




# Initial growth of *Coffea canephora*: Simulation of organomineral system and *Limnoperna fortunei* as soil improvement

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## ABSTRACT

The use of residues can be an alternative to mineral fertilization. Manures, sources of nutrients and organic matter, are successfully used in family farming. Another residue that can be used for soil acidity amelioration is the shell of the golden mussel (*Limnoperna fortunei*), which is rich in CaCO<sub>3</sub>. This work evaluated the contribution of ground golden mussel shells (GMS) in combination with cattle (CTM) and sheep (SHM) manure to the initial growth of Conilon coffee in an acidic sandy soil. The experiment was carried out in protected cultivation in pots (30 kg) with two Conilon coffee cultivars. The treatments consisted of two doses of CTM (0 or 67 t ha<sup>-1</sup>) and four doses of GMS (0, 1, 2 and 3 t ha<sup>-1</sup>), with three replications. Each treatment was incorporated into the soil before the introduction of the seedlings in the experimental units, and 60 days later, a dose of SHM was applied on the soil surface (20 t ha<sup>-1</sup>). After 180 days, the soil fertility was analysed, and the seedling height, stem diameter, root volume and fresh and dry mass of the aerial parts and roots were measured. Furthermore, the Dickson Quality Index (DQI) was calculated. Both the sheep and the bovine manure increased the pH, the contents of P, MO, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>, and the cations exchange capacity (CEC) of the soil. The addition of GMS increased the pH and the Ca<sup>2+</sup> content while reducing the potential acidity of the soil. A pH above 6.0 reduced the levels of micronutrients in the soil. The use of CTM and SHM shows promise in the initial growth of coffee seedlings (height - H, FMAP, fresh and dry mass of the aerial parts - DMAP, FAR, fresh and dry mass of the root system - DMR diameter, RV root volume and Dickson Quality Index). Ipiranga 501 surpassed Verdebras G35 in aerial fresh and dry mass, root volume and IQD, with or without the addition of 3 t ha<sup>-1</sup> of ground GMS.

**Key-words:** Cattle manure; Sheep manure; Golden mussel; Robusta coffee; Conilon coffee.

## 1 INTRODUCTION

Coffee is one of the most widely consumed beverages in the world, and it is among the most cultivated crops in Brazil, which makes it one of the world's largest coffee exporters. As of January 2021, its productivity was 2,460 kg ha<sup>-1</sup> (41 bags) for *Coffea canephora* and 1,440 kg ha<sup>-1</sup> /24 bags) for *Coffea arabica*; because these coffees are in positive bienniality, the year in which production is higher is represented here (Companhia Nacional de Abastecimento - CONAB, 2021).

Awareness of environmental issues has caused coffee growers to search for techniques aimed at sustainable agriculture, that is, with positive and simultaneous financial and environmental responses (Amorim; Oliveira, 2022), reducing environmental damage and ensuring the continuous supply of the product. Among the measures discussed is the use of no-tillage systems and the management of organic matter and the use of waste that helps in the recycling of nutrients (Amaral et al., 2012).

According to Matiello et al. (2005), coffee has a high nutritional demand (120 to 479 kg ha<sup>-1</sup> for N, 120 to 400 kg ha<sup>-1</sup> for K<sub>2</sub>O and 15 to 80 kg ha<sup>-1</sup> for P<sub>2</sub>O<sub>5</sub>), and its production in sandy soil is a challenge, arising from the chemical aspects of the soil (Macedo et al., 1998) and reduced moisture retention (Cordeiro et al., 2020).

Sandy soils usually have a reduced organic matter content, possibly as low as approximately 2% in some situations (Brady; Weil, 2013; Balbinot Junior et al., 2016), along with low moisture retention and cation exchange capacity (CEC) (Costa et al., 2020) due to the small amount of clay and the predominance of quartz, a mineral that does not contribute to soil CEC or to moisture retention, in the sand fraction (Brady; Weil, 2013). As a consequence of low CEC, the retention of mobile nutrients is low (Cordeiro et al., 2020; Salviano et al., 2016), which leads to leaching (Costa et al., 2020).

Therefore, coffee production in sandy soils demands more attention to management, and such attention should be directed towards introducing and maintaining organic matter/organic carbon in the soil system and searching for economic, environmental and social benefits (Cordeiro et al., 2015; Kluthcouski, Cordeiro; Marchão, 2015; Salton et al., 2015).

Organic farming allows the use of organic compounds and manure as a source of nutrients and organic matter, in addition to other alternative and natural materials (vegetable and animal waste, compost, humus, vermicompost) that can contribute to improving the soil chemical condition (Moura; Lopes; Silva, 2019).

To be used in organic production, the selected material must be of animal or vegetable origin and have several main functions: providing nutrients to plants, association with soil

correction, the introduction of organic matter, and improving nutrient cycling, among others (Mahmood et al., 2017).

The use of manure as fertilizer in this system is a possibility, as manure is organic and rich in nutrients such as nitrogen (N) and phosphorus (P) (Nascimento et al., 2020). Some manures have a C/N ratio favourable to seedling establishment, that is, a low C/N ratio (less than 20), leading to quick nutrient release (Moreira; Siqueira, 2007), in addition to its use as residue. As Brazil is a major producer of dairy and beef cattle (Matos et al., 2017) and a growing sheep cattle producer (Costa et al., 2019), the waste produced is easily accessible.

Golden mussel shells (*Limnoperna fortunei* Dunker, 1857), rich in calcium carbonate, can also be a low-cost alternative in soil correction (Maltoni et al., 2020); this mussel is an invasive species that has spread rapidly in Brazilian waters (Morton, 2015), causing economic and environmental problems because it adheres to water catchment surfaces, public water supply pipes, fish-cage meshes, ship hulls, and hydroelectric equipment, which compromise the maintenance and generation of electrical energy (Wachholz et al., 2017).

Golden mussel shells can add calcium carbonate to the soil and promote soil acidity correction, in addition to adding calcium (Ca), while manure contributes to improving the chemical conditions and introducing organic matter into the soil. It is thus assumed that the combined addition of these elements (manure and mussels) to sandy soils can improve their chemical conditions and allow the sustainable cultivation of coffee trees.

Within this context, the objective of this work was to evaluate the contribution of cattle and sheep manure, in combination with golden mussel shells, to the initial growth of coffee seedlings, simulating an organic system in acidic and sandy soil.

## 2 MATERIAL AND METHODS

The research began in June 2019 in a greenhouse at the University of Engineering, UNESP-Campus of Ilha Solteira (SP), where the average annual temperature is 25.5 °C (São Paulo State University - UNESP, 2020).

The experiments were carried out in pots (25 L, height 0,32 m, top 0,37 and base diameter 0,37 and 0,32 m, respectively containing 30 kg of soil with a sandy texture (sand = 883 g kg<sup>-1</sup>, silt = 17 g kg<sup>-1</sup>, clay = 100 g kg<sup>-1</sup>) as determined by the pipette method, according to Donagemma et al. (2017).

Soil was collected in the municipality of Selvíria (MS) at the Teaching, Research and Extension Farm (FEPE) of the São Paulo State University (Unesp), School of Engineering, Ilha Solteira at a depth of 0.0 to 0.40 m. The soil was analysed for fertility according to the methodology of Raj et al. (2001). The soil was acidic and had low fertility, typical of a Typic Haplustox, which is common throughout the region (Table 1).

The established treatments were arranged in a completely randomized design, with three replications; organic fertilization was evaluated (with and without cattle manure at planting, incorporated on the day of transplanting, and with sheep manure added as a topdressing 60 days after transplanting) for two Conilon coffee varieties (*Coffea canephora* cv. Verdebras G35 and *C. canephora* cv. Robusta, Ipiranga 501), and four doses (0, 1, 2 and 3 t ha<sup>-1</sup>) of ground golden mussel shells (GMS) as a substitute for liming (Table 2) were incorporated on the day of transplanting.

Experimental units (pots) were prepared, and the seedlings were transplanted immediately. To avoid any possible loss of soil, the bottoms of the pots were covered with filter paper (quick filtering), allowing the excess water to drain away (Figure 1). After implementation, the experimental units received two manual daily irrigations, avoiding waterlogging.

The coffee cultivars selected were clone G35 (Conilon variety), with lower productivity, lower vigour and production, and relatively late maturation (Partelli et al., 2014) compared to the Robusta coffee variety, and the clone 501 (Robusta variety), also known as Ipiranga 501, which presents high vigour, super-late maturation and a nutrient-demanding genotype due to intense vegetative branch growth. The first measurement was taken 30 days after implantation, so that the plants could stabilize due to the stress suffered (Figure 2).

**Table 1:** Initial analysis of soil chemical attributes.

P mg dm <sup>-3</sup>	OM g dm <sup>-3</sup>	pH CaCl <sub>2</sub>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	H+Al mmol <sub>c</sub> dm <sup>-3</sup>	Al <sup>3+</sup>	SB	CEC
1	10	4.4	0.4	2	2	16.3	2	4.4	20.7
V %	m %	B %	Cu mg dm <sup>-3</sup>	Fe %	Mn %	Zn %			
21.7	31	0.63	0.6	13.7	7.6	1.17			

P = phosphorus, OM = organic matter, pH = soil reaction, K<sup>+</sup> = potassium, Ca<sup>2+</sup> = calcium, Mg<sup>2+</sup> = magnesium, H+Al = potential acidity, Al<sup>3+</sup> = aluminium, SB = sum of bases, CEC = cation exchange capacity, V% = bases saturation, m% = aluminium saturation, B = boron, Cu = copper, Fe = iron, Mn = manganese, Zn = zinc.

**Table 2:** Description of treatments (Treat) with cattle manure (CTM) and ground golden mussel shells (GMS) applied to sandy soil.

Treat	CTM (t ha <sup>-1</sup> )	GMS	SHM*
1	67	0	20
2	67	1	20
3	67	2	20
4	67	3	20
5	00	0	20
6	00	1	20
7	00	2	20
8	00	3	20

\* Sheep manure applied as a topdressing 60 days after the introduction of seedlings to the treatments.

Seedlings donated by the Eldorado Coffee Nursery, municipality of Adamantina (Figure 1), were transplanted into pots in June 2019.

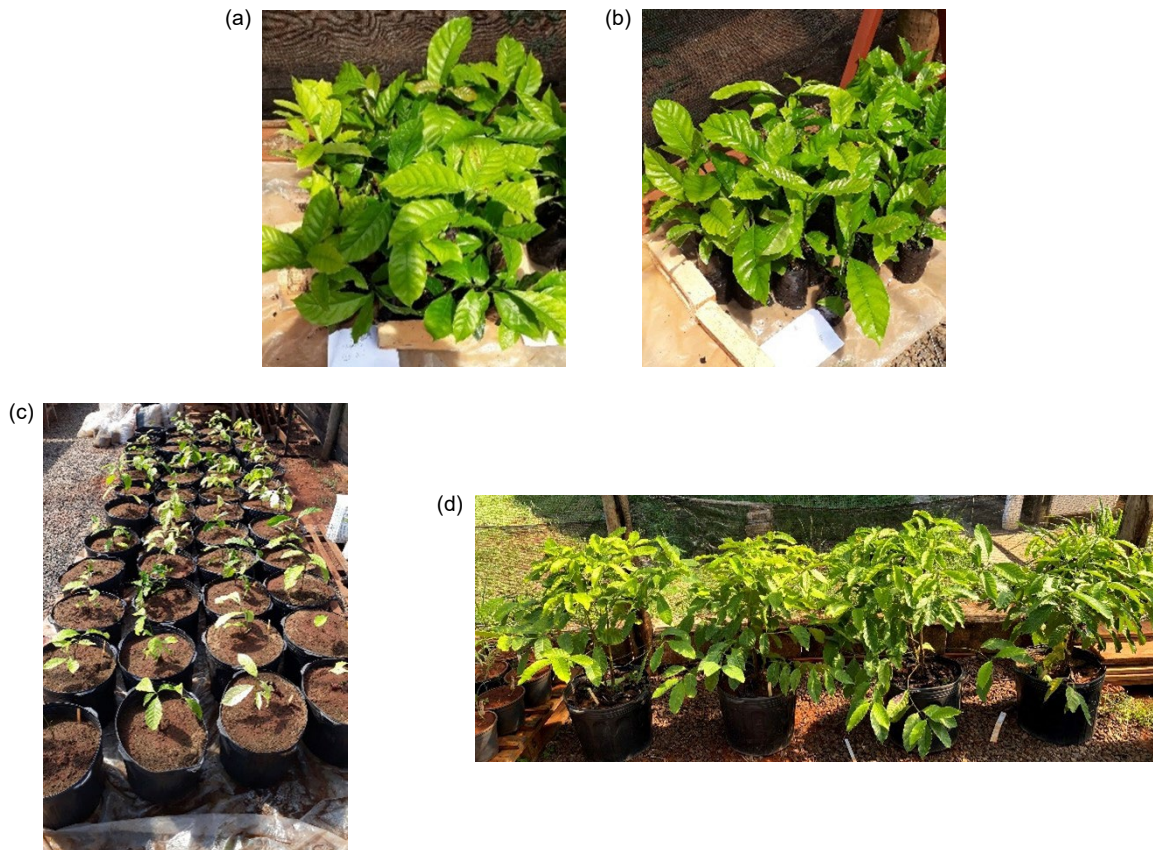
The golden mussel (*Limnoperna fortunei* Dunker, 1857) shells used were collected in net tanks located in the

São José dos Dourados River (SP). The shells were removed from the meshes of the tanks, then air-dried in a covered area. After 120 days, only the shells remained, which were ground (Wiley-type mill), passed through a 0.25 mm sieve, chemically analysed (Table 3) and used as an alternative to liming in doses equivalent to 0, 1, 2 and 3 t ha<sup>-1</sup>. The manure used (cattle and sheep) was obtained at FEPE - UNESP and chemically analysed (Table 4) following the methodology of Malavolta, Vitti and Oliveira (1997).

Cultivar growth was evaluated for all plants. Height was measured with a millimetre ruler in the region between the apical bud and the cervix. The diameter of the stem was measured with a measuring tape 10 cm above the collar.

The cultivars were evaluated 180 days after transplanting for shoot height (H) and stem diameter (SD) and collected to determine shoot and root system fresh and dry mass (Carneiro, 1995) and root system volume in cm<sup>3</sup>, determined by the displacement of water in a graduated container (Zenzen et al., 2007).

The Dickson Quality Index (DQI) (Dickson; Leaf; Hosner, 1960) was also calculated as a function of H, the SD, the dry mass of the aerial parts (DMAP), the dry mass of the root system (DMR) and the total dry mass (TDM), using Equation (1).



**Figure 1:** Coffee seedlings of *Coffea canephora* cv. Verdebras G35 (a) and cv. Robusta, Ipiranga 501 super-late (b), introduced to the treatments, at beginning of the experiment (c) and at the end of the experiment (d).

Source: Author

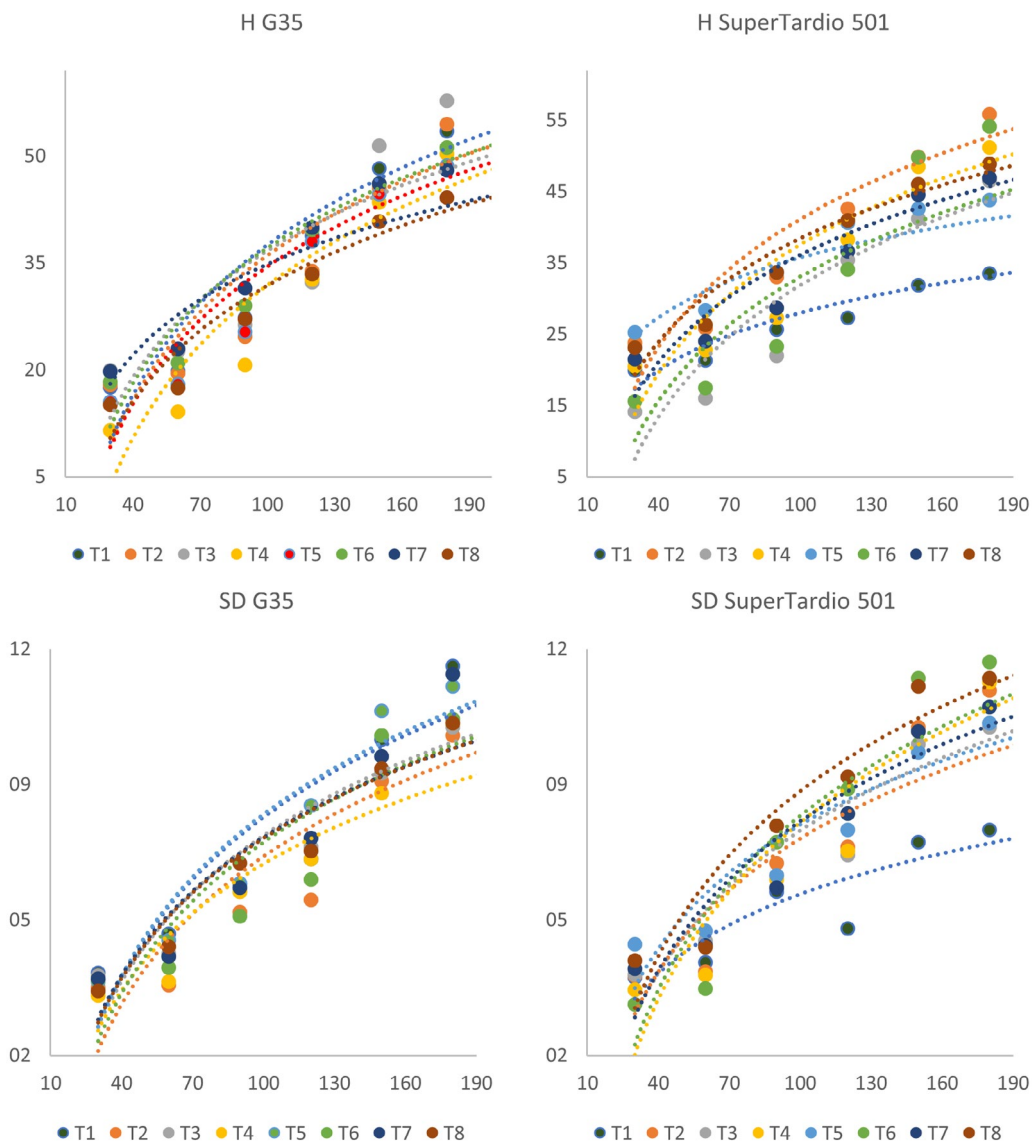


Figure 2: Trend line of the 180 days of experiment.

$$DQI = \frac{TDM}{\frac{H}{SD} + \frac{DMAP}{DMR}} \quad (1)$$

The data obtained were tested for normality by the Shapiro and Wilk (1965). Once the assumptions were met, analysis of variance (ANOVA) was performed, applying the F test at 5% probability to determine differences among all treatments. When a significant difference was found for two different GMS doses, regression analysis of variance was performed. Statistical analyses were performed using SISVAR software (Ferreira, 2019).

### 3 RESULTS

The addition of manure (cattle (CTM) and sheep (SHM)) in combination with ground golden mussel shells

(GMS) altered some soil chemical properties (Tables 5, 6 and 7) compared to initial conditions (Table 1).

The addition of SHM, CTM and GMS increased the contents of P, OM, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Zn, CEC and pH and reduced the contents of H+Al, Al<sup>3+</sup>, Mn and B, while Fe and Cu remained at concentrations close to the initial soil conditions (Table 1).

However, notably, the addition of SHM increased the contents of P (6,460%), OM (28%), K<sup>+</sup> (575%), Ca<sup>2+</sup> (1,915%), Mg<sup>2+</sup> (510%), and CEC (268%) and reduced the potential acidity (45%), Al<sup>3+</sup> (100%), B (87%), Cu (25%), Fe (34%), Mn (44%) and Zn (17%), relative to initial soil conditions.

CTM increased the concentrations of P (4020%), OM (24%), Ca<sup>2+</sup> (235%), Mg<sup>2+</sup> (140%), B (94%), Cu (130%), Fe (71%), Mn (83%) and Zn (17%) and reduced H+Al (12%) compared to the addition of SHM alone; furthermore, H+Al decreased linearly with increasing GMS.



**Table 3:** Physicochemical analyses of the golden mussel shells.

Parameter	Units	Result	Parameter	Units	Result
pH (water 1:10)	--	7.7	B	mg B kg <sup>-1</sup>	<3.2 <sup>(2)</sup>
Moisture	% (m m <sup>-1</sup> )	0.9	Cd	mg Cd kg <sup>-1</sup>	<0.4 <sup>(2)</sup>
Total solids	% (m m <sup>-1</sup> )	98.9	Ca	g Ca kg <sup>-1</sup>	297
Volatile Solids	% (m m <sup>-1</sup> )	16.6	Pb	mg Pb kg <sup>-1</sup>	3.3
Organic C	g C kg <sup>-1</sup>	71.9	Cu	mg Cu kg <sup>-1</sup>	10.7
Kjeldahl N	g N kg <sup>-1</sup>	23.0	Cr	mg Cr kg <sup>-1</sup>	3.2
Ammoniacal N	mg N kg <sup>-1</sup>	84.5	S	g S/kg <sup>-1</sup>	2.1
Nitrate-Nitrite N	mg N kg <sup>-1</sup>	42.8	Fe	mg Fe kg <sup>-1</sup>	1360
Ba	mg Ba kg <sup>-1</sup>	140	P	g P kg <sup>-1</sup>	1.6
Na	mg Na kg <sup>-1</sup>	1822	Mn	g Mg kg <sup>-1</sup>	0.46
K	mg K kg <sup>-1</sup>	551	Mg	mg Mn kg <sup>-1</sup>	140
Ar	mg As kg <sup>-1</sup>	15.9	Mo	mg Mo kg <sup>-1</sup>	1.4
Se	mg Se kg <sup>-1</sup>	5.3	Ni	mg Ni kg <sup>-1</sup>	<2.4 <sup>(2)</sup>
Hg	mg Hg kg <sup>-1</sup>	<1.0 <sup>(2)</sup>	Zn	mg Zn kg <sup>-1</sup>	22.4
Al	mg Al kg <sup>-1</sup>	508			

Analysis was performed at the Instituto Agronômico de Campinas (IAC) based on the EPA-SW-846-3051a method, with determination by ICP-AES, according to EPA-SW-846-6010c for metals, the Kjeldahl method for nitrogen, mass loss at 60 and 500 °C for moisture and volatile solids, respectively, and determination in aqueous extract at a 1:10 ratio (residue:water) for pH.

**Table 4:** Results of the chemical analysis of sheep (SHM) and cattle (CTM) manure.

Manure type	N	P	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	S	B	Cu	Fe	Mn	Zn	
												g kg <sup>-1</sup>
Sheep	20.4	8.0	21.9	14.7	6.8	2.7	13	50	3026	239	96	
Cattle	9.7	2.5	9.2	7.2	3.1	1.1	8	13	1038	107	43	
	N <sub>%</sub>	C <sub>%</sub>	C:N									
Sheep	2.04	41.9	20.5									
Cattle	0.96	46.1	47.7									

N = nitrogen, P = phosphorus, K<sup>+</sup> = potassium, Ca<sup>2+</sup> = calcium, Mg<sup>2+</sup> = magnesium, S = sulfur, B = boron, Cu = copper, Fe = iron, Mn = manganese, Zn = zinc, C = carbon and C:N = C ratio N.

The addition of GMS to the soil linearly increased the pH and the concentration of Ca<sup>2+</sup> and reduced the Fe concentration and the potential acidity of the soil (Tables 5 and 7). The total acidity (H+Al) was significantly reduced for the interaction of MNR x GMS only in the presence of CTM + SHM (Table 6).

Analysing the parameters selected for the coffee seedlings (Table 8) revealed that the ST501 variety had higher values of DMR (19%), root volume (30%) and DQI than the G35 variety.

The association of CTM and SHM shows promise for use in the initial growth of coffee seedlings, with higher H, FMAP, DMAP, FMR, DMR, VR and DQI provided by this combination (Table 8). Relative to SHM alone, the association of SHM+CTM contributed to increases in height (9%), FMAP (58%), DMAP (64%), FMR (28%), DMR (49%), VR (49%) and the DQI (42%).

Both varieties have a late behaviour; however, in the Var x Manure comparison (CTM+SHM and SHM), the

ST501 variety showed greater growth in the evaluated plant parameters (FMAP, DMAP, DMR, VR, DQI - Table 9) than did G35. Only ST501 had a positive significant result for manure, indicating that the combined use of SHM and CTM favours seedling development; in contrast, for G35, there is no difference between combined and separate use.

Although the incorporation of GMS contributed to improving the chemical conditions of the soil, no clear effects on seedling growth were observed. However, in the Var x GMS comparison (Table 10), the ST501 variety was verified to have higher DMR, VR and DQI in the absence of GMS or with the highest dose tested (3.0 t ha<sup>-1</sup> GMS); the values measured for ST501 surpass those measured for the G35 variety (Table 10). The G35 variety had higher DMR and VR when a dose of 2 t ha<sup>-1</sup> GMS was used; however, this behaviour was not observed for DQI.

**Table 5:** Average values of labile phosphorus (P), organic matter (OM), hydrogenic potential (pH), exchangeable potassium (K<sup>+</sup>), calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>) and aluminium (Al<sup>3+</sup>) and potential acidity (H+ Al) for treatments (Treat) with the application of manure (MNR), i.e., 20 t ha<sup>-1</sup> sheep manure (SHM) and 0 or 67 t ha<sup>-1</sup> cattle manure (CTM) and increasing doses of ground golden mussel shells (GMS), as well as p value, general averages, regression equations and coefficients of variation (CV) and determination (R<sup>2</sup>).

Treat	P	OM	pH	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	H+Al	Al <sup>3+</sup>
	mg dm <sup>-3</sup>	g dm <sup>-3</sup>	CaCl <sub>2</sub>		----- mmol <sub>c</sub> dm <sup>-3</sup> -----			
	<i>p</i> value							
MNR	0.0001	0.0000	0.0628	0.1001	0.0001	0.0004	0.0000	1.0000
GMS	0.4628	0.2239	0.0014	0.8265	0.0000	0.9743	0.0000	1.0000
MNRxGMS	0.5294	0.8335	0.3703	0.4169	0.2368	0.8056	0.0014	1.0000
General average	86.2	14.0	6.7	3.2	43.6	14.6	9.2	0.0
CV (%)	22	7	2	29	8	18	4	0
MNR	Averages							
Initial soil	1	10	4.4	0.4	2	2	16.3	2
CTM+SHM	106.8A	15.2A	6.7A	3.4A	47.0A	17.0A	8.8A	0.0A
SHM	65.6B	12.8B	6.6A	2.7A	40.3B	12.2B	9.6B	0.0A
GMS (Doses t ha <sup>-1</sup> )								
0.0	75.5	13.5	6.5	3.2	27.8	14.7	10.0	0.0
1.0	92.2	13.8	6.6	2.9	39.2	14.7	9.0	0.0
2.0	89.2	14.7	6.8	2.9	51.3	14.8	9.0	0.0
3.0	88.0	13.8	6.8	3.3	56.2	14.2	8.7	0.0
Regression Equations for GMS Doses						R <sup>2</sup>	Pr/F	
pH	$\hat{y}^{**} = 6.4500 + 0.0900 x$					0.9287	0.000	
Ca <sup>2+</sup>	$\hat{y}^{**} = 19.3333 + 9.7167 x$					0.9714	0.000	
H+Al	$\hat{y}^* = 10.1700 - 0.4000 x$					0.8000	0.000	

Averages followed by the same letter in the column do not differ statistically from each other at  $p > 0.05$ . Pr/F=probability of F (\*\*, \* = significant values at 1% and 5%, respectively).

**Table 6:** Average values, p value, correlation coefficient R<sup>2</sup> and regression equation for potential acidity (H+Al) in the comparison between treatments (Treat), with sheep manure (SHM) and with and without cattle manure (CTM), x increasing doses of ground golden mussel shells (GMS).

Treat	Doses de GMS (t ha <sup>-1</sup> )				Regression Equations	R <sup>2</sup>	p value
	0	1	2	3			
H + Al (mmol <sub>c</sub> dm <sup>-3</sup> )							
CTM + SHM	11.00 A	9.33 A	9.00 B	9.00 A	$\hat{y}^{**} = 11.17 - 0.63 x$	0.7293	0.0000
SHM	9.00 B	8.67 A	9.00 A	8.33 B	$\hat{y}^{ns}$		0.1003
p value	0.0000	0.0346	1.0000	0.0346			

Average values followed by the same letter in the column do not differ statistically from each other at  $p > 0.05$ . Pr/F=probability of F (\*\*, \* = significant values at 1% and 5%, respectively).

**Table 7:** Average values for treatments (Treat) with manure incorporation (CTM = 67 t ha<sup>-1</sup> and SHM = 20 t h<sup>-1</sup>) and increasing doses of ground golden mussel shells (GMS), for CEC = exchange capacity cationic, N = nitrogen, S = sulfur, B = boron, Cu = copper, Fe = iron, Mn = manganese and Zn = zinc, as well as *p* values and general averages.

Treat	CEC mmol <sub>c</sub> dm <sup>-3</sup>	N g kg <sup>-1</sup>	S mg dm <sup>-3</sup>	B	Cu	Fe	Mn	Zn
----- mg dm <sup>-3</sup> -----								
<i>p</i> value								
MNR	0.7705	0.3569	0.3993	0.0494	0.0000	0.0003	0.0000	0.0000
GMS	0.0792	0.6159	0.3157	0.2016	0.6175	0.0073	0.0526	0.8542
MNRxGMS	0.3466	0.6534	0.2082	0.1062	0.3953	0.8889	0.1207	0.9214
General Average	75.8	0.6	8.3	0.10	0.5	11.0	4.9	1.4
CV (%)	32.67	27.44	16.97	51.30	9.76	19.12	9.76	23.15
Averages								
Initial Soil	20.7	-	-	0.63	0.60	13.7	7.6	1.17
CTM+SHM	77.3A	0.58A	8.58A	0.12A	0.58A	13.00A	5.55A	1.94A
SHM	74.3A	0.65A	8.08A	0.08B	0.45B	9.08B	4.24B	0.97B
GMS (Doses t ha <sup>-1</sup> )								
0.0	58.6	0.63	8.17	0.14	0.53	14.00	5.42	1.45
1.0	70.8	0.55	7.50	0.09	0.50	10.83	4.75	1.38
2.0	75.4	0.68	8.67	0.09	0.50	10.00	4.70	1.43
3.0	98.4	0.63	9.00	0.08	0.52	9.33	4.72	1.55
Regression Equations for GMS Doses						R <sup>2</sup>	Pr/F	
Fe	$\hat{y}^{**} = 14.7500 - 1.4833x$					0.8596	0.0014	

Averages followed by the same letter in the column do not differ statistically from each other at *p* > 0.05.

## 4 DISCUSSION

Although we are aware that the composition of manure can vary according to the animal's feed and location (corral, pasture), according to Carneiro and Vieira (2020), this factor does not represent an impediment to its use, as it commonly benefits the soil and the plant.

Manure is usually used in family farming, where it is adopted mainly due to the concentration of N and P and because it can add OM and other nutrients to the soil (Amanullah et al., 2016; Fagwalawa; Yahaya, 2016; Najafi-Ghiri et al., 2017; Souto; Souto; Nascimento, 2013).

The results of the application of manure, both cattle and sheep combined, are promising relative to the initial soil conditions for P, OM, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, and CEC, as they increase the concentration of these elements and the pH in the soil solution, consequently reducing the potential acidity and Al<sup>3+</sup>. On the other hand, with increasing pH, the micronutrient contents (Cu, Fe and Mn) were reduced.

CTM, in turn, contributes to the addition of OM, P, Ca, Mg and micronutrients B, Cu, Fe, Mn and Zn, as has also been observed in other experiments (Alani; Mohammed; Al-Shaheen, 2019; Chatzistathis et al., 2020). Although the association of CTM with SHM increases the levels of micronutrients (B, Cu, Fe, Mn and Zn) relative to the addition of SHM alone (Chatzistathis et al., 2020), there is a reduction in micronutrients relative to the

initial soil conditions (Table 1) and an unexpected effect when observing the composition of manure (Table 2).

This behaviour with respect to micronutrients (B, Cu, Fe, Mn and Zn) can be attributed to the pH values (approximately 6.5): with increasing pH, the availability of these elements decline (Souza et al., 2007; Malavolta, 1989; Lehmann; Kleber, 2015).

The increase in soil pH to the range of 6.5 to 6.8, from pH = 4.4 in the initial soil conditions, was achieved by the combined application of SHM, CTM (Ye et al., 2021) and GMS, with a pH=6.6 achieved in the absence of GMS. In view of this result, soil correction is not necessary when manure is applied. Another important factor is the pH range reached, as it reduces the availability of micronutrients for plants.

However, the contribution of GMS linearly increased until pH = 6.8, which is attributed to the calcium carbonate present in the mussel shells, and GMS acted as a soil corrector, replacing limestone (Spångberg; Jönsson; Tidåker, 2013; Maltoni et al., 2020), raising the pH, and reducing the Al<sup>3+</sup> contents and the potential soil acidity (Kang; Wang; Zhang, 2018).

As reported, both SHM and CTM contributed to increasing the concentrations of P, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> in the soil, which is associated with the composition of each element (Table 4). This behaviour of SHM and CTM with respect to soil fertility was previously reported by Costa et al. (2015), Cai et al. (2019), and Chatzistathis et al. (2020).

**Table 8:** Average values for height (H), diameter (SD), fresh (FMAP) and dry (DMAP) mass of the aerial parts, fresh and dry mass of the root system (FMR) and (DMR), root volume (VR) and Dickson Quality Index (DQI) for two varieties of Conilon coffee (VAR) in treatments (Treat) with manure (MNR) application, i.e., 20 t ha<sup>-1</sup> sheep (SHM) and with or without application of 67 t ha<sup>-1</sup> of cattle manure (CTM), and increasing doses of ground golden mussel shells (GMS), as well as *p* values, general averages and coefficients of variation (CV).

Treat	H cm	SD mm	FMAP ----- g -----	DMAP ----- g -----	FMR ----- g -----	DMR ----- g -----	VR mL	DQI
	<i>p</i> value							
VAR	0.5061	0.5138	0.0546	0.0951	0.5867	0.0252	0.0034	0.0047
MNR	0.0371	0.6498	0.0000	0.0000	0.0246	0.0000	0.0001	0.0000
GMS	0.2227	0.9200	0.4615	0.6447	0.3740	0.8634	0.1987	0.6717
VARxMNR	0.3777	0.8331	0.0108	0.0243	0.3684	0.0129	0.0063	0.0019
VARxGMS	0.2865	0.3086	0.7285	0.4085	0.0728	0.0038	0.0009	0.0230
MNRxGMS	0.9516	0.9730	0.1313	0.3017	0.4246	0.5752	0.1495	0.3793
General average	50.35	10.66	252.85	93.96	271.23	79.10	425.00	28.97
CV (%)	13.43	15.42	22.00	21.97	36.34	28.04	29.02	24.42
VAR	Averages							
ST501	49.69 A	10.50A	268.88A	99.08A	279.04A	86.62A	481.25A	32.07A
G 35	51.00 A	10.82A	236.83A	88.83A	263.42A	72.58B	368.75B	25.86B
MNR								
CTM+ SHM	52.47A	10.55A	309.75A	116.83A	304.79A	94.79A	508.33A	33.94A
SHM	48.22 B	10.77A	195.96B	71.08B	237.67B	63.42B	341.67B	23.99B
GMS (Doses t ha <sup>-1</sup> )								
0.0	49.05	10.92	243.92	87.83	230.42	74.75	475.00	28.48
1.0	53.91	10.64	253.50	93.50	270.83	79.00	366.67	27.46
2.0	49.81	10.45	273.83	97.67	285.00	80.50	416.67	28.95
3.0	48.62	10.64	240.17	96.83	298.67	82.17	441.67	30.97

Averages followed by the same letter in the column do not differ statistically from each other at *p* > 0.05.

GMS contains calcium carbonate, which contributes to raising the pH and the Ca<sup>2+</sup> levels in the soil and consequently causing decreases in Fe<sup>3+</sup>, Al<sup>3+</sup> (Maia et al., 2015) and the potential acidity, with increasing effects as the dose applied increases (Spångberg; Jönsson; Tidåker, 2013). Assuming that liming is done with magnesium or calcium silicates or carbonates, the use of GMS could be used in its replacement, so GMS can also reduce or eliminate the toxic effects of Mn and Cu from the soil (Chatzistathis; Alifragis; Papaioannou, 2015), preventing quantities that would harm plant development (Rodrigues Filho et al., 2020). Depending on the demand of the culture and the micronutrient content, GMS may promote deficiency.

As indicated in table 4, it was expected that addition of CTM increased the levels of micronutrients such as B, Cu, Fe, Mn and Zn (Chatzistathis et al., 2020) in the soil, while GMS, used as a substitute for liming, increased the soil pH, leading to a reduction in the levels exchangeable for Al, Fe, Mn and Cu. The residues used contain amounts of Fe, Mn,

Cu and Zn that could increase the content of these elements in the soil, which did not occur due to the increase in pH to values above 6.0, a behaviour also reported by Lehmann and Kleber (2015) and Zong et al. (2021), both of whom verified the existence of a negative correlation between pH and these elements.

The addition of manure promoted gains in coffee seedling height, total fresh mass, dry mass and DQI (Dickson Quality Index), as observed by Partelli and Espindula (2019) for plants of the Robusta variety (ST501) and corroborated in this work, where the variety ST501 outperforms the variety G35 in the same parameters and in root volume, as a consequence of the lower vigour of the G35 (Partelli, et al., 2014), whereas ST501 has intense initial growth.

The use of manure was promising in the initial growth of Conilon coffee seedlings ST501 and G35, with higher values for H, FMAP, DMAP, FMR, DMR, VR and DQI when manure was added, especially for ST501. Bashir



et al. (2020) also reported increases in height, dry mass and production in wheat when fertilized with cattle manure, showing the possibility of using manure in other crops as well.

The incorporation of GMS had no clear effects on seedling growth; however, the Var x GMS comparison shows that the ST501 variety had higher DMR, VR and DQI in the absence of GMS or in the highest dose tested (3.0 t ha<sup>-1</sup> of GMS), in which these values surpassed those of the G35 variety (Table 10). As noted by Oliveira et al. (2018), Robusta coffee clones (ST501) tend to have better agronomic characteristics than Conilon coffee clones (G35), suggesting that the ST501 variety may be less demanding than G35 in the absence of soil correction.

As reported by Cai et al. (2019), the addition of manure to improve the fertility and physical and chemical attributes of the soil positively influences the initial growth of seedlings (Kar et al., 2017), especially of the ST501 variety, which responded with higher values for FMAP, DMAP, DMR, RV and DQI when compared to the G35 variety.

However, the association of manure with GMS may have reduced micronutrient availability, making it necessary

to evaluate the doses before application to the crop so as not to interfere with plant nutrition. In future studies, it is suggested to evaluate the seedlings for a longer period of time and evaluate the use of GMS alone to verify its effects on micronutrients.

## 5 CONCLUSION

Manure, both sheep and cattle, contributed to increase the soil pH and the levels of P, MO, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and CEC, indicating the positivity of its use over soil chemical quality.

The golden mussel shells contributed to the pH and levels of Ca<sup>2+</sup> elevation and reduced the potential acidity, contributing to the correction of soil acidity.

Thus, the association of manure (cattle and sheep) with golden mussel shells brought an organic perspective of improved soil health and guaranteed the initial growth of coffee seedlings, by their association (CTM and SHM) in the absence or with at least 3 t ha<sup>-1</sup> of GMS, for the clone ST501 that, in this condition, outperformed the clone G35 in DMR, VR and DQI.

**Table 9:** Evaluation of the variety (VAR) x cattle (CTM) and sheep (SHM) manure interaction for shoot fresh (FMAP) and dry mass (DMAP), root system dry mass (DMR), root volume (VR) and Dickson quality index (DQI), as well as *p* values.

VAR	CTM + SHM	SHM	p value
FMAP (g)			
ST501	347.50 Aa	272.00 Ab	0.0022
G35	190.25 Ba	201.67 Ba	0.6187
p value	0.0000	0.0041	
DMAP (g)			
ST501	129.00 Aa	104.67 Ab	0.0069
G35	69.17 Ba	73.00 Ba	0.6523
p value	0.0000	0.0007	
DMR (g)			
ST501	110.75 Aa	78.83 Ab	0.0013
G35	62.50 Ba	64.33 Aa	0.8409
p value	0.0000	0.1192	
VR (mL)			
ST501	616.67 Aa	400.00 Ab	0.0001
G35	345.83 Ba	337.50 Aa	0.8696
p value	0.0000	0.2235	
DQI			
ST501	40.49 Aa	27.39 Ab	0.0001
G35	23.64 Ba	24.34 Aa	0.8095
p value	0.0000	0.2998	

Averages followed by the same uppercase letter in the column and lowercase letter in the row do not differ statistically from each other by the F test at 5% probability.

**Table 10:** Evaluation of the variety interaction (VAR) x doses of ground golden mussel shells (GMS) for the variables root dry mass (DMR), root volume (RV) and Dickson quality index (DQI) and the respective regression equations, as well as correlation coefficients ( $R^2$ ), F probabilities (Pr/F) and  $p$  values.

VAR	GMS Doses (t ha <sup>-1</sup> )				Regression Equations	$R^2$	Pr/F
	0	1	2	3			
	DMR (g)						
ST501	91.50 A	86.33 A	67.00 B	101.67 A	$\hat{y}^{**} = -11.25x^2 + 59.88x$	0.7466	0.0697
G35	58.00 B	71.67 A	94.00 A	62.67 B			
$p$ value	0.0135	0.2606	0.0429	0.0046			
	VR (mL)						
ST501	625.00 A	433.33 A	341.67 B	525.00 A	$\hat{y}^{**} = 93.75x^2 - 507.92x + 1047.92$	0.9655	<b>0.0026</b>
G35	325.00 B	300.00 A	491.67 A	358.33 B			
$p$ value	0.0002	0.0703	0.0431	0.0256			
	DQI						
ST501	33.54 A	30.03 A	26.85 A	37.85 A			0.0627
G35	23.42 B	24.90 A	31.05 A	24.09 B			0.2381
$p$ value	0.0186	0.2184	0.3114	0.0020			

Averages followed by the same letter in the column do not differ statistically from each other at  $p > 0.05$  in the F test. Pr/F=probability of F (\*\*, \* = significant values at 1% and 5%, respectively).

## 6 AUTHORS' CONTRIBUTION

MCB wrote the manuscript and performed the experiment, KLM supervised the experiment, co-work the manuscript and approved the final version of the work, KNS review and conducted all statistical analyses.

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