by litter, grass, and herbaceous plants, were measured monthly by visual observation on 1.0 m^2 plots at the canopy projection of six-months-old *P. regnellii* trees.

Arthropods. The arthropods counting was done biweekly in the mornings (7:00 to 11:00 AM) by visual observations on the adaxial and abaxial faces (12 leaves/tree/survey), on apical, middle, and basal canopy parts in the northerly, southerly, easterly, and westerly directions, totalizing 27,648 leaves during all experimental period. At least three specimens per arthropod species were captured with aspirator, put in glass flasks with 70 % ethanol, and sent for identification.

Data analysis. The mean data (i.e. 12 leaves/tree/survey) from single trees was used. Data on leaf mass production (leaves per branch and branches per tree) and ground cover (percentages of cover by litter, grass, and herbaceous plants) were submitted to analysis of variance (one-way ANOVA) and means were compared using the Tukey range test (P < 0.05). Ecological indices (abundance, diversity, and species richness) were calculated per group of arthropods (i.e. hemipteran, trophobiont-tending ants, and natural enemies) and treatment (fertilization or not with dehydrated sewage sludge) using BioDiversity Professional, Version 2. The diversity was calculated with Hill's formula and species richness with Simpson indices. The abundance, diversity, and species richness of phytophagous Hemiptera, trophobiont-tending ants, and natural enemies were submitted to the non-parametric statistical hypothesis Wilcoxon signed-rank test (P < 0.05). Throughout the text, means ± standard errors (S.E.) are presented. All analyses were conducted in "Sistema para Análises Estatísticas e Genéticas" (Sistema para Análises Estatísticas 2007), version 9.1. Regression analysis was performed for ecological indices of phytophagous Hemiptera against trophobiont-tending ants and natural enemies in SAEG (P < 0.05).

Results

Leaf mass production and ground cover. *Platycyamus regnellii* trees fertilized with dehydrated sewage sludge produced significantly (P < 0.05) more leaves per branch, branches per tree, as well as more percentage of ground cover (leaf litter, herbaceous plants, and grasses) compared to unfertilized ones (Table 1).

Arthropods. No differences (P > 0.05) in abundance, diversity, and species richness of phytophagous Hemiptera were observed between fertilized and unfertilized trees. However, *Phenacoccus* sp. (Hemiptera: Pseudococcidae) was the most abundant phytophagous insect collected and was found exclusively on fertilized trees (1.46 ± 1.01). Trophobiont-tending ants presented a higher abundance (P = 0.02) and species richness (P = 0.02) on leaves of fertilized *P. regnellii* trees (0.63 ± 0.18 and 0.58 ± 0.15, respectively) compared to those unfertilized (0.25 ± 0.13 and 0.21 ± 0.10, respectively). *Camponotus* sp. (Hymenoptera: Formicidae) was the most abundant (P = 0.11) trophobiont-tending ants on fertilized *P. regnellii* trees, regardless of the treatment (fertilized = 0.21 ± 0.08 and unfertilized = 0.08 ± 0.05). Natural enemies presented the highest indices of species richness (P = 0.03) on fertilized trees (1.63 ± 0.36) compared to unfertilized (0.75 ± 0.16) ones; no other ecological indices were affected by tree fertilization (P > 0.05). Although there was no significant difference (P > 0.05) between treatments, Aranae and Dolichopodidae (Diptera) were the most abundant natural enemies on fertilized (2.75 ± 1.89 and 0.21 ± 0.13, respectively) and on unfertilized (0.58 ± 0.16 and 0.13 ± 0.06, respectively) *P. regnellii* trees (Table 2). The increase in abundance of trophobiont-tending ants positively affected the abundance of phytophagous Hemiptera ($y' = 3.19 + 8.66x$, $R^2 = 0.14$, $F = 7.26$, $P = 0.01$).

Discussion

We found that fertilization with dehydrated sewage sludge improved *P. regnellii* trees leaf mass and ground cover and increased the diversity of trophobiont-tending ants and natural enemies. To our knowledge, this is the first study on the arthropods community associated with this tree species. Dehydrated sewage sludge fertilization favored the development of *P. regnellii* trees as this organic waste product is rich in nitrogen (Nogueira *et al.* 2007). Trees fertilized with

Table 1. Mean ± SE of leaf mass (leaves per branch and branches per tree) and percentage of ground cover (leaf litter, herbaceous plants, and grasses) of *Platycyamus regnellii* Benth. (Fabaceae) trees fertilized and unfertilized with dehydrated sewage sludge.

	Sewage sludge		ANOVA	
	fertilized	Control	F	P
Leaves per branch	7.56 ± 0.29 a	5.58 ± 0.23 b	16.7	0.00
Branches per tree	4.54 ± 0.14 a	3.06 ± 0.11 b	16.8	0.00
Ground cover (%)	25.09 ± 1.20 a	6.32 ± 0.46 b	62.6	0.00

Means followed by the same letter (± standard error) per line are not different by the test of Tukey. F and P values were obtained by ANOVA. Degrees of freedom of treatments and errors were 1 and 23, respectively. N = 24 per treatment.

Table 2. Mean ± SE of abundance (Abun.), diversity (D.), and species richness (S.R.) of phytophagous Hemiptera (Hem.), trophobiont-tending ants (Ants) and natural enemies (N.E.), and numbers of species of insects on *Platycyamus regnellii* Benth. (Fabaceae) trees fertilized and unfertilized with dehydrated sewage sludge. Ecological indices (abundance, diversity, and species richness) were calculated per group of arthropods (i.e. hemipteran, trophobiont-tending ants, and natural enemies) and treatment (fertilization or not with dehydrated sewage sludge).

	Sewage sludge		Wilcoxon test	
	Fertilized	**Control**	**VT***	**P**
Abund. Hem.	1.96 ± 1.00	1.08 ± 0.51	0.5	0.31
D. Hem.	0.18 ± 0.09	0.28 ± 0.17	0.1	0.48
S.R. Hem.	0.58 ± 0.16	0.38 ± 0.11	0.7	0.25
Aleyrodidae	0.13 ± 0.09	0.88 ± 0.51	0.9	0.18
Cicadellidae: *Balclutha hebe* (Kirkaldy, 1906)	0.04 ± 0.04	0.04 ± 0.04	0.0	0.50
Erythrogonia sexguttata (Fabr., 1803)	0.04 ± 0.04	0.00 ± 0.00	1.0	0.16
Nogodinidae: *Bladina* sp.	0.04 ± 0.04	0.00 ± 0.00	1.0	0.16
Pentatomidae	0.25 ± 0.10	0.17 ± 0.07	0.4	0.34
Pseudococcidae: *Phenacoccus* sp.	1.46 ± 1.01	0.00 ± 0.00	1.4	0.08
Abun. Ants	0.63 ± 0.18	0.25 ± 0.13	2.1	0.02
D. Ants	0.33 ± 0.27	0.12 ± 0.08	0.0	0.48
S.R. Ants	0.58 ± 0.15	0.21 ± 0.10	2.1	0.02
Abun. N.E.	3.67 ± 1.90	0.96 ± 0.23	1.5	0.07
D. N.E.	2.33 ± 0.95	1.02 ± 0.34	0.5	0.31
S.R. N.E.	1.63 ± 0.36	0.75 ± 0.16	1.9	0.03
Araneae	2.75 ± 1.89	0.58 ± 0.16	0.9	0.19
Diptera: Dolichopodidae	0.21 ± 0.13	0.13 ± 0.06	0.1	0.48
Hymenoptera: Braconidae	0.04 ± 0.04	0.00 ± 0.00	1.0	0.16
Formicidae: *Brachymyrmex* sp.	0.04 ± 0.04	0.08 ± 0.08	0.03	0.49
Camponotus sp.	0.21 ± 0.08	0.08 ± 0.05	1.2	0.11
Cephalotes sp.	0.08 ± 0.08	0.00 ± 0.00	1.0	0.16
Ectatoma sp.	0.08 ± 0.05	0.00 ± 0.00	1.4	0.08
Pheidole sp.	0.04 ± 0.04	0.00 ± 0.00	1.0	0.16
Pseudomyrmex termitarius (Smith, F., 1855)	0.17 ± 0.07	0.08 ± 0.05	0.9	0.19

N = 24 per treatment. *VT* (*) = value of the test.

dehydrated sewage sludge had larger canopy width (> BGI) that results in more food resources for phytophagous insects (Ferrier and Price 2004; Leite *et al.* 2017). In our study, the most abundant herbivorous insect was found exclusively on fertilized plants, attracting more trophobiont-tending ants and natural enemies (Auslander *et al.* 2003) compared to unfertilized trees (as seen in the positive regression).

Platycyamus regnellii trees fertilized with sewage sludge developed better, i.e. they had a higher number of leaves (> 1.4) and branches (> 1.5), and this reflected in a higher percentage of ground cover (> 3.9) compared to unfertilized trees. This confirms the first assumption that fertilization with dehydrated sewage sludge on *P. regnellii* trees would speed up the recovery process of degraded areas by increasing soil fertility and decrease laminar erosion (Franco *et al.* 2002). The recovery of degraded areas is a slow process (Ferreira *et al.* 2007), so the use of fertilization such as organic waste products on *P. regnellii* trees is promising. In fact, sewage sludge has been broadly used for fertilization of arboreal species such as *Araucaria angustifolia* (Bertol) Kuntze (Araucariaceae), *Cedrela fissilis* Vell (Meliaceae), *Eucalyptus grandis* Hill (Myrtaceae), *Lafoensia pacari* Saint-Hilaire (Lythraceae) and *Senna spectabilis* Schrad (Fabaceae) (Silva *et al.* 2011; Abreu *et al.* 2017) and also for natural regeneration in riparian forests (Campos and Landgraf 2001).

The fact that twice as many insects were observed on fertilized *P. regnellii* trees (in absolute numbers) probably was due to the higher amount and quality of leaves, which are food resources to phytophagous insects. This confirms the second assumption that the abundance of hemipterans is usually higher in trees with higher leaf mass (Ferrier and Price 2004; Leite *et al.* 2017). Furthermore, the amount of free amino acids and protein in leaf sap usually increases with nitrogen fertilization on trees, thus favoring sucking insects (Taiz *et al.* 2017) such as *Phenacoccus* sp. (≈ 1.5 insects per tree). Sewage sludge used as biofertilizer is also helpful in the ecological recovery of macrofauna, including larvae and adults of Scarabaeidae (Coleoptera) in degraded areas of Cerrado (Kitamura *et al.* 2008).

The highest abundance and species richness of trophobiont-tending ants and species richness of total natural enemies, including spiders, were observed on fertilized *P. regnellii* trees compared to those unfertilized, with a positive correlation between phytophagous (sucking) insects. The results confirm the third assumption that trees with larger canopy width (> BGI) would have more trophobiont-tending ants (e.g. *Camponotus* sp. and *Cephalotes* sp.) and natural enemies attracted by sucking insects. This tritrophic interaction among plants => sap-sucking insects => trophobiont-tending ants is one of the most critical interactions found in nature (Stadler and Dixon 2005; Araujo *et al.* 2016). The presence of trophobiont-tending ants on *P. regnellii* trees is likely due to a mutualistic relationship with phytophagous insects such as Hemiptera (Stadler and Dixon 2005; Araujo *et al.* 2016). Beyond the ecological importance, ants are also used as bioindicators of ecological restoration of degraded areas as they respond quickly to environmental changes (Chomicki *et al.* 2015).

Platycyamus regnellii fertilized with dehydrated sewage sludge had larger canopy width (higher number of leaves and branches) that favors ground cover. Bigger and leafy trees could present a better resource for phytophagous (sucking) insects, such as hemipterans, which in turn attract a greater abundance and richness of trophobiont-tending ants and richness of species of natural enemies. To our knowledge, this is the first study on arthropods communities associated with this tree species. It suggests that, upon fertilization, *P regnellii* is an adequate option for the recovery of degraded areas as it developed faster and favored the local biodiversity.

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Author Contribution

Gezilene Fernandes de Souza, Farley William Souza Silva and Júlia Letícia Silva carried out the experiments.

Gezilene Fernandes de Souza and Germano Leão Demolin Leite analyzed the data and performed the interpretation of the results.

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Germano Leão Demolin Leite and Gustavo Leal Teixeira translated to English.

Germano Leão Demolin Leite, Regynaldo Arruda Sampaio, and José Cola Zanuncio supervised the project.