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ORIGINAL RESEARCH ARTICLE

Soil micromorphology and CO_2 exchange in initial *Atta sexdens* (Hymenoptera: Formicidae) nests

Kátia Kaelly Andrade Sousa[1] · Roberto da Silva Camargo[1] · Nadia Caldato[1] · Adriano Pimentel Farias[1] · Carlos Alberto Oliveira de Matos[2] · José Cola Zanuncio[3] · Julian Alberto Sabattini[4] · Luiz Carlos Forti[1]

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Abstract

Ants build underground nests to protect their colonies and to improve conditions for their offspring. The excavation of nests, by ants, modifies the soil structure, facilitating gas exchange and circulation, which needs further studies. Do initial nests modify the initial chamber soil matrix and gas exchange? The objective was to study the soil micromorphology of the wall of the chamber of initial nests of the leaf-cutting ant *A. sexdens* and how the CO_2 diffuses into the soil matrix. The CO_2 concentration in initial four-month-old *A. sexdens* nests was measured for 24 h using a respirometric system with a gas meter and closed nest holes (obliterated). After this period, they were opened and the CO_2 concentration measured again. In addition, 15 cm deep holes were drilled into the ground 15 and 60 cm away from the ant nest hole. The CO_2 was measured in these orifices and then they were sealed for 24 h and new ones made after this period. The contents of the nest chambers were removed, after the CO_2 measures and the soil micromorphology of the walls of the initial chamber analyzed. The CO_2 concentration in the nest chamber was greater than that in the soil at 15 and 60 cm distant from it. The CO_2 accumulation did not increase with the obliteration of the nest entrance for 24 h. Coarse material, mainly quartz and charcoal fragments, besides fine material of clay, organic matter and iron oxides composed the soil of the wall of initial nests. The soil porosity in the chamber walls of the initial nests was lower than that of the matrix of the adjacent soil and differed between those of the nests. Initial nests of *A. sexdens* modifies the soil matrix of their walls, allowing to CO_2 exchange between its chamber and adjacent soil.

Keywords Attine · CO_2 · Diffusion · Porosity

Introduction

Nests of the leaf-cutting ants *Atta*, *Acromyrmex* and *Amoimyrmex*, are elaborated during their construction and, normally, formed by chambers connected by galleries or channels opening on the soil surface (Cristiano et al. 2020). These chambers house the symbiotic fungus, the main food source for their larvae and adults. The excavation of digging the soil protects and allow to maintain favorable environmental conditions for the development of ant brood (Silva et al. 2003; Halley et al. 2005; Mota et al. 2021).

The nest initially excavated by a recently fertilized queen, which builds the first chamber (Camargo et al. 2011; Fujihara et al. 2012). This queen performs activities inside the nest until the first workers emerge and start foraging (Camargo et al. 2011), around four months after its foundation and then they made digging of channels and fungus and refuse chambers (Autuori 1942). The ants, when digging

✉ Roberto da Silva Camargo
camargosauva@gmail.com; camargobotucatu@yahoo.com.br

[1] Departamento de Proteção Vegetal, Faculdade de Ciências Agronômicas. Botucatu, Universidade Estadual Paulista (UNESP), São Paulo 18603-970, Brasil

[2] Instituto de Ciências e Engenharia, Universidade Estadual Paulista. Itapeva, São Paulo 18409-010, Brasil

[3] Departamento de Entomologia/BIOAGRO, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-900, Brasil

[4] Department of Ecology, School of Agriculture Science, National University of Entre Rios, National Council for Scientific and Technical Research, Route No. 11 Km 10.5, Entre Rios, Oro Verde 3100, Argentina

the nests, build chambers and tunnels, modifying the soil to facilitate gas exchange inside them. The internal architectural system created in the construction of these nests can also facilitate gas exchange and circulation (Bollazzi et al. 2012; Hasin et al. 2014; Halboth and Roces 2017). Soil micromorphology is suitable to study modifications by ants, identifying porosity, permeability and visualizing structural changes caused by soil compaction and densification (Cosarinsky and Roces 2012; Halboth and Roces 2017). However, the micromorphology gradually changed during the nest growth (Cosarinsky et al. 2021).

The nest architecture of *Atta vollenweideri* Forel (Hymenoptera: Formicidae), with ventilation towers over the central openings improving the wind-induced circulation, differs from that of other *Atta* species (Cosarinsky and Roces 2012; Halboth and Roces 2017). These openings are used as entrances connecting the foraging trails and, at the top of the nest by conspicuous towers facilitating gas exchange between the interior of the nest and the environment (Kleineidam and Roces 2000; Kleineidam et al. 2001; Halboth and Roces 2017).

Modifications in the soil structure by leaf-cutting ants, when digging their nests, increase gas exchange and gas circulation within them (Bollazzi et al. 2012), which needs further studies. Do initial nests modify the soil matrix of the initial chamber and do these modifications improve gas exchange in them? The soil micromorphology of the wall of the initial chamber of the leaf-cutting ant *A. sexdens* and the CO_2 rate emitted by its initial nests were studied to answer these questions.

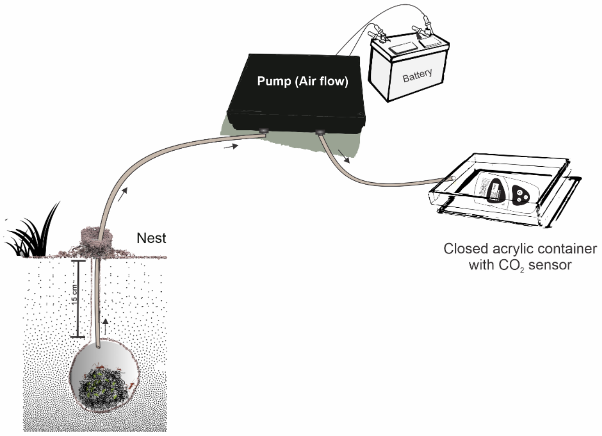

Fig. 1 Scheme of the respirometric system to measuring CO_2 in nests of *Atta sexdens* (Hymenoptera: Formicidae)

Methods and materials

Initial nests of *atta sexdens*

Initial nests of *A. sexdens*, four months old, were marked at Fazenda Santana, Botucatu, São Paulo, Brazil in 2018 (22°50'29.9"S 48°25'22.8"W).

Measuring the CO_2 level in the nests

A respirometric system (Fig. 1) with a Bacharach brand gas meter using a fixed probe (http://www.bacharach-32inc.com) was used to measure the CO_2 concentration in the initial *A. sexdens* nests. Ten consecutive readings of the CO_2 concentration in the respirometric container were taken to obtain an average. These measurements were performed by introducing a heat exchanger into the inlet orifice and the air removed by a peristaltic pump (Camargo et al. 2016).

The holes in the nests were sealed (obliterated) for 24 h, after measuring the CO_2 in them, and again opened when a new measurement was made. 5 cm deep holes in the ground, 15 and 60 cm away from the nest hole, were drilled. The CO_2 concentration was measured in these holes, which were also sealed for 24 h, after which new measurements were taken. We followed the methodology of our previous study (Sousa et al. 2021).

Soil micromorphology of the wall of initial chamber nests

Three undisturbed soil samples were collected, air-dried for 15 days and subsequently in an oven at 40 °C for 24 h. Then, they were impregnated with polyester resin diluted in styrene monomer and fluorescent pigment (Tynopal OB) to observation and to define the pore space using ultraviolet light (UV) (Murphy 1986). Soil thin Sect. (5 × 9 cm) were obtained from the impregnated and dried blocks and described (Bullock et al. 1985; Castro and Cooper 2019). Small areas (0.8 × 0.6 cm) in these thin sections were selected, photographed and processed in the Noesis Visilog program, determining the total porosity (sum of the pore type areas/sum of the total analyzed area), pore morphology (rounded, irregular, elongated) and pore size classes 39–3900; 3900–39,000; >390,000 µm (Cooper and Vidal-Torrado 2005). Pedological features of the longitudinal section of the walls of the leaf-cutting ant fungus chambers were obtained by micromorphological description (Table 1).

Statistical analysis

The best fit of the CO_2 concentration data to the model was obtained by the maximum likelihood estimation method of

Table 1 Microstructure (Mi.), coarse material (MG), fine material (MF), pores (Po.), relative distribution C/F (DR), fabric-b (Fb) and pedofeatures (Pe) of the soil and chamber wall (PC) of three nests of *Atta sexdens* (Hymenoptera: Formicidae) compared to *Atta vollenweideri* (Hymenoptera: Formicidae) (Cosarinsky and Roces 2007)

	PC nest 1	PC nest 2	PC nest 3	*Atta vollenweideri*
Mi.	Subangular blocks	Subangular blocks	Subangular blocks	Microporous and massive, intergrain cemented structures alternated with pellicular and intergrain microaggregated structures
MG	Quartz (99%); Charcoal fragments (1%)	Quartz (97%); Charcoal fragments (3%)	Quartz (98%); Charcoal fragments (2%)	Quartz, hematite e feldspar (50–70%)
MF	Clay, organic matter and iron oxides	Clay, organic matter and iron oxides	Clay, organic matter and iron oxides	Clay and iron oxides
Po.	Chambers, cavities, channels e fissures	Chambers, cavities, channels e fissures	Chambers, cavities, channels e fissures	-
DR	Porphyric	Porphyric	Porphyric	-
Fb	Speckled	Speckled	Speckled	-
Pe	Incomplete infillings; roots	Incomplete infillings; roots	Incomplete and complete infillings, roots and excrements	-

(Intercept)	X	(phi)
-4.431708	-0.036743	**0.03550**

Table 2 Total porosity (%) of the fungus chamber wall (Fungus Chamber) and the soil matrix (Soil matrix) adjacent to nests of *Atta sexdens* (Hymenoptera: Formicidae)

	Fungus chamber	Soil matrix
Nest 1	14.1	27.9
Nest 2	5.9	7.9
Nest 3	2.9	4.9

β and ϕ and its estimated coefficients related to the linear predictor in the transformed scale:

For example, the predicted expected value when $X = 1$ is:

$$g(E[Y|X = 1] = - 4.431708 - 0.36743*1 = - 4.468451$$

$$E[Y|X = 1] = \frac{e^{- 4.468451}}{1 + e^{- 4.468451}} = 0.0113351$$

The plyr, tidyverse, betareg, lmtest, emmeans, plotly, htmlwidgets and extrafont packages from the R free software environment for statistical computing and graphics version 4.0.4 were used.

Results

The soil fabric is defined by the total organization between the coarse, fine material and the pores (Bullock et al. 1985). The coarse material is composed of quartz and charcoal fragments varying in percentage of occurrence among the three studied nests, being 99, 97 and 98% for quartz in nests 1, 2 and 3, respectively; and 1, 3 and 2% for charcoal fragments in nests 1, 2 and 3. The fine material was characterized by the occurrence of clay minerals, organic matter and iron oxides inferred by matrix coloration. The relative distribution in the three nests is characterized as porphyric, with moderately developed subangular blocks microstructure and speckled b-fabric. Pedofeatures such as incomplete infillings and roots were found in nests 1 and 2, and complete and incomplete infillings, roots and excrements observed in nest 3 (Table 1).

The pores observed in the soil matrix and in the wall of the fungus chamber of *A. sexdens* nests were chambers, cavities, channels and fissures, with higher values in nest chamber 1 than in 2 and 3, these with predominance of elongated pores and irregular and presence of roots (Fig. 2d). The total soil porosity of the chambers walls of the initial ant nests was lower than the adjacent soil matrix (Table 2; Fig. 2a,b,c).

The total area occupied and the distribution of pores of the nest chambers, showed elongated and irregular pores in the soil matrix of the chambers of nests 2 and 3 and those of the nest 1 elongated and irregular (Fig. 3).

The significance of the negative coefficient estimate (β2= -0.036743, $pval < 2e\text{-}16$) indicated a decrease in CO_2 values with increasing distance from the nest chamber of the leaf-cutting ant *A. sexdens* nests (Fig. 4; Table 3) without effect of the 24-hour obliteration on this gas emission (β1= -0.057043, $pval = 0.737$) (Table 3).

Discussion

The soil fabric with coarse material composed of quartz and charcoal, and fine material composed of clay, organic matter and iron oxides, related to the relative distribution of the porphyric type with microstructure in subangular blocks in nests 1, 2 and 3 was similar to the one reported

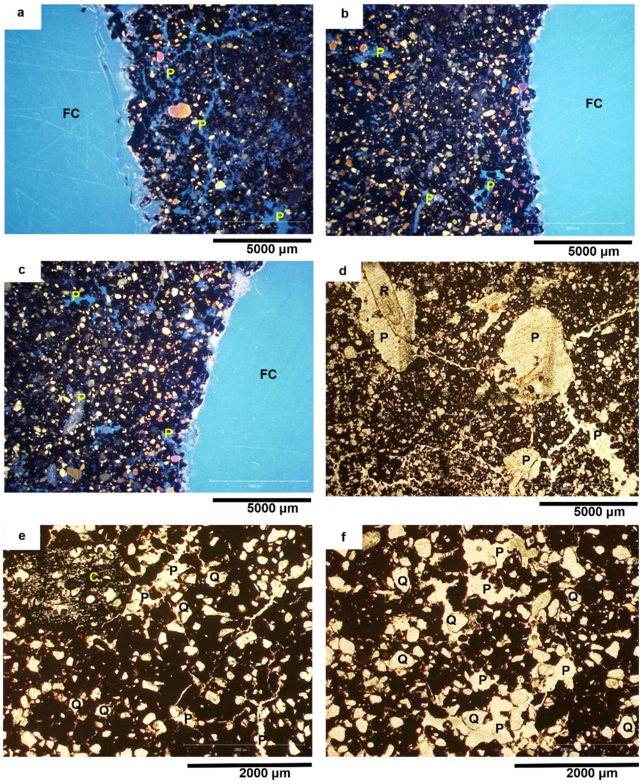

Fig. 2 Photomicrographs of the soils of the walls of the chambers of initial nests of *Atta sexdens* (Hymenoptera: Formicidae). (a) Boundary between the soil matrix and the nest chamber 1 (FC– fungus chamber) (XPL (cross polarized light) + UV (UV light) (b) do 2 (FC) (XPL + UV); (c) and 3 (FC) (XPL + UV); d, e, f). Detail of the dominant pore type (P; chambers and cavities) in the soil matrix of the chamber of nests 1, 2 and 3 (PPL (plane polarized light)); Q = quartz; P = pore; FC = fungus chamber; R = root; C = charcoal fragment. Figure 2a, b and c, blue colored pores, black/brown soil matrix and quartz grains as bright/yellow spots

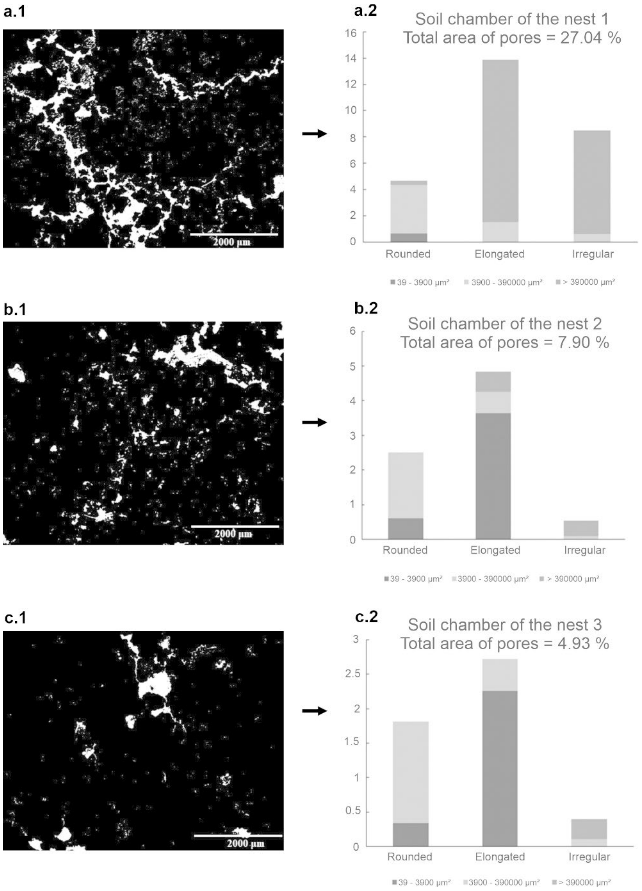

Fig. 3 Total porosity of the soil matrix of nests 1 (a), 2 (b) and 3 (c). (1) Binary images used in their analysis (pores with white color and the soil matrix in black); (2) Pore distribution according to their area and morphology

those of *Solenopsis invicta* Buren (Hymenoptera: Formicidae) and *A. vollenweideri* (Green et al. 1999; Cosarinsky 2006). The absence of coatings and the presence of serrated and irregular edges of the walls of the fungus chambers of nests 1, 2 and 3, together with their lower porosity, indicate an incipient compaction of the walls of the nest chambers when excavating the first fungus chambers, leading to a more compact structure, which stabilizes the chamber walls of the three nests of *A. sexdens* (Humphreys 1994; Castro and Cooper 2019). However, the soil micromorphology of the underground structure of the initial nest chamber of *A. sexdens* differs from that of adult nests of *A. vollenweideri* with cemented microporous and massive intergrain structures alternating with pellicular and intergrain microaggregate structures (Table 1) (Cosarinsky and Roces 2007). In *A. vollenweideri*, based on morphological data, their surrounding soil profile is a Typic Haplustalf (Alfisol), while in *A. sexdens* is oxisol. This difference in structure is correlated with the maturity of the nests, the older and more mature the nest the greater reworking of soil particles by the ants generating complex structures such as those observed by (Cosarinsky and Roces 2007). As the original ferralsol is medium

Table 3 Summary of estimates of model coefficients $CO_2 = f(\text{hour, dist})$ varying with predictors

		Estimate	Std. Error		Z	Pr(>|z|)
Coefficients (mean model with logit link):	(Intercept)	-4.431708	0.150195		-29.506	< 2e-16
	Fator (hora)	-0.057043	0.170072		-0.335	0.737
	Dist	-0.036743	0.003568		-10.297	< 2e-16
Phi coefficients (precision model with log link):	(Intercept)	5.90963	0.36929	16.003	< 2e-16	
	Dist	0.03550	0.01036	3.426	0.000612	

Log-likelihood: 142.8 on 5 Df Pseudo R-squared: 0.7817. Pr- probality

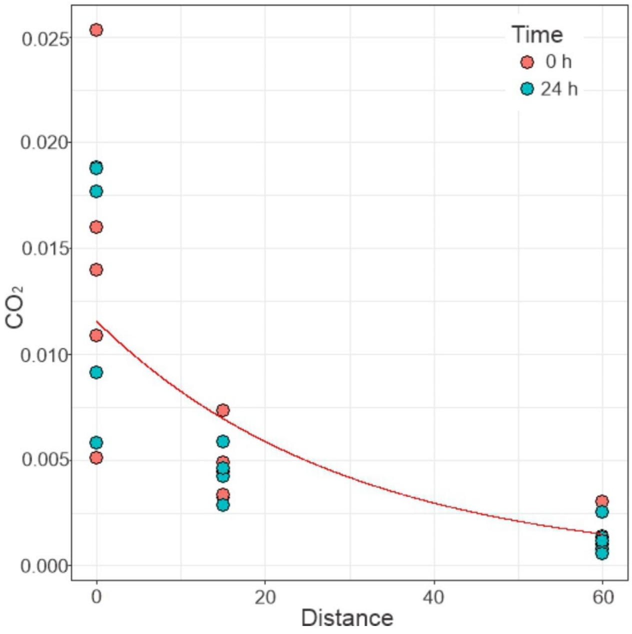

Fig. 4 CO_2 concentration in the chamber of the initial nests (0) of *Atta sexdens* (Hymenoptera: Formicidae) and at 15 and 60 cm away from it

texture (sandy loamy) quartz is the mineral with the highest percentage in nests of *A. sexdens* and *A. vollenweideri*, but charcoal fragments were reported for those of *A. sexdens* and hematite grains (nodules) and some feldspar in those of *A. vollenweideri* (Cosarinsky and Roces 2007) formed over a less wheathered and poorly drained Planosol. The abundance of quartz grains provides larger stacking porosity, facilitating the diffusion of CO_2 (Hillel 1998). Furthermore, clay particles and iron oxides (grains, coatings), were found in nests of these two species besides organic matter in the initial chamber wall of those of *A. sexdens* (Cosarinsky and Roces 2007). Micromorphological differences between *A. sexdens* and *A. vollenweideri* nests must be related to the nesting environments of these species, because, unlike *A. sexdens*, *A. vollenweideri* nests are often found in areas with flooded soil. Furthermore, as mentioned above, nest maturity can interfere with these micromorphological differences due to greater reworking of soil particles (Cosarinsky et al. 2021).

The greater porosity of the wall of the initial chamber of nest 1, of *A. sexdens*, than those of 2 and 3, is due to the differences during the nest excavation process by the workers, increasing them and the behavior to isolate the chamber with the matrix of adjacent soil (Cosarinsky and Roces 2007). The porosity of chambers in *A. sexdens* nests with very wide variation in pore area, from 39 μm^2 to 390,000 μm^2 differs from the microstructure of fungus chambers, garbage and tunnels in *A. vollenweideri* nests, very porous with equal or smaller voids than 100 µm (Cosarinsky and Roces 2007). This high variation in chamber wall porosity of *A. sexdens* nests may be mainly related to the fact that this ant initial nests are more superficial, approximately 15 cm deep and more exposed to roots (Fernandez-Bou et al. 2019). For this reason, differences in porosity between the wall of the nest chamber and the adjacent soil may be related to gas exchange in the nest, as they store CO_2 from the soil and because they are not totally isolated, this gas diffuses from the outside to the inside of the chamber and vice versa (Fernandez-Bou et al. 2019). The larger spaces and porosity of the nest chamber than the adjacent soil facilitates the CO_2 entry from the soil as reported for *A. vollemweideri*, with mounds of soil excavated during colony increase and reinforcement to stabilize structures with tower as ventilation structures (Halboth and Roces 2017) to underground chambers by passive mechanism driven by the wind (Kleineidam and Roces 2000; Kleineidam et al. 2001). Gas exchange in initial nests of *A. sexdens* should be by passive diffusion, similar to that of most terrestrial substrates (Evans 1966), as structures such as towers were not reported in their soil mounds.

The rounded and elongated pores of nests 2 and 3 and those elongated and irregular in nest 1 of *A. sexdens* are due to variations during the excavation process by the ant, increasing its initial chamber and also to isolate the chamber from the matrix of adjacent soil (Cosarinsky and Roces 2007). These underground structures, resulting from the excavation to form chambers (for fungus and residues) and the tunnels in *A. vollenweideri* nests, are simple excavations. Furthermore, the micromorphology of these structures does not differ from the adjacent soil, with a maximum frequency of coarse components in the soil pellets of 30% and 50% in other regions such as the Bt Horizon (20–200 cm excavation limit). This difference may result from later incorporation of clay into the soil removed or from the way the pellets were formed, as reported for the digging behavior of ants in a natural environment with original microstructure and unknown fine and coarse components (Cosarinsky and Roces 2007).

The significance of the negative coefficient estimate, indicating a decrease in CO_2 values with increasing distance from the nest chamber of the leaf-cutting ant *A. sexdens*, is due to the accumulation of this gas reducing its concentrations in the adjacent soil (Fernandez-Bou et al. 2019). Therefore, the lower concentration of this gas in more distant soils, even with similar depth, facilitates its penetration, by diffusion, into the initial nest chamber, reducing its concentration in the adjacent soil. The higher concentration of CO_2 in the initial chamber makes wind-induced ventilation mechanisms necessary (Kleineidam et al. 2001) as reported for gas exchange in adult *A. vollenweideri* nests. Three mathematical models of gas diffusion have been studied to explain it in terrestrial substrates, probably by passive diffusion (Evans 1966). These models used empirical data and the physical and behavioral attributes of fossorial animals in simple cylindrical underwater and underground burrows (a) and spherical nest chamber and cavities (b) in impermeable medium, besides spherical nest chamber and cylindrical cavities in permeable medium (c). Ectothermic animals, in impermeable burrows, cannot depend on diffusion to meet their metabolic CO_2 excretion needs, unless they if small in size (Withers 1978) and underground ectotherms may depend on gaseous diffusion, except when in large numbers (Withers 1978). Early nests of *A. sexdens* are much smaller with only one chamber, which possibly aids in gas exchange diffusion compared to adult ones with thousands of chambers.

Modifications in the soil matrix of the wall chamber of initial *A. sexdens* nests can help in the gas exchange between them and the adjacent soil by diffusion.

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Author Contribution R.S.C. and L.C.F. conceived the experiment(s), K.K.A.S., R.S.C., N.C., J.A.S. and A.P.F. conducted the experiment(s). All authors analysed the results and wrote the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

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