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Biomass production and leaf gas exchange of perennial legumes associated with bananas

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The search management for alternatives that reduce the use of mineral fertilizers is important for agricultural sustainability. Therefore, we sought to identify two legume species that are grown as cover crops with banana (*Musa spp*.) cultivar Prata-anã and spontaneous plant compositions that enable further reduction in the use of inputs. A randomized block design in a split plot arrangement with five replications was used. The plots were formed at four irrigation depths: 50, 75, 100 and 125% of crop evapotranspiration. Subplots were formed by three plant cover types associated with bananas: Tropical kudzu (*Pueraria phaseoloides* Benth.), calopo (*Calopogonium mucunoides* Desv.) and spontaneous vegetation, which primarily consist of *Panicum maximum* Jacq. The biomass production of the cover crops was measured 105, 200 and 400 days after planting (DAP); the total input of N was also measured (105 DAP). The measurements of leaf gas exchange and relative index of chlorophyll were recorded at 200 DAP. Tropical kudzu is better acclimated to a shaded environment, as demonstrated by evaluations of dry matter production, leaf gas exchange and the input of N. Thus, this crop may be a good alternative for supplying N to bananas irrigated under Brazilian semi-arid conditions.

Key words: Water management, *Musa* sp., cover crops, photosynthesis.

INTRODUCTION

High yields of banana crop are dependent on a high amount of mineral nutrients extracted from the soil, especially potassium and nitrogen (Nyombi et al., 2010; Wairegi and Asten, 2011). Nitrogen deficiencies cause a reduction in chlorophyll levels and significantly interfere in biological processes such as photosynthesis, ion absorption, respiration, cell multiplication, and differentiation (Liu et al., 2013; Peng et al., 2014).

However, the high application rate of nitrogen fertilizers, as recommended for bananas, increases

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production costs and the risk of groundwater contamination by nitrate leaching, especially if irrigation is performed beyond the requirements of crops or if high rainfall occurs (Zhu et al., 2005). Furthermore, fertilization with nitrogen can increase emissions of nitrous oxide (N_2O) (Ding et al., 2013) and reduce soil pH, which affects microbial activity (Hong-qi et al., 2013) and increases the availability of aluminum and micronutrients such as Mn and Fe.

Cover crops, such as types of legumes, can be an alternative to adding nitrogen for agronomic crops. These species have been successfully integrated into conservation agriculture and other systems in many parts of the world to provide surface coverage and improve soil fertility by fixing biological nitrogen. Some studies have shown that the use of cover crops increases the supply of N to subsequent crops (Campiglia et al., 2011; Kramberger et al., 2014), reduces impacts on soil microbiota (Ferreira et al., 2011), and ensures the suppression of weeds (Brennan et al., 2011; Flowera et al., 2012). However, successful management using cover crops greatly depends on the species used. In the case of fruit plants, such as banana, the choice should consider characteristics beyond the capacity of nitrogen fixation and dry matter production, including weed competition, shade tolerance imposed by the larger fruit bowl (Espindola et al., 2006b) and competition for nutrients and water.

Pueraria phaseoloides Benth. (tropical kudzu) and *Calopogonium mucunoides* Desv. (calopo) have developed very well in the semi-arid conditions of the Brazilian Northeast and are known to increase nitrogen and soil organic carbon and contribute other macronutrients such as K, P, Ca and Mg (Espindola et al., 2005; Teodoro et al., 2011; Xavier et al., 2013). Tropical kudzu is also effective in reducing the density and number of weed species and soil seed banks; in addition, it is resistant to shady conditions (Ekeleme et al., 2004; Rocha et al., 2007; Chikoye et al., 2008). Another advantage is that tropical kudzu and calopo are perennial herbaceous legumes. These legumes are best known as cover crops in areas of orchards because they allow periodic cuts after seeding, which circumvents the need to buy new seeds or seedlings in subsequent cycles of the main crop (Bryan et al., 2001).

Given the above information, the objective of this paper was to identify, among three types of cover crops that are associated with banana prata-anã (*Musa spp*.), which crop provides the most efficient use of water and nutrient resources in the production of bananas.

MATERIALS AND METHODS

This research was conducted between November 2010 and November 2011 at the experimental farm of the Vale do Curú, which belongs to the Federal University of Ceará in the municipality of Pentecoste, Ceara, Brazil (3°48'S, 39º19'W at an altitude of 47 m). The climate is semi-arid (BSw'h') according to Koppen's classification and is associated with erratic rainfall. The average annual rainfall is 797 mm, the potential evapotranspiration is 1847 mm, and the critical water deficit period extends from June to January.

The soil from the experiment area is classified as Fluvents (Soil Survey Staff, 2006) and has the following chemical characteristics: pH (water) = 7.1, EC = 0.36 dS m^{-1}, 0.75 g kg^{-1} of N, 24 mg kg^{-1} P, 13.3 g kg^{-1} OM; $cmol_c$ 12.95 kg CTC^{-1}, 92% of V, and 9.1, 2.2, 0.27, 0.99, 0.05, 0.38 $cmol_c$ kg^{-1} Ca^{2+}, Mg^{2+}, K^+, H^+ + Al^{3+}, Al^{3+} and Na^+, respectively.

The experimental design was a randomized block split plot with five replications. The plots were formed at four irrigation depths: 50, 75, 100 and 125% of the crop evapotranspiration (ETc). Subplots were formed by cover crops: tropical kudzu (*Pueraria phaseoloides* Benth.), calopo (*Calopogonium mucunoides* Desv.) and spontaneous vegetation. Each plot (12 × 40 m) consisted of four rows of banana cultivar Prata-anã planted in a single-row system with spacing of 3 × 2 m for a total of 80 plants. The subplots had dimensions of 12 × 10 m and contained twenty plants; the six central plants were used in the assessment for a total of eighty experimental units.

The perennial legumes of tropical kudzu and calopo were planted one month after transplanting the banana and were distributed in seven rows spaced 0.25 m apart between the lines of banana. Spontaneous vegetation is considered an entire set of plants that was naturally born in the selected subplots between the lines of banana, and *Panicum maximum* Jacq. grass was predominant.

The transplanting of the micropropagated banana plantlets was conducted in early November 2010 in pits with dimensions of 40 × 40 cm. At the foundation, 10 Mg ha^{-1} of cattle manure, 833 kg ha^{-1} of dolomitic limestone and 25 kg ha^{-1} FTEBR12 (micronutrients) were added. Macronutrients (NPK) were added in the form of urea, superphosphate and potassium chloride in three applications totaling 733, 150 and 770 kg ha^{-1} $year^{-1}$ of NPK, respectively. Note that in the subplots with cover crops, the total N applied to the foundation was only 32 kg ha^{-1} $year^{-1}$.

The management of irrigation in the plots, based on the daily data of reference evapotranspiration (ET0) and crop coefficients (Kc), was initiated at 54 days after planting (DAP) and stopped at 71 DAP due to the onset of rain, which prevented the differentiation of the water depths. This management was reinstated at 245 DAP during the intense period of banana fruiting and continued until the end of the 1st cycle. The sum of each water depth applied during both periods was L1 = 390.7 mm, L2 = 586.1 mm, L3 = 781.5 mm, and L4 = 976.9 mm. However, due to the high total rainfall in 2011 (1095.9 mm), the total water in the first cycle was much higher (L1 = 1486.6 mm, L2 = 1682.0 mm, L3 = 1877.4 mm, and L4 = 2072.8 mm).

To determine the biomass of legumes and spontaneous vegetation, samples were taken in a 0.25 m^2 area of each subplot with the aid of a wooden frame (Armecin et al., 2005) at 105, 200 and 400 DAP. The fresh biomass was determined immediately after the cut, and the samples were then dried in an oven with forced air circulation at 65°C for further dry biomass determination.

Part of the dry biomass obtained at 105 DAP was used in determining the N concentration of the plants based on the methodology described by Kjeldahl. The input of N was estimated by multiplying the N concentration by the dry matter production of the cuts made at 105 and 200 DAP.

Measurements of the CO_2 assimilation rate (A) (µmol $m^{-2}s^{-1}$), stomata conductance (gs) (mol $m^{-2}s^{-1}$) and transpiration (E) (mmol $m^{-2}s^{-1}$) were recorded at 200 DAP in fully expanded leaves of the tropical kudzu and calopo using an infrared gas analyzer (IRGA model LI-6400XT, Licor) in an open system with an air flow of 300 ml min^{-1} under ambient temperature and CO_2 values. The readings were taken between 10 and 12 h using natural and artificial sources of radiation with an intensity of 1800 µmol $m^{-2}s^{-1}$. The average radiation measurements with the natural light did not exceed 140

Table 1. Production of fresh and dry weight of plants for roofs in consortium with banana cv Prata Anã, at 105, 200 and 400 days after planting (DAP).

Treatments	Fresh mass	Dry mass
	Mg ha^{-1}	
	105 DAP	
Calopo	21.99[a†]	4.13[ab]
Tropical kudzu	22.28[a]	3.93[b]
Spontaneous vegetation	23.62[a]	4.78[a]
200 DAP		
Calopo	4.26[c]	1.55[c]
Tropical kudzu	6.26[b]	2.01[b]
Spontaneous vegetation	12.06[a]	3.09[a]
400 DAP		
Calopo	10.66[a]	2.62[a]
Tropical kudzu	11.74[a]	2.75[a]
Spontaneous vegetation	11.26[a]	2.81[a]

[†]Means followed by the same letter in the column do not differ by Tukey test at 5% probability.

µmol m^{-2}s^{-1} due to shading by the banana plants. The data of leaf gas exchange determined the instantaneous (A/E) and intrinsic (A/gs) water use efficiency.

The relative chlorophyll index was measured in the same leaves used for determining the leaf gas exchange by using a portable chlorophyll meter SPAD-502 (Minolta Camera Co. Ltd.). Measurements occurred between 9 and 11 h on the adaxial and median parts of the leaf blade. The average of five readings of two plants on each subplot was used as a replication.

The data for each evaluated characteristic were analyzed using analysis of variance. If the values were significant based on the F test, then they were subjected to Tukey's test and regression analysis to assess the effects of cover crops and irrigation depths, respectively.

RESULTS

Biomass of legumes and spontaneous vegetation

The water depth did not affect the biomass production, but the cover crops showed significant differences at 105 and 200 DAP. In general, the highest dry matter was produced by spontaneous vegetation (Table 1). Among the legumes, there were no differences in plant dry matter in the first cut (105 DAP); however, the tropical kudzu reached a higher value than calopo at 200 DAP. In the third cut, no differences were observed in the fresh and dry masses between the cover crops.

Content and nitrogen input of perennial legumes and spontaneous vegetation

The concentration of N increased linearly with the increase in the irrigation depth (Figure 1). However, the cover crops showed different results at 105 and 200 DAP (Table 2). In these cuts, the legumes presented an N concentration that was significantly higher than that of the spontaneous vegetation, which was attributed to the ability of legume plants to fix atmospheric N_2 through symbiosis with diazotrophic bacteria (Espindola et al., 2006b; Tueche and Hauser, 2011).

The input of N differed between the first and second cuts, and the superiority of the tropical kudzu was observed at 200 DAP. At the end of the first cycle of the bananas, the supply of N was observed to decrease in the following order: tropical kudzu > calopo > spontaneous vegetation (Table 2).

Leaf gas exchange and chlorophyll index of perennial legumes

The legume cover, radiation, and interaction of cover crops with radiation affected leaf gas exchange and water use efficiency. The calopo exhibited significantly higher values than the tropical kudzu for all traits (Table 3). For both species, under saturating light (1800 µmol m^{-2}s^{-1}), the values of all variables were higher than those observed under low levels of natural radiation.

The interaction of cover crops with radiation was significant for A and gs (Figure 2). Under a high intensity of radiation, higher values of A and gs in the leaves of calopo were observed. However, under a low level of radiation, the rates of photosynthesis were similar for the two cover crops, which resulted in higher intrinsic water efficiency for kudzu under natural radiation conditions.

The relative index of chlorophyll was only affected by cover crops, and it was higher in the leaves of calopo than in those of tropical kudzu (Figure 3).

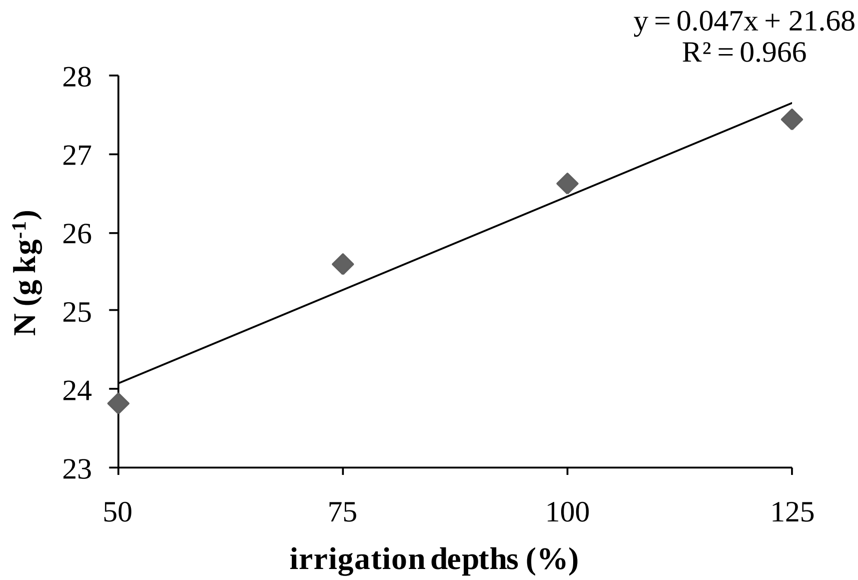

Figure 1. Nitrogen (N) content of cover plants associated with banana prata anã as a function of irrigation depth.

Table 2. Content of N, N input of the first and second harvest and total N contributed in treatments with plants associated with banana cv Prata Anã.

Treatments	N	Contribution of N		
		1º trench	2º trench	Total
	g kg^{-1}		kg ha^{-1}	
Calopo	28.40[a†]	119.61[a]	45.22[b]	164.83[ab]
Tropical kudzu	30.80[a]	124.35[a]	61.57[a]	185.92[a]
Spontaneous vegetation	18.39[c]	89.68[b]	59.02[a]	148.70[b]

[†]Means followed by the same letter between species do not differ by Tukey test at 5% probability.

Table 3. Comparison of photosynthesis (A), stomatal conductance (gs), transpiration (E), intrinsic water uses efficiency (EUAi) and momentary efficiency of water use (EUAm) between legumes calopo and tropical kudzu and among two different intensities light.

Species	A	gs	E	EUAi	EUAm
	μmol m^{-2}s^{-1}	mol m^{-2}s^{-1}	mmol m^{-2}s^{-2}		
Calopo	10.775[a†]	0.320[a]	7.325[a]	31.578[b]	1.402[b]
Tropical kudzu	8.707[b]	0.214[b]	5.276[b]	41.923[a]	1.639[a]
Radiation					
Artificial light [(2)]	14.331[a]	0.337[a]	7.194[a]	44.740[a]	2.024[a]
Natural light[(3)]	5.150[b]	0.197[b]	5.406[b]	28.761[b]	1.017[b]

[†]Means followed by the same letter between species do not differ by Tukey test at 5% probability ; [(2)]Artificial light radiation 1800 μmol m^{-2}s^{-1}; [(3)]Natural light recorded at the time of the measurements, which average values were 140 μmol m^{-2} s^{-1}, this period was observed a high degree of shading banana on legumes.

DISCUSSION

Dry mass production of legumes in this study is consistent with the data observed by Armecin et al., (2005), who found a productivity of 3 to 5 Mg ha^{-1} in calopo plants growing in consortium with abaca (*Musa textilis* Née). For tropical kudzu, Perin et al. (2004) found the productivity equal to 3.28 Mg ha^{-1} in the first cut for a

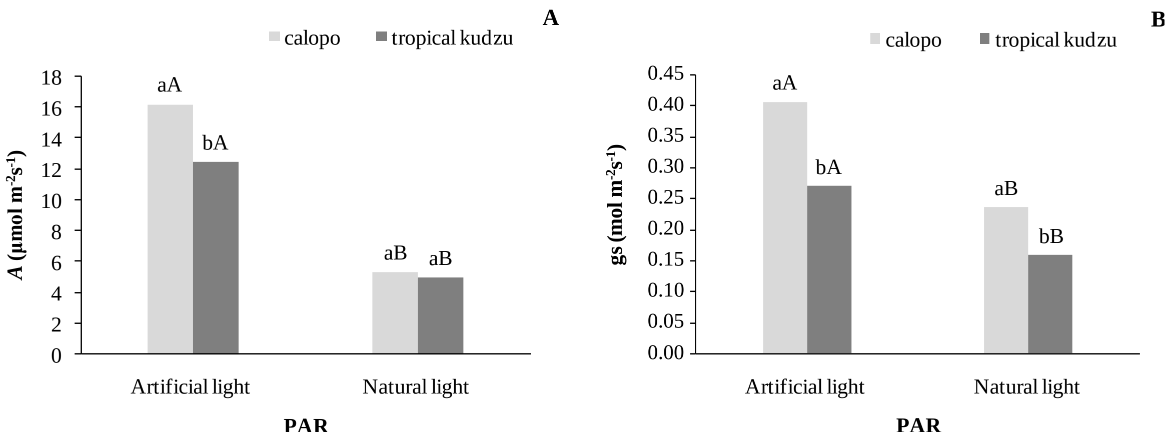

Figure 2. Photosynthesis - *A* (A) and stomatal conductance - gs (B) of calopo and tropical kudzu as a function of photosynthetically active radiation (PAR). Mean values followed by the same letter for each PAR and a capital letter between different PAR values do not differ based on the Tukey test at a 5% probability.

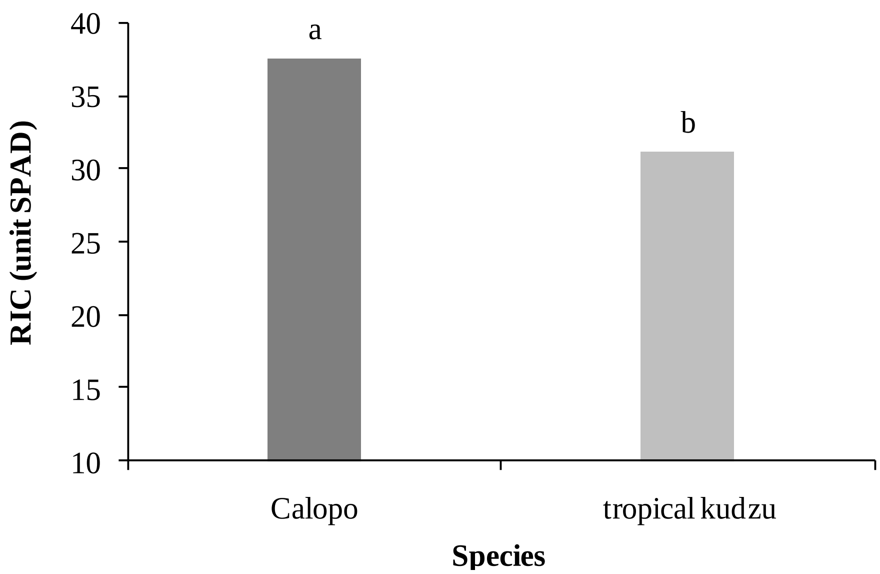

Figure 3. Relative index of chlorophyll (RIC) in leaves of calopo and tropical kudzu legumes 200 days after planting (DAP). Mean values followed by the same letter in the column and a capital letter between columns do not differ based on the Tukey test at a 5% probability.

density of 20 plants per meter, which is similar to the results found in the current study. Higher yields were reported by Espindola et al. (2006a) and Kaho et al. (2009): up to 5.0 and 5.87 Mg ha^{-1}, respectively.

There were significant differences in the dry mass production among harvests, which is most likely a consequence of the changes in the level of radiation and the restoration capacity of each cover species. For example, there was a clear difference in the dry mass production of cover crops at 200 DAP, and the values were consistently lower than those observed at the first cut. This difference may be attributed to the shadows from the bananas at the time of the second cut; at the first cutting, the height of the main crop was approximately 1 m, whereas at the second cutting, the height was greater than 2.3 m, which results in a reduction of radiation. The negative effect of the radiation reduction was more pronounced for calopo, which suggests a low capacity of this species to grow under low radiation conditions.

According to Rocha et al. (2007), calopo needs a greater amount of light (≈70%) to achieve a higher dry matter production, whereas tropical kudzu needs only 50% light to reach its maximum production. These results show that calopo has the lowest tolerance to shade, and they explain the better performance of tropical kudzu.

The dry mass of calopo at 400 DAP suggests the species required a higher level of brightness: after the second legume cut occurred, the banana harvest ensured greater light penetration between the rows of banana, which possibly contributed to the increased calopo dry mass of this section compared with the previous cut.

In case of spontaneous vegetation, Santos et al. (2003) found fresh and dry masses of 27.30 Mg ha^{-1} and 5.63 Mg ha^{-1}, respectively, for *Panicum maximum* Jacq. cv Mombasa and 25.03 Mg ha^{-1} and 5.23 Mg ha^{-1}, respectively, for cv Tanzania, which are consistent with those obtained in the present work. All studies on the shade tolerance of cultivars of *Panicum maximum* Jacq. indicate good tolerance and a high productive capacity, which is a relevant fact when considering the management of these plants.

By analyzing the content of N in cover crops, Espindola et al. (2006b) found higher concentrations of N in tropical kudzu, siratro (*Macroptilium atropurpureum* Urb.) and forage peanut (*Arachis glabrata* Benth.) compared to vegetation formed by guinea grass, which is a variety of *Panicum maximum* Jacq. and was predominant among the weeds in the present study. Teodoro et al. (2011) found concentrations of N equal to 25.55 g kg^{-1} in calopo, and Perin et al. (2004) found levels equal to 25 and 31.19 g kg^{-1} in the first and second cuts of tropical kudzu, respectively; these findings are consistent with the values obtained for kudzu in this study. Kaho et al. (2009) also reported that after twelve months, tropical kudzu accumulated a total of 124.03 Mg ha^{-1} of biomass.

The legumes experienced a higher input of N than the spontaneous vegetation. This result highlights the importance of the N concentration and the amount of dry matter produced by each species to comprise the total intake of the nutrient. For example, calopo's lowest input of N in the 2^{nd} cut was a result of the reduced production of dry mass. Espindola et al. (2006b) obtained total N inputs of 375 and 132 kg ha^{-1} for the first three cuts of tropical kudzu and spontaneous vegetation, respectively. Perin et al. (2004) achieved N inputs of 75.66 and 116.32 kg ha^{-1} in the first and second cuts of tropical kudzu, respectively, to yield a total of 191.98 kg ha^{-1} N, which is close to the value observed in this study.

Referring to Figure 1, a linear increase in the N content of the cover crops occurred when the irrigation depth increased. These results may indicate that rather than a greater loss of N in higher water depth, the presence of cover crops favors the absorption of this nutrient, possibly due to the deep root system and more favorable absorption of N by the mass flow.

By comparing the two species, it was observed that under high radiation conditions, tropical kudzu maintained a lower photosynthetic rate than did calopo. This result may be related to the high chlorophyll index of calopo and to the adaptation of the first species to shade, as shown by Rocha et al. (2007). In general, plants adapted to shade have lower photosynthetic rates (Lei et al., 2014) due to saturation at low levels of irradiance. In addition, the larger leaves of the tropical kudzu in relation to the calopo may increase the air resistance of the sheet boundary layer, which reduces stomata conductance and consequently reduces the exchange of gases (Stokes et al., 2006; Defraeye et al., 2013).

Under low-intensity radiation, photosynthesis rate was low and similar for calopo and tropical kudzu (Figure 1A). However, tropical kudzu appears to control g_s more efficiently, which ensures greater water use efficiency. This fact explains the higher dry matter production of this species compared to calopo (Table 1) at 200 DAP, which was a period of higher shading of the legumes and consequently reduced the availability of radiation. These results corroborate the statement that the satisfactory growth of some species in environments with different rates of photosynthetically active radiation can be attributed to their ability to quickly adjust and effectively use their photosynthetic apparatus to maximize resource acquisition (Zanella et al., 2006).

Conclusion

Tropical kudzu is better acclimated to a shaded environment, as demonstrated by evaluations of dry matter production, leaf gas exchange and the input of N. Thus, this crop may be of a good alternative for supplying N to bananas irrigated under Brazilian semi-arid conditions.

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Conflict of interests

The authors have not declared any conflict of interest.

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