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CROP PROTECTION

Strategies for reducing the impact of clubroot on broccoli cultivation in tropical mountain regions

Carlos Antônio dos Santos[1*], Nelson Moura Brasil do Amaral Sobrinho[2], Rafael Gomes da Mota Gonçalves[3], Jéssica de Oliveira Lima[1], Laura Carine Candido Diniz Cruz[1] and Margarida Goréte Ferreira do Carmo[1]

[1]Departamento de Fitotecnia, Instituto de Agronomia, Universidade Federal Rural do Rio de Janeiro, Rod. BR-465, km 07, 23897-000, Seropédica, Rio de Janeiro, Brazil. [2]Departamento de Agrotecnologias e Sustentabilidade, Instituto de Agronomia, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil. [3]Departamento de Solos, Instituto de Agronomia, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil. *Author for correspondence. E-mail: carlosantoniokds@gmail.com

ABSTRACT. *Brassica* spp. production can be negatively affected by clubroot, which is caused by the protozoan *Plasmodiophora brassicae* Woronin. Most of the information on clubroot control is derived from studies in temperate regions. Here, management strategies were evaluated to reduce broccoli (*Brassica oleracea* L. var. *italica* Plenck) crop losses owing to clubroot in tropical mountain regions. The first experiment revealed the effect of green manure from coriander (*Coriandrum sativum* L.), sunn hemp (*Crotalaria juncea* L.), sweet corn (*Zea mays* L.), and spontaneous vegetation (control) associated with broccoli seedlings of 4 different sizes. In the second experiment, the effect of soil amendments (limestone and steel slag) in conjunction with poultry litter (fresh or composted for 45 days) and without poultry litter (control), was assessed. Both field experiments sought to evaluate the disease intensity, plant development (root growth, biomass, and nutrient accumulation), and yield. Sunn hemp and coriander biomass resulted in higher healthy root volumes and dry weights of broccoli. However, such benefits were not derived from corn treatment. Compared to smaller seedlings (10 mL cell and 20 days of age, and 16 mL cell and 24 days of age), the use of larger seedlings (35 mL cell and 28 days of age, and 50 mL cell and 32 days of age) resulted in lower intensity of clubroot and increased the average yield by 143% in summer crops. Steel slag, like limestone, corrected soil acidity and resulted in plant growth; however, clubroot intensity was not significantly affected. Fresh and composted poultry litter increased the percentage of diseased roots compared with the control; however, broccoli yield was not affected by the treatments. Using green manure (sunn hemp or coriander) and well-developed seedlings is recommended as a strategy to reduce losses induced by clubroot during broccoli cultivation.

Keywords: *Brassica oleracea* var. *italica*; *Plasmodiophora brassicae*; green manure; composting; liming; steel slag; silicon.

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Introduction

Clubroot, which is caused by *Plasmodiophora brassicae* Woronin, a biotrophic soil-inhabiting protozoan, reduces cultivation yield in broccoli (*Brassica oleracea* L. var. *italica* Plenck) and other *Brassica* spp. in Brazil (Bhering, Carmo, Matos, Lima, & Amaral Sobrinho, 2017; Irokawa, Zambolim, & Parreira, 2020; Santos, Amaral Sobrinho, Lima, & Carmo, 2022) and worldwide (Dixon, 2014; Botero et al., 2019; Botero, Hwang, & Strelkov, 2021). The pathogen infects the roots of host plants, causing galls (clubroots), ultimately resulting in the underdevelopment of plants and reducing yield (Dixon, 2014; Gossen, Deora, Peng, Hwang, & McDonald, 2014).

Germination of the resting spore and infection and colonization of *Brassica* spp. roots by *P. brassicae* often occur in acidic or slightly acidic (pH < 6.2) conditions, with moist soil and temperatures ranging from 20°C to 25°C providing ideal conditions for infection. Intensive cultivation of *Brassica* spp. increases the inoculum potential of *P. brassicae* in the soil (Gossen et al., 2013; Dixon, 2014; Gossen et al., 2014; Bhering et al., 2017).

Disease management is hampered by the lack of resistant cultivars and limited chemical control (Donald & Porter, 2009; Botero et al., 2019; Irokawa et al., 2020). Strategies to reduce pathogen-induced damage are restricted to crop rotation with non-host species and liming (Donald & Porter, 2009; Gossen et al., 2014;

Santos, Carmo, Bhering, Costa, & Amaral Sobrinho, 2020). Crop rotation of *Brassica* with certain non-host species of *P. brassicae* may stimulate the germination of resting spores of the pathogen without completing its life cycle, leading to a reduction in inoculum potential and disease intensity in subsequent crops (Hasse, Mai-De-Mio, & Lima Neto, 2007; Friberg, Lagerlof, & Ramert, 2006; Donald & Porter, 2009; Ahmed et al., 2011; Hwang et al., 2015; Chen, Zhou, Yu, & Wu, 2018). Crop rotation with green manure is also beneficial as it promotes nutrient cycling and increases soil organic matter content (Araújo et al., 2011; Cordeiro et al., 2018; Castro & Devide, 2018).

Limestone is commonly applied for increasing soil pH and providing calcium, which influences the life cycle of *P. brassicae* (Donald & Porter, 2009; Santos et al., 2020). In addition, steel slag is a low-price industrial residue from steel production that contains high concentrations of calcium and magnesium silicate. Steel slag is used to increase soil pH and enrich the soil with silicon, which can reduce damage caused by certain pathogens (Romero, Munévar, & Cayón, 2011; Prezotti & Martins, 2012). However, information on the residual effects of this treatment on the growth of *Brassica* spp. and the reduction in clubroot remains scarce.

Another recommended strategy for managing clubroot in broccoli is the use of organic compounds. However, the results of their use vary depending on multiple factors (Dixon, 2014; Gossen, Kasinathan, Deora, Peng, & McDonald, 2016; Bonanomi, Lorito, Vinale, & Woo, 2018). Poultry litter, often without prior composting, is the most widely used organic fertilizer for *Brassica* production in Brazil (Adami et al., 2012; Bhering et al., 2017; Bhering et al., 2020; Santos et al., 2022). The use of untreated poultry litter is critical because of the potential risks to human health caused by pathogens, such as *Salmonella* spp., *Staphylococcus* spp., and *Escherichia coli* (Chen & Jiang, 2014; Kyakuwaire, Olupot, Amoding, Kizza, & Basamba, 2019). This practice has been associated with increased clubroot intensity in cauliflower (*Brassica oleracea* var. *botrytis* L.) grown in acidic soils in the mountainous region of Rio de Janeiro (Bhering et al., 2017; Bhering et al., 2020); however, additional studies are required to guide prescriptions for its use.

In tropical mountainous regions, broccoli and other *Brassica* spp. are regularly cultivated, with frequent fertilization with poultry litter, little or no adherence to crop rotation, no correction of soil acidity, and the use of small seedlings (Assis & Aquino, 2018; Bhering et al., 2017; Santos et al., 2020; Santos et al., 2022). In addition to losses caused by clubroot, another major challenge is cultivation during spring/summer, which is the most profitable period for broccoli cultivators; however, this period is also associated with high temperatures and frequent rainfall (Assis & Aquino, 2018; Santos et al., 2022), which are unfavorable to the crop (Melo, 2015) and conducive to clubroot development (Dixon, 2014).

In this study, field experiments were conducted to assess management strategies to reduce losses caused by clubroot using a) green manure, b) larger broccoli seedlings, c) correction of soil acidity with steel slag, and d) application of fresh and composted poultry litter.

Material and methods

The study was performed in the mountainous region of Rio de Janeiro, the main *Brassica* spp. producing region in Brazil. This region is characterized by a tropical climate, with an intermediate temperature of 18.4°C, high rainfall of 1,372 mm per year, and sloping and acidic soils (Bhering et al., 2017; Assis & Aquino, 2018; Bhering et al., 2020; Santos et al., 2022).

Two field experiments were conducted in the municipality of Petrópolis, Rio de Janeiro State, Brazil, at 22°25′41.64″ S and 43°02′54.49″ W (altitude: 1,274 m) and 22°25′36.48″ S and 43°02′54.22″ W (altitude: 1,261 m), in areas with a history of clubroot occurrence and intensive cultivation of *Brassica* crops. Preliminary tests using soil samples from both areas indicated that the incidence of this disease in arugula (*Eruca sativa* Miller) ranged from 73 to 86%. The soil in the two areas was classified as a Haplic Cambisol type. Weather records for the two growing periods were obtained from the Brazilian National Institute of Meteorology (INMET).

Use of green manure and larger broccoli seedlings

The first experiment was performed from November 2017 to May 2018 in a depression in the landscape that was more favorable for the accumulation of water and *P. brassicae* spores from crops in higher areas. This area was previously cultivated with cauliflower (*B. oleracea* L. var. *botrytis*) and had the following initial soil characteristics at 0-20 cm: $pH_{(water)}$ = 5.95; Al^{3+} = 0.02 $cmol_c$ kg^{-1}; Ca^{2+} = 5.13 $cmol_c$ kg^{-1}; Mg^{2+} = 2.30 $cmol_c$ kg^{-1}; cation exchange capacity (T) = 11.27 $cmol_c$ kg^{-1}; potential acidity (H+Al) = 3.75 $cmol_c$ kg^{-1}; base saturation (V%) = 67.0%; K^+ = 107 mg kg^{-1}; and extractable P = 34 mg kg^{-1}.

The experimental design was a 4 x 4 factorial scheme in a completely randomized block design with five replications in a split-plot. The plots consisted of areas with green manure and the subplots consisted of seedling size. The effects of green manure from coriander (*Coriandrum sativum* L.), sunn hemp (*Crotalaria juncea* L.), sweet corn (*Zea mays* L.), spontaneous vegetation (control), and four broccoli seedling sizes were assessed to determine clubroot intensity, broccoli development, and nutrient accumulation.

The soil was initially prepared using a plow and a rotary hoe. Each species was sown in 1 m wide beds with inter-rows spaced 0.30 m perpendicular to them at plant densities of 45, 20, and 10 plants per row for coriander, sunn hemp, and corn, respectively. The control beds were maintained free from cultivation and without weeding, allowing the unrestricted development of spontaneous vegetation. The predominant spontaneous plant species in these plots was identified according to Lorenzi (2014).

Plants were irrigated by a sprinkler system every 3 days and by rainfall during the experimental period. Weeds were controlled by manual weeding 30 days after transplantation (DAT), except those in the control plots (spontaneous vegetation).

The production of fresh and dry biomass from green manure was assessed 75 days after sowing (DAS) when corn had flowered. Random samples were retrieved with a 0.25 m^2 frame, with subsequent drying in an oven via forced air circulation to a constant weight. The plants were mowed at the stem base and maintained on the soil surface until 82 DAS; thereafter, they were incorporated into the soil using a rotary hoe.

Dry biomass samples from green manure were ground and digested according to method 3050 (USEPA) to measure the macronutrient concentrations (Santos et al., 2022). The K concentration was determined using flame emission spectrometry (DM-62, Digimed, Brazil), and the P concentration was determined using metavanadate colorimetry (Sousa, Carmo, Lima, Souza, & Amaral Sobrinho, 2020). Ca and Mg were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (IP1101M100, Agilent Technologies, Santa Clara, USA).

Total N, S, and C concentrations were obtained from dry combustion in a CHNO-S Vario Macro Cube analyzer (Elementar, Langenselbold, Germany), with subsequent determination of the C/N ratio. Accumulated macronutrient values in the green manure samples and estimates of nutrient contributions were calculated based on the concentrations and dry weights.

Four broccoli seedling sizes of cv. Avenger (Sakata®) were assessed (Size 1–10-mL cell and 20 days of age; Size 2: 16-mL cell and 24 days of age; Size 3: 35-mL cell and 28 days of age; and Size 4: 50-mL cell and 32 days of age) (Table 1). Seedlings were produced in a greenhouse by sowing in trays with different cell volumes that were associated with different sowing times. The trays were filled with a commercial substrate, Multiplant® Hortaliças (Terra do Paraíso, Holambra, Brazil). Seedlings were irrigated daily in the morning using sprinklers (approximately 400 mL per tray). Seedling samples were collected before transplanting to determine the number of expanded leaves, shoot height, leaf area, fresh and dry weights of shoots and roots, and root length and volume. Descriptive statistics were obtained using Microsoft Office Excel® (Microsoft Corporation, Redmond, WA, USA) to characterize the different types of seedlings used (Table 1).

Table 1. Attributes of the shoot and roots of the 4 sizes of broccoli seedlings developed in trays with 4 different volumes (10, 16, 35, and 50 mL) and 4 ages counted after the date of sowing (20, 24, 28, and 32 days).

	Shoot					Root			
Size and age	Nº. of leaves	Height (cm)	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Volume (mL)	Length (cm)	Fresh weight (g)	Dry weight (g)
					Size 1				
10-mL cell and 20 days of age	1.83±0.40	4.38±0.51	13.69 ± 3.00	0.54±0.11	0.07±0.01	0.28±0.07	6.87±0.84	0.31±0.10	0.018±0.005
					Size 2				
16-mL cell and 24 days of age	2.30±0.50	6.17±0.88	28.28 ± 5.61	1.33±0.13	0.14 ± 0.03	0.31±0.11	8.25±1.17	0.39±0.10	0.021±0.007
					Size 3				
35-mL cell and 28 days of age	3.00±0.50	7.78±0.91	53.54±14.37	2.50±0.69	0.26±0.11	0.61±0.16	11.11±2.71	0.56±0.21	0.036±0.010
					Size 4				
50-mL cell and 32 days of age	4.00±0.50	11.43±1.50	71.69±28.33	3.87±1.39	0.34 ± 0.16	0.98±0.47	14.96±3.27	1.37±0.81	0.067±0.028

As the seedlings developed, the soil was prepared using a rotary hoe and bed former. Broccoli seedlings were manually transplanted in the field on February 16, 2018, 12 days after the incorporation of green manure biomass. Soil samples were collected from a depth of 0-20 cm to determine the chemical attributes of the soil according to Donagema, Campos, Calderano, Teixeira, and Viana (2011). The total C, N, and S concentrations and soil C/N ratios were determined using dry combustion with an elemental CHNO-S analyzer. Fresh poultry litter was incorporated directly into planting pits at 11.11 Mg ha^{-1}, and 25.2 kg ha^{-1} N (urea), 120 kg ha^{-1} P_2O_5 (simple superphosphate), and 48 kg ha^{-1} of K_2O (potassium chloride) were added. Seedlings were transplanted at a spacing of 0.60 m (rows) x 0.60 m (plants). Each subplot contained six experimental plants and four additional plants at the borders.

Plants were irrigated using a sprinkler system every three days. Topdressing fertilization was performed 20, 40, and 60 days after transplanting (DAT) by applying 25.2 kg ha^{-1} of N (urea) and 48 kg ha^{-1} of K_2O (potassium chloride). Boric acid (1 g L^{-1}) and sodium molybdate (0.5 g L^{-1}) were applied to the leaves. Weeds were manually controlled at 40 DAT.

Harvesting was performed 86 and 96 DAT, which are the timepoints when most plants had inflorescences at the point of commercial harvest, with good uniformity, compactness, and tightly closed granules. Plants were collected by cutting close to the base of the stem.

The roots of all experimental plants were collected using a straight blade, washed, and then used to quantify clubroot severity (%) using a scale composed of the scores (0, 8, 20, 42, 68, 87, and 95% of roots with galls) (Santos et al., 2020), and to quantify the volume and fresh weight of the healthy fraction and fraction with galls (Bhering et al., 2017). Clubroot intensity is expressed as the percentage of diseased roots (DR%), calculated based on the relationship between the fresh weight of galls and the total fresh weight of the roots (Santos et al., 2020).

The fresh weights of the leaves, stems, and inflorescences and the longitudinal diameter of the inflorescences were determined. The total yield was estimated from the fresh weight of the inflorescences. The dry weights of the organs were determined after drying in an oven with forced air circulation at 65°C until a constant weight was reached. The values were summed to obtain total plant dry weight.

Macronutrient concentrations in each plant organ were quantified in dry samples following the same methodology and equipment as previously mentioned. These concentrations were used to calculate the accumulated values in different organs and in the entire plant.

Steel slag and fertilization with poultry litter

The second experiment was performed from December 2017 to June 2018 in an area previously cultivated with parsley [*Petroselinum crispum* (Mill.) Nym.] and radish (*Raphanus sativus* L.), with the following soil characteristics at 0-20 cm: $pH_{(water)}$ = 5.80; Al^{3+} = 0.10 $cmol_c$ kg^{-1}; Ca^{2+} = 4.20 $cmol_c$ kg^{-1}; Mg^{2+} = 1.40 $cmol_c$ kg^{-1}; T = 14.32 $cmol_c$ kg^{-1}; H+Al = 8.66 $cmol_c$ kg^{-1}; V(%) = 39.5%; K^+ = 217 mg kg^{-1}; and extractable P = 20 mg kg^{-1}.

The experimental design was 2 x 4 factorial in a completely randomized block design with four replications in a split-plot. The main plots received steel slag and limestone amendments, and subplots had fresh (non-composted) poultry litter, poultry litter composted for 45 days, and a control (only mineral fertilizer, without use of poultry litter). Each subplot contained 24 plants. Steel slag was compared to limestone, and its use was associated with fertilization using fresh (not composted) and composted poultry litter to determine its effects on clubroot intensity and the development of broccoli plants.

Steel slag, composed of calcium and magnesium silicate (Agrosilício Plus®, Timóteo, Brazil), was composed of 25.0% Ca, 6.0% Mg, and 10.5% Si. Dolomitic agricultural limestone (Mibita®, Castelo, Brazil) was composed of 28.0% Ca and 6.6% Mg. The soil amendment doses were determined using an incubation curve of soil samples from the area with the respective materials (Santos et al., 2020) to achieve a pH of 6.5, which is ideal for broccoli (Melo, 2015). This pH value was reached by applying 3.5 Mg ha^{-1} steel slag and 3.4 Mg ha^{-1} limestone. Soil amendments were applied on December 20, 2017, and incorporated using a rotary hoe.

Poultry litter was obtained from a local chicken farm. Fresh samples (non-composted) and composted poultry litter over 45 days were characterized (Table 2). Fresh and composted poultry litter were applied and incorporated into pits the day before transplanting at a dose equivalent to 400 g per plant (11.11 Mg ha^{-1}), expressed on a dry basis (Santos et al., 2022).

Table 2. Attributes of fresh (non-composted) and composted poultry litter at 45 days.

	Moisture	pH	EC	C/N ratio	N	Ca	Mg	P	K	S
Poultry litter	%		dS m^{-1}		g kg^{-1}					
Fresh	21.2	7.92	2.13	16.88	19.00	41.23	3.16	5.72	17.75	2.31
Composted	37.3	7.70	3.21	17.75	16.13	61.57	7.15	9.07	26.81	4.51

Broccoli seedlings were manually transplanted on March 17, 2018, 87 days after the application of soil amendments. Broccoli seedlings of cv. Avenger (Sakata®), which was 28 days old and produced in 128-cell trays with 35 mL of substrate per cell, were employed. The inter-row spacing was 0.60 m and the spacing between plants was 0.60 m.

Soil samples were collected from each plot for chemical analysis during transplanting. All plants were fertilized with mineral fertilizers, including the control plants. Planting and top-dressing fertilization, doses, application times, and mineral fertilizers were similar to those used in the first experiment. Irrigation and management practices were performed according to the methodology used in the first experiment. Harvesting and assessments were performed at 74, 79, and 86 DAT, and the same variables measured in the first experiment were used.

Data obtained in both experiments were analyzed using analysis of variance. Means were compared with Tukey's test ($p \leqslant 0.05$) using the SISVAR statistical software (Ferreira, 2011). The isolated effects (main effects) and interactions between factors (green manure × seedling size and soil amendments × poultry litter) were considered for variables related to clubroot intensity, biomass accumulation, yield, concentrations, and nutrient accumulation in broccoli. For results that did not show a significant interaction between the two factors, the means of the main effects were presented separately.

Results

Use of green manure and larger broccoli seedlings

The predominant spontaneous plant species in plots free from cultivation and without weeding were *Amaranthus retroflexus* L., *Bidens pilosa* L., *Brachiaria decumbens* Stapf, *Eleusine indica* (L.) Gaertn, *Galinsoga ciliata* (Raf.) Blake, *Melampodium perfoliatum* (Cav.) Kunth., *Panicum maximum* Jacq., *Polygonum persicaria* L., *Portulaca oleracea* L., *Sonchus oleraceus* L., and *Spergula arvensis* L. (Lorenzi, 2014).

Corn produced high amounts of fresh (124.33 Mg ha^{-1}) and dry biomass (14.50 Mg ha^{-1}), with higher amounts ($p \leqslant 0.05$) than the other species, which were like each other in terms of these attributes (Table 3). Compared to the other species, the dry biomass of corn had a higher ($p \leqslant 0.05$) C/N ratio (36.48), lower N (11.08 g kg^{-1}), Mg (2.62 g kg^{-1}), and S concentrations (1.10 g kg^{-1}), high P values (5.79 g kg^{-1}), and intermediate Ca (37.46 g kg^{-1}) and K concentrations (45.48 g kg^{-1}). The dry biomass of sunn hemp and coriander had high N concentrations (22.93 and 19.90 g kg^{-1}, respectively) and low C/N ratios (18.08 and 16.20, respectively). Corn resulted in high ($p \leqslant 0.05$) extraction and incorporation of N, Ca, Mg, P, K, and S into the soil owing to the high biomass (Table 3).

Table 3. Characterization of the green manures (corn, coriander, sunn hemp, and spontaneous vegetation) and the amount of fresh and dry biomass and nutrients incorporated into the soil before the transplantation of broccoli seedlings.

Characterization of green manure								
Green manure[1]	C/N ratio	C	N	Ca	Mg	P	K	S
		g kg^{-1}						
Corn	36.48 a	404 a	11.08 b	37.46 bc	2.62 c	5.79 a	45.48 b	1.10 b
Coriander	16.20 b	322 a	19.90 a	45.43 ab	5.70 ab	6.54 a	90.40 a	2.40 a
Sunn hemp	18.08 b	415 a	22.93 a	31.59 c	5.52 b	3.17 b	26.40 c	2.11 a
Spontaneous	20.66 b	367 a	17.75 ab	47.24 a	6.59 a	4.70 ab	59.86 b	2.71 a
CV%	19.70	14.03	20.93	9.61	8.79	23.78	14.95	18.68
Estimated amount incorporated in the soil								
Green manure[1]	Fresh biomass	Dry biomass	N	Ca	Mg	P	K	S
	Mg ha^{-1}		kg ha^{-1}					
Corn	124.33 a	14.50 a	160.58 a	543.16 a	38.01 a	83.93 a	659.45 a	15.91 a
Coriander	28.50 b	2.10 b	41.79 b	95.40 b	11.98 c	13.72 b	189.85 b	5.04 c
Sunn hemp	18.70 b	3.55 b	8.38 b	112.14 b	19.58 b	10.06 b	93.71 c	7.48 bc
Spontaneous	29.76 b	3.13 b	55.56 b	147.64 b	20.60 b	14.70 b	187.07 b	8.49 b
CV%	15.29	15.57	23.31	14.51	7.64	16.99	13.65	13.88

[1]For each parameter and in each column, means followed by the same letter did not differ statistically by Tukey's test ($p \leqslant 0.05$).

Green manure influenced soil attributes, which were evaluated at the time of broccoli seedling transplantation (Table 4). The highest pH was observed in soils containing spontaneous vegetation (pH = 5.84) and coriander (pH = 5.80), and the lowest values were found in areas with corn (pH = 5.61). Areas with sunn hemp had the highest Ca^{2+}, Mg^{2+}, and K^{+} concentrations and cation exchange capacity (T). Treatment with green manure had no significant effect on potential acidity (H + Al), base saturation (V%), and C and P concentrations (Table 4). The highest total N concentration in the soil was observed in areas previously cultivated with sunn hemp (4.60 g kg^{-1}), followed by areas cultivated with coriander and spontaneous vegetation. A low total N concentration was previously observed in the soil of the plots with corn. Areas previously cultivated with coriander and sunn hemp had the highest S values, whereas those cultivated with corn had the lowest S values (Table 4).

Table 4. Soil chemical attributes at the time of broccoli seedling transplantation, 12 days after incorporating residues of the predecessor plants (corn, coriander, sunn hemp, and spontaneous vegetation).

Green manure [1]	pH	C	N	S	C/N ratio	Ca	Mg	H+AL	T	V	K	P
	(water)	g kg^{-1}				cmol$_c$ kg^{-1}				%	mg kg^{-1}	
Corn	5.61 c	39.34 a	3.86 b	4.34 d	10.19 a	6.58 c	2.38 c	2.60 a	11.62 c	77.64 a	95.61 ab	22.56 a
Coriander	5.80 ab	40.98 a	4.30 ab	8.00 a	9.51 a	7.10 bc	2.88 c	2.94 a	12.99 c	77.54 a	101.78 ab	26.30 a
Sunn hemp	5.73 b	44.18 a	4.60 a	6.61 b	9.58 a	9.20 a	4.86 a	3.58 a	17.72 a	79.66 a	118.68 a	28.46 a
Spontaneous	5.84 a	40.58 a	4.10 ab	5.70 c	9.87 a	8.04 b	3.58 b	3.62 a	15.31 b	76.61 a	92.13 b	24.92 a
CV(%)	1.06	9.58	6.65	4.53	6.52	7.93	9.25	21.03	6.16	4.74	13.66	17.59

[1]In each column, means followed by the same letter did not differ statistically by Tukey's test ($p \leq 0.05$). Al^{3+} = 0.00 cmol$_c$ kg^{-1}. T = cation exchange capacity. V = base saturation.

Medium temperatures and frequent rainfall occurred, particularly at the beginning of the broccoli cycle. The temperatures ranged from 12.2 to 24.2°C, with a mean value of 14.7°C. Accumulated precipitation during the crop cycle was high (624 mm), with 384 mm concentrated during the first 30 days. These conditions, especially high precipitation at the beginning of the crop cycle, favored the early development of clubroot, which was diagnosed at 26 DAT.

The interaction between the green manure crop and seedling size had no significant effect ($p \leq 0.05$) on any variable. The main effects of both factors were used to explain the results (Table 5).

Treatments involving green manure had no influence on disease severity, percentage of diseased roots (DR%), total root dry weight, fresh weight, diameter of broccoli inflorescences, and yield. Green manure with sunn hemp, coriander, and spontaneous vegetation resulted in broccoli plants with a higher healthy root volume and drier biomass than green manure with corn (Table 5).

Table 5. Main effects of green manure from corn, coriander, sunn hemp, and spontaneous vegetation, and broccoli seedling size (size 1 = 10 mL cell and 20 days old; size 2 = 16 mL cell and 24 days old; size 3 = 35 mL cell and 28 days old; size 4 = 50 mL cell and 32 days old) on the severity of clubroot (caused by *Plasmodiophora brassicae*) based on note scale, percentage of diseased roots (DR%), healthy root volume, total root dry weight, total dry weight of plant, fresh weight of inflorescence, inflorescence diameter, and estimated yield.

Green manure[1]	Clubroot and root growth				Plant growth and production			
	Severity (%)	DR%	Healthy root volume (mL)	Root dry weight (g)	Dry weight of plant (g)	Inflorescence Fresh weight (g)	Inflorescence Diameter (cm)	Yield (kg ha^{-1})
Corn	42.62 a	43.81 a	24.30 b	9.60 a	44.67 b	73.96 a	7.86 a	2,110 a
Coriander	42.47 a	41.57 a	31.26 ab	10.41 a	60.77 a	118.71 a	6.55 a	3,230 a
Sunn hemp	40.04 a	40.28 a	43.67 a	11.94 a	61.24 a	123.25 a	12.56 a	3,300 a
Spontaneous	42.93 a	41.92 a	30.99 ab	10.67 a	60.14 ab	111.14 a	6.50 a	3,080 a
CV1(%)	11.52	8.78	22.45	22.55	15.29	33.46	33.56	25.15
Seedling size [1]								
Size 1	49.92 a	47.83 a	26.65 b	7.08 b	40.08 b	57.31 b	4.71 c	1,590 b
Size 2	47.57 a	45.46 ab	30.74 ab	9.89 ab	50.23 b	70.37 b	5.96 bc	1,830 b
Size 3	35.78 b	38.21 bc	35.53 a	12.57 a	67.58 a	139.52 a	9.09 ab	3,850 a
Size 3	34.78 b	36.08 c	37.92 a	13.09 a	68.92 a	159.86 a	13.71 a	4,450 a
CV2(%)	13.49	12.78	17.37	20.00	17.18	33.24	30.63	24.16

[1]For each factor (green manure or seedling size) and in each column, means followed by the same letter did not differ statistically by Tukey's test ($p \leq 0.05$). No significant effect of the interaction between green manure × seedling size was observed.

Corn residue resulted in lower concentrations and the accumulation of N and S and a higher C/N ratio (10.96) in broccoli plants than sunn hemp (9.76) and coriander (9.26) (Table 6). High plant dry weight

(Table 5), high N concentration, and low C/N ratio were observed in broccoli plants from areas previously cultivated with coriander and sunn hemp (Table 6). High S concentrations were observed in broccoli plants grown in areas with spontaneous vegetation, coriander, and sunn hemp (Table 6). High Ca concentrations were observed in broccoli plants from areas cultivated with coriander and sunn hemp (5.74 and 4.26 g plant^{-1}, respectively). The highest Mg accumulation occurred in plants from plots previously cultivated with coriander, sunn hemp, and spontaneous vegetation and low Ca and Mg concentrations were found in broccoli plants from plots previously cultivated with corn. No differences were observed in P and K accumulation in the broccoli plants.

Table 6. Main effects of green manure from corn, coriander, sunn hemp, and spontaneous vegetation, and broccoli seedling size (size 1 = 10 mL cell and 20 days old; size 2 = 16 mL cell and 24 days old; size 3 = 35 mL cell and 28 days old; size 4 = 50 mL cell and 32 days old) on the carbon to nitrogen (C/N) ratio, mean N and S concentrations, and N, S, Ca, Mg, P, and K accumulation in broccoli plants.

Green manure[1]	C/N ratio	N	S	N	S	Ca	Mg	P	K
		g kg^{-1}		g plant^{-1}					
Corn	10.96 a	29.20 c	5.57 c	1.29 b	0.249 b	3.16 b	0.35 b	0.52 a	4.27 a
Coriander	9.26 b	36.15 a	7.25 ab	2.20 a	0.435 a	5.74 a	0.53 a	0.69 a	5.70 a
Sunn hemp	9.76 b	33.12 ab	6.94 b	2.04 a	0.417 a	4.26 ab	0.43 ab	0.64 a	5.43 a
Spontaneous	10.02 ab	32.98 b	8.28 a	2.00 a	0.493 a	3.69 b	0.44 ab	0.68 a	5.78 a
CV1(%)	10.60	10.07	19.12	32.42	29.17	38.56	39.37	33.89	35.36
Seedling size[1]									
Size 1	10.14 a	32.64 a	6.90 ab	1.31 b	0.276 a	3.27 b	0.33 b	0.49 b	4.20 b
Size 2	9.53 a	33.26 a	7.57 a	1.68 ab	0.384 ab	4.00 ab	0.41 ab	0.61 ab	5.04 ab
Size 3	10.13 a	31.47 a	6.80 ab	2.26 a	0.479 a	5.15 a	0.53 a	0.76 a	6.37 a
Size 4	10.20 a	32.63 a	6.53 b	2.29 a	0.455 a	4.43 ab	0.47 ab	0.66 ab	5.55 ab
CV2(%)	10.28	13.73	16.46	41.38	40.19	37.44	37.15	41.40	39.53

[1]For each factor (green manure or seedling size) and in each column, means followed by the same letter did not differ statistically by Tukey's test ($p \leqslant 0.05$). No significant effect of the interaction between green manure × seedling size was observed.

Seedling size affected all variables related to disease intensity, plant development, and yield (Table 5). Low disease severity, DR%, and a high volume of healthy roots and root dry weight were found in plants obtained from larger seedlings, that is, sizes 3 (35 mL cell and 28 days old) and 4 (50 mL cell and 32 days old). Consequently, seedlings of sizes 3 and 4 showed better development, which was expressed as high plant dry weight, fresh weight of inflorescence, inflorescence diameter, and yield (Table 5). Seedling size did not affect the N concentration (mean = 32.5 g kg^{-1}) and C/N ratio (mean = 10.0) of broccoli plants. Seedling size influenced only S concentrations, with the highest value in plants from size 2 seedlings (7.57 g kg^{-1}). The lowest N, Ca, Mg, P, and K accumulation occurred in plants of the smallest size (size 1 = 10 mL cell and 20 days old); the highest accumulation occurred in plants of sizes 3 and 4, followed by size 2 seedlings. The accumulated S values varied little as a function of the seedling size (Table 6).

Steel slag and fertilization with poultry litter

The two soil amendments did not differ statistically ($p \leqslant 0.05$) in terms of their effects on soil chemical attributes, which were assessed 87 days after application, except for pH. Both steel slag and limestone increased the initial pH, which was 5.80. Limestone application resulted in a statistically higher pH ($p = 0.0062$) than that obtained with steel slag, with values of 6.23 and 6.00, respectively. The Ca^{2+}, Mg^{2+}, K^{+}, and extractable P concentrations varied from 7.9 to 8.1 cmol$_c$ kg^{-1}, 4.09 to 4.10 cmol$_c$ kg^{-1}, 185.4 to 205.4 mg kg^{-1}, and 18.6 to 20.3 mg kg^{-1}, respectively. The cation exchange capacity (T) ranged from 17.7 to 17.9 cmol$_c$ kg^{-1}. Further, Al^{3+} was not present in both treatments, H+Al ranged from 5.6 to 5.7 cmol$_c$ kg^{-1}, and base saturation (V%) ranged from 68.50 to 68.84%. The soil fertility resulting from both soil amendments was within the recommended range for broccoli (Melo, 2015).

Temperatures varied from 13.4 to 23.0 °C during the crop cycle, with reduced and well-distributed rainfall totaling 277 mm. The interaction between soil amendments and organic compounds had no significant effect ($p \leqslant 0.05$) on any variable, and the main effects of both factors are presented separately (Tables 7 and 8). The soil amendments did not affect disease development or broccoli yield (Table 7). The application of fresh or composted poultry litter for 45 days significantly increased ($p \leqslant 0.05$) the DR% (7.27 to 13.78%) compared to the control (2.97%) (Table 8).

Table 7. Main effects of applying the soil amendments (limestone and steel slag), poultry litter (fresh or non-composted and composted for 45 days), and control (without poultry litter) on the severity of clubroot (caused by *Plasmodiophora brassicae*) based on note scale, percentage of diseased roots (DR%), healthy root volume, total dry weight of plant, fresh weight of inflorescence, diameter of inflorescences, and broccoli yield.

Soil amendments[1]	Clubroot and root growth				Plant growth and production			
						Inflorescence		
	Severity (%)	DR %	Healthy root volume (mL)	Root dry weight (g)	Dry weight of plant (g)	Fresh weight (g)	Diameter (cm)	Yield (kg ha^{-1})
Limestone	12.67 a	7.97 a	50.50 a	10.44 a	100.01 a	354.34 a	13.67 a	8,720 a
Steel slag	9.63 a	8.05 a	53.39 a	11.28 a	102.99 a	357.19 a	14.16 a	9,310 a
CV1(%)	16.51	9.37	14.86	19.96	13.43	22.08	21.71	18.98
Poultry litter [1]								
Fresh	14.06 a	13.78 a	54.93 a	11.75 a	105.00 a	352.65 a	13.18 a	8,780 a
Composted	9.77 a	7.27 ab	55.42 a	11.12 a	98.92 a	367.52 a	14.24 a	9,200 a
Control (without poultry litter)	9.68 a	2.97 b	45.48 a	9.71 a	100.59 a	347.16 a	14.32 a	9,060 a
CV2(%)	27.12	44.81	10.98	30.29	24.96	25.14	22.49	20.65

[1]For each factor (soil amendments or compounds) and in each column, means followed by the same letter did not differ statistically by Tukey's test ($p \leqslant 0.05$). The interaction between soil amendments × compounds had no significant effect.

The interaction between soil amendments and the application of poultry litter had no significant effect ($p \leqslant 0.05$) on any variable related to the concentration and accumulation of nutrients in broccoli plants. These two factors are presented separately in Table 8. Soil amendments had no significant effects on any of the measured characteristics, except for S levels, which were higher in plants cultivated in areas with limestone application. The use of poultry litter influenced the C/N ratio and N concentration of broccoli ($p \leqslant 0.05$). Fresh poultry litter resulted in broccoli plants with a low C/N ratio (8.18) compared with composted poultry litter (8.26) and the control (8.50), which did not differ from each other. The highest mean N concentration was found in plants fertilized with fresh poultry litter, followed by those fertilized with composted poultry litter (Table 8).

Table 8. Main effects of applying the soil amendments (limestone and steel slag), poultry litter (fresh or non-composted and composted for 45 days), and control (without poultry litter) on the carbon to nitrogen (C/N) ratio, mean N and S concentrations, and accumulation of N, S, Ca, Mg, P, and K in broccoli plants.

Soil amendments[1]	C/N ratio	N	S	N	S	Ca	Mg	P	K
		g kg^{-1}		g $plant^{-1}$					
Limestone	8.03 a	44.84 a	10.37 a	4.49 a	1.03 a	2.75 a	0.424 a	0.646 a	4.94 a
Steel slag	8.15 a	43.63 a	9.59 b	4.51 a	0.99 a	2.54 a	0.426 a	0.666 a	5.62 a
CV1(%)	7.15	6.74	4.87	9.41	9.00	9.91	4.25	7.2	13.44
Poultry litter[1]									
Fresh	8.18 b	46.48 a	9.55 a	4.87 a	1.01 a	2.90 a	0.491 a	0.682 a	5.35 a
Composted	8.26 a	43.40 ab	10.09 a	4.32 a	0.99 a	2.45 a	0.377 a	0.642 a	5.18 a
Control (without poultry litter)	8.50 a	42.84 b	10.31 a	4.31 a	1.03 a	2.59 a	0.407 a	0.645 a	5.31 a
CV2(%)	7.21	6.40	11.00	27.86	26.28	30.89	27.78	27.78	27.42

[1]For each factor (soil amendments or compounds) and in each column, means followed by the same letter did not differ statistically by Tukey's test ($p \leqslant 0.05$). The interaction between soil amendments × compounds had no significant effect.

Discussion

Use of green manure and larger broccoli seedlings

The high biomass supply with low N concentration and high C/N ratio from corn may immobilize nutrients, mainly N, during the initial stage of the broccoli crop cycle (Giacomini et al., 2003; Silva, 2008; Veras et al., 2016; Castro & Devide, 2018), negatively affecting the accumulation of biomass and nutrients in broccoli plants. The chemical composition and decomposition rate of organic residues in the soil affect the release of nutrients from plants (Silva, 2008). In general, N-rich materials with low C/N ratios typically decompose more quickly. Grass residues are poorer in N, more lignified, and more slowly decomposed by soil microorganisms (Giacomini et al., 2003).

Broccoli plants from areas previously with coriander and sunn hemp had the highest dry biomass and nitrogen accumulation (Tables 5 and 6). Coriander and sunn hemp biomass had the lowest C/N ratio, indicating faster decomposition and higher release rate than corn (Giacomini et al., 2003; Veras et al., 2016). However, sunn hemp decomposition may occur more slowly because of its high fiber content, which can influence its decomposition in soil (Castro & Devide, 2018).

The lowest growth of broccoli plants was observed in areas with corn biomass, indicating an inadequate synchrony between the release of nutrients from corn residues and absorption by the crop (Araújo et al., 2011; Melo, 2015). In this study, in addition to the N from green manure, the N supply for broccoli plants was complemented by mineral fertilization at planting and application of topdressing. However, N loss might have been enhanced by the high volume of precipitation during the broccoli cycle (Yuan et al., 2020) and the sloping relief in the region (Bhering et al., 2017; Assis & Aquino, 2018; Santos et al., 2020; Santos et al., 2022). N loss was not measured.

Some species can stimulate the germination of *P. brassicae* resting spores, thereby reducing disease severity in subsequent cultivations (Friberg et al., 2006; Hasse et al., 2007; Hwang et al., 2015; Chen et al., 2018). These plants can cause changes in soil pH, which may explain some of the results (Friberg et al., 2006). However, the underlying mechanism remains unclear. In the present study, the effects were directly related to improved healthy root development in broccoli, which were caused by the high supply/availability of nutrients, especially N (Melo, 2015; Bhering et al., 2017), in treatments with sunn hemp, followed by coriander; corn treatment proved least useful.

Relevant results were obtained by increasing seedling size under the adverse and limiting conditions of the present study. Using trays with a high number of cells, such as 200 and 288 cells, has become a common practice to produce various vegetables where seedling production is outsourced and decreases in production costs are sourced (Melo, 2015). Notably, using larger seedlings with a better-structured root system reduces the cycle or critical period of exposure to pathogens, reducing the loss caused by clubroot (Hwang et al., 2011; Hwang et al., 2012).

The use of larger seedlings may be recommended for summer crops to reduce losses due to clubroot disease and ensure broccoli production under conditions less favorable to the crop (Melo, 2015) or more favorable to the disease (Dixon, 2014; Gossen et al., 2014; Bhering et al., 2017). This growing period corresponds to the off-season broccoli production in Brazil, where producers are paid the highest prices for the crop. The use of larger seedlings resulted in an increase of more than 143% in yield, weight, and inflorescence diameter.

Steel slag and fertilization with poultry litter

The increase in soil pH, supply of Ca^{2+}, and decrease in Al^{3+} phytotoxicity are important strategies for managing clubroot and improving broccoli production (Donald & Porter, 2009; Bhering et al., 2017; Santos et al., 2020). The effect of steel slag was similar to that of limestone on soil chemical attributes (Prezotti & Martins, 2012; Caetano, Prezotti, Pacheco, & Guarçoni, 2016; Oliveira et al., 2020), clubroot intensity, biomass accumulation, and broccoli yield. However, the presence of calcium silicate was not demonstrated to control clubroot.

Fresh or composted poultry litter application supplied nutrients and increased the DR%; however, it did not affect biomass accumulation or broccoli yield. Fresh poultry litter is widely used by *Brassica* producers in the mountainous region of Rio de Janeiro (Grisel & Assis, 2012; Bhering et al., 2017; Sousa et al., 2020; Santos et al., 2022). This practice, associated with the non-correction of soil acidity, was suggested to be related to the high intensity of clubroot in the region by Bhering et al. (2017; 2020). This hypothesis was reinforced by the results of the present study; the increase in DR% was linked to fresh poultry litter application. When mineralized, soil organic matter can increase temperature and acidity by releasing organic and inorganic acids, depending on the material, dose, season, and soil attributes (Haynes & Mokolobate, 2001; Bhering et al., 2020).

Owing to the wide use of poultry litter for vegetable production (Grisel & Assis, 2012; Bhering et al., 2017; Bhering et al., 2020; Santos et al., 2022), studies are needed to elucidate the interactions among the type, quantity, and application method of this compound and its effects on the infection process of *Brassica* roots by *P. brassicae*. Furthermore, studies are required to assess the biological and chemical risks to human health posed by the application of poultry litter in *Brassica* crop production.

Conclusion

Green manure from sunn hemp and coriander is associated with many benefits, including increased root and broccoli plant growth, and it can improve broccoli production in tropical mountain regions. The use of larger seedlings is recommended for summer broccoli, as they reduce losses caused by clubroot and increase biomass accumulation and broccoli yield. Steel slag application resulted in soil acidity correction and plant growth similar to that observed with limestone application; however, its application did not affect clubroot intensity. The use of either fresh or composted poultry litter for 45 days increased the severity of clubroot on roots. Owing to the importance of poultry litter for broccoli cultivation in tropical mountain agroecosystems, further studies are required to evaluate its contribution to the establishment of *P. brassicae* in roots.

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