

Research Article

Cavalcade Legume (*Centrosema pascuorum***) Used as Soil Amendment in RD41 Rice Fields: Short-term Effects on the Soil Nematode Community, Soil Properties, and Yield Components**

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Abstract

Numerous phytoparasitic nematodes have been identified in Thailand's paddy fields, which routinely cause substantial reductions in rice crop yields. However, effective strategies for their management have yet to be documented. In this study, cavalcade legume was used as a soil amendment in RD41 rice fields to examine its effects on the soil nematode community, soil properties, and yield components compared to untreated control plots. The results demonstrated that the population densities of plant-parasitic nematodes (PPNs) in the order Tylenchida, primarily *Hirschmanniella* sp., significantly decreased in cavalcade-treated plots across all soil sampling periods. Moreover, there was an increase in the populations of beneficial nematodes within the orders Dorylaimida and Araeolaimida. In contrast, greater PPN populations were observed in the control plots compared to the initial nematode population. In addition to reducing PPN populations, this legume showed other benefits, specifically increased soil properties (available P) and rice plant growth (plant height and number of tillers). While there was no statistically significant difference in soil organic matter (SOM) content, the application of this legume tended to increase SOM content, in contrast to a decrease in SOM content observed in the control plots. Overall, this study provides valuable insights into the substantial advantages of using cavalcade legumes in RD41 rice fields.

Keywords: Beneficial nematodes, Leguminous crops, Metabarcoding, Rice-parasitic nematodes, Soil amendment, Soil-borne disease

1 Introduction

Plant-parasitic nematodes (PPNs) have been a significant biotic constraint in rice production, and their impacts, biology, and management have been extensively researched in Asia [1], [2]. Presently, twenty-nine species of PPNs associated with rice have been identified, but not all pose potential economic impacts [3]. Economic losses in rice production attributed to PPNs are estimated to be as much as 20% globally [4]. In field surveys conducted in Thailand,

five genera of PPNs have been observed [5], [6]. However, according to Kyndt *et al*. [7], only three nematode genera—*Hirschmanniella* spp., *Meloidogyne* spp., and *Pratylenchus* sp.—have been identified as the major species that attack rice. Of these nematodes, *Hirschmanniella* spp. is the dominant PPN found in rice fields that are regularly flooded, as it can endure in an oxygen-deprived environment [8]. Symptoms of damage caused by this nematode are not specific but include stunting, a decrease in culm height, delayed tillering, fewer shoots, and discoloration [9]. Varying

reductions to crop yield caused by *Hirschmanniella oryzae* have been reported, ranging between 12% and 39% in India and Thailand, depending on extent of nematode infestation and population density [10], [11]. Overall, it is well-documented that if nematodes are not properly managed, they can cause significant harm to rice crops.

Compared to other crop pests, managing PPNs is more challenging because they generally inhabit the soil and attack the plant's subterranean parts, primarily the roots [12]. Although many control measures are available, the effectiveness of methods applied in different locations/circumstances and to various crop types needs to be studied and updated [13], [14]. Recently, numerous studies have investigated the use of leguminous crops, either as cover crops or soil amendments, to combat PPNs in economic crops [15]–[18]. In addition to reducing PPN populations, certain legumes help reduce soil erosion, boost soil organic matter, and enhance yield benefits [19].

Wang *et al*., [20] noted various mechanisms that nematodes are suppressed by *Crotalaria* soil amendments: 1) poor or non-host effects, 2) allelopathic effects, and 3) enhancement of nematode antagonists. Masson *et al*., [21] demonstrated that introducing *Stylosanthes guianensis* (cv. Nina) to a conservation agriculture system in a Cambodian rice field, with no tillage and as a cover crop, significantly enhanced the microbial richness and diversity of rhizosphere communities, resulting in a 92% reduction of Pratylenchidae nematodes (*Hirschmanniella* spp.) in the rhizosphere. Similarly, cavalcade (*Centrosema pascuorum*), another leguminous species belonging to the Family Fabaceae [22], has been widely used as a cover crop or crop rotation to minimize the number of PPNs in Cambodian rice and Nigerian maize fields [18], [23]. This legume acts as a non-host for riceparasitic nematodes (*Meloidogyne graminicola* and *H. mucronata*) and contains bioactive compounds (quercetin, kaempferol, and rutin), which have a toxic effect on PPNs [24]. However, in Thai rice fields, no information has been documented regarding the utilization of cavalcade legume for controlling PPNs. Therefore, this study was conducted to examine the effects of cavalcade, when applied as a soil amendment, on the soil nematode community through metabarcoding analysis of 18S rRNA amplicons. Additionally, soil properties and rice yield components were assessed.

2 Materials and Methods

2.1 *Site description and experimental design*

This field experiment, conducted from May to December 2023, was established in paddy fields located at the Department of Rice, Bangkok, Thailand (13°50'59.0"N 100°34'26.4"E). The soil texture is clay \approx 17% sand, 27% silt, and 56% clay). The experiment area was partitioned into plots, each plot was 6.5 m $(\text{length}) \times 7 \text{ m}$ (width). The distance between each plot was 0.5 m, with 7.5 m distance between treatments (Figure 1). The experiment was set up in a Randomized Complete Block Design (RCBD) with three replicates for each of the soil plots amended with cavalcade legume and untreated control plots.

Initial populations of PPNs were determined prior to cavalcade planting time. Near the end of June 2023, cavalcade seeds were sown in each plot (at a rate of 5 kg per 0.16 hectare) and allowed to grow for six weeks. Subsequently, cavalcade plants were incorporated into the soil at a depth of 5–10 cm from the soil surface using a hoe and left to decompose for three weeks. Meanwhile, the RD41 rice seedlings were prepared at the rate of 0.35 kg per plot in 434 well seeding trays, followed by the transplantation of 3-week-old rice seedlings into each block under flooded conditions. Fertilizer (N-P-K) was applied three times: 46-0-0 (300 g/block) at week 2 after transplant, and 16-20-0 (600 g/block) at weeks 4 and 6 after transplanting. During the rice cycle, weed control was conducted by hand every two weeks.

Figure 1: A schematic representation of the map displaying the treatment blocks.

2.2 *Data collection*

Soil and root samples were collected randomly from each block at five different times: before planting, three weeks after cavalcade incorporation into the soil, one month after rice transplanting (2-month-old rice plants), two months after rice transplanting (3-monthold rice plants), and seven days after harvesting. In each block, six composite soil or root samples were randomly collected (X shaped) and homogenized in a bucket. One representative sample (containing 500 cc soil and 5 g root) was then selected, placed in labeled plastic bags and transported to the nematology laboratory for nematode extraction. Also, plant growth measurements of plant height and number of tillers were taken $(n = 18)$ when the rice plants were 3months old. Subsequently, the weight of panicles, 1000-seed weight, percentage of filled grains, and actual yield (kg/0.16 hectare) were assessed after the rice harvest ($n = 3$ for actual yield and $n = 18$ for other parameters).

Additionally, 1 kg of soil was collected twice during the experiment, just prior to planting and at completion (rice harvest), and sent to the Department of Soil Sciences at the Faculty of Agriculture, Kasetsart University, for analysis of various soil properties. These properties included soil pH, soil organic matter, available phosphorus (P), and exchangeable potassium (K), magnesium (Mg), and calcium (Ca).

2.3 *Nematode extraction*

Nematodes were extracted from 200 cc soil using Cobb's Sieving and Decantation and the Modified Baermann's Funnel techniques [25] and 1 g root (for population dynamic examination) using Baermann's Funnel technique [26]. Nematodes were collected from the bottom of the funnel after 48 h, examined, and enumerated using a compound microscope (Olympus BX50).

2.4 *Nematode metabarcoding*

The effects of soil amended with cavalcade legume on the soil nematode community were studied. The nematodes isolated from 200 cc of soil and collected at each period (before planting, three weeks after

incorporating cavalcade into the soil, and after harvesting), were used for DNA extraction using lysis buffer consistent with the method outlined by Holterman *et al*. [27]. DNA quality and quantity were measured using a NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific). The NanoDrop thresholds of 1.8–2.2 for OD260/280 indicate the purity of the DNA sample. The pooled DNA of each sample was sent to F.N. Science Company Limited for sequencing using Illumina Novaseq SP(PE250). The 18S rDNA (eukaryotic) amplicons were amplified using V4 primer sets: 18S forward (5′- CCAGCASCYGCGGTAATTCC-3′) and reverse (5′-ACTTTCGTTCTTGAT-3′). The average read count per sample was 100,000 after demultiplexing.

The data was analyzed using the Galaxy platform which is available online at https://usegalaxy.eu/. Paired-end reads were checked using FastQC [28] and MultiQC [29]. Assembled reads (mean length 424 bp) were merged using FastP [30] and further processed, including quality filtering, chimera checking, and clustering, using DaDa2. Representative sequences were filtered to remove other microbial sequences and assigned taxonomic identities based on the SILVA v138 rRNA database using the software package QIIME2 [31].

2.5 *Diversity and differential abundance analyses*

Order-level taxonomic abundances were utilized to compute diversity metrics. Alpha diversity metrics including Shannon's diversity index, Simpson's diversity index, and Chao 1 diversity index, Venn diagram, and Beta diversity to determine Bray-Curtis dissimilarity were calculated for each sample using R version 4.3.1 [32]. PCoA was performed using Phyloseq v1.36.036 [33]. STAMP analysis was performed to identify differentially abundant nematodes between treatments with the criteria *p-*value < 0.05 [34].

2.6 *Statistical analysis*

Data were statistically analyzed using the SPSS software (version 23.0; SPSS Inc.; Chicago, IL, USA). Differences between treatments in *Hirschmanniella* population dynamics, soil properties and rice yield components were assessed using the Student's pairedplot design test at the 0.05 significance level.

3 Results

3.1 *Population dynamics of Hirschmanniella sp.*

Following nematode extraction from soil, *Hirschmanniella* sp. was identified as the predominant PPN in the studied rice field, with its presence exceeding 90% of all samples. As a result, this study focused on evaluating the population dynamics of this nematode.

The *Hirschmanniella* populations in the soil and rice roots were monitored monthly throughout the experiment (Figure 2). This study determined that the *Hirschmanniella* populations in the soil were significantly reduced in the cavalcade-treated plots compared to the untreated control plots and initial populations (first soil sampling). Subsequently, nematode populations in the soil in both treatments gradually declined (third and fourth soil sampling), and a greater number of nematodes were observed in the rice roots. Fewer nematodes were found in the rice roots grown in the cavalcade-treated plots than in the untreated control plots throughout the study (Figure $2(b)$).

Figure 2: Population dynamics of *Hirschmanniella* sp. in soil (a) and rice roots (b) from RD41 rice fields amended with cavalcade legume (C1) compared with untreated control plots (F1). The asterisk (*) indicated that means are significantly different according to the Student's paired-plot design test at the 0.05 significance level.

Figure 3: Relative abundance of nematodes in 200 cc soil samples collected from RD41 rice plots, both amended with fresh cavalcade legume (C) and non-amended control (F), at three different time periods: before planting (C1 and F1), three weeks after the legume incorporation into the soil (C2 and F2), and seven days after rice harvest (C3 and F3). (a) Relative abundance of all nematode groups classified by order; and (b) relative abundance of plant-parasitic nematodes categorized in the order Tylenchida.

Figure 4: Different abundance of soil nematode order in 200 cc soil samples collected from RD41 rice plots, both amended with fresh cavalcade legume (C) and non-amended control (F), at three different time periods: (a) before planting (C1 and F1), (b) three weeks after legume incorporation into the soil (C2 and F2), and (c) seven days after rice harvest (C3 and F3).

3.2 *Composition of the soil nematode community*

The relative abundance of groups (orders) of soil nematodes found in different treatment plots at various periods of soil samplings was studied (Figure 3). At the beginning of the experiment, the dominant orders in the cavalcade-treated plots (C1) were Triponchida (59.4%) and Tylenchida (34.1%), while in the untreated control plots (F1), the dominant orders were Tylenchida (41.4%), Rhabditida (32.1%), and Araeolaimida (23.4%). In the second soil samplings, taken three weeks after treatments with the cavalcade legume (C2), the number of nematodes in the orders Dorylaimida and Araeolaimida had increased abundantly, while those in the orders Tylenchida (plant-parasitic nematodes) and Triplonchida decreased threefold compared to the populations at the beginning of the experiment. In contrast, in the untreated control plots (F2), the populations of PPNs doubled from the initial population count. At the rice harvest stage, fewer populations of PPNs were observed in the cavalcade treatment (C3) compared to

the untreated control plots (F3), while the population of Triplonchida was greater (Figure 3(a)).

When focusing solely on the order Tylenchida, *Hirschmanniella* sp. emerged as the dominant nematode, with an abundance exceeding 80% in the early stage and reaching 100% at later stages. In addition to promoting the diversity of beneficial and free-living nematodes, cavalcade legume soil amendments decreased the population of *Hirschmanniella* in the soil. Moreover, it increased the number of *Filenchus* sp. and *Aphelenchus* sp. (C2), which are categorized as both weak plant parasites and fungal-feeding nematodes (Figure 3(b)), leading to a more diverse range of nematode groups in the paddy fields.

Different abundance of each nematode order was compared between the two treatments at different periods of soil samplings (Figure 4). At all soil sampling periods, the population of PPNs in the order Tylenchida was lower in soil treated with the cavalcade than in the untreated plot controls, while a significantly higher number of beneficial nematodes in

the orders Dorylaimida and Triplonchida was observed in the cavalcade-treated plots (Figure 4(b) and (c)).

The indices of Chao 1, Inverse Simpson, and Shannon were utilized to determine soil nematode diversity between the treatments at all soil sampling periods (Figure 5). These indices can reflect the $α$ diversity of nematodes across various habitats and have been effectively employed to describe nematode diversity in natural forest ecosystems [35] as well as in a National Park in Sweden [36]. In the current study, no significant differences were observed in all indices; however, the highest values of the Chao 1 and Shannon indices were observed at C2 (three weeks after cavalcade treatment). This suggests that the soil nematode diversity was somewhat affected by the cavalcade legume soil amendment.

Although the Chao 1, Inverse Simpson, and Shannon indices showed no significant differences, the Venn diagram indicated that the similarity of amplicon sequence variants (ASVs) between the two treatment plots was relatively low (only 5 and 10%), and the total number of ASVs was similar, except for the third soil sampling (C3:F3) (Figure 6). This result revealed that different nematode communities were observed between the two plots. However, when considering the relative abundance in Figure 3, the cavalcade amendments led to a 70% increase in beneficial nematodes (Dorylaimida and Triplonchida), while in the untreated plots, the dominant group was Tylenchida (PPNs), which accounted for over 80%.

PCoA of the soil nematode sequencing data was collected at different treatments (with or without cavalcade amendments) and soil sampling periods, and then analyzed using the Bray-Curtis distance principal coordinate analysis. The two principal component axes accounted for 86.09% of the overall variation, with the first axis (PC1) explaining 48.84% and the second axis (PC2) explaining 37.25%. These figures indicated that there were three diverse groups of nematodes: 1) F1 and F2, 2) C1, C3, and F3, and 3) only C2. Based on the findings observed, incorporating cavalcade legume into the soil (C2) significantly affected soil nematode diversity compared to the initial population (C1), while similar levels of nematode diversity in the control plot were observed between the first two soil sampling periods (F1 and F2) (Figure 7).

This study suggests that amending soil with the cavalcade legume enhanced beneficial nematode diversity, leading to a reduction in the number of PPNs, particularly during stages after the legume had been incorporated into the soil.

Figure 5: Chao 1, Inverse Simpson, and Shanon indices of soil nematode community in 200 cc soil samples collected from RD41 rice plots, both amended with cavalcade legume (C) and nonamended control (F), at three different time periods: before planting (C1 and F1), three weeks after the legume incorporation into the soil (C2 and F2), and seven days after rice harvest (C3 and F3).

Figure 6: Venn diagram showing overlap of amplicon sequence variants (ASVs) between cavalcade-treated plots (C) and untreated control (F) at three different time periods: before planting (C1 and F1), three weeks after the legume incorporation into the soil (C2 and F2), and seven days after rice harvest (C3 and F3). The size of the circles is proportional to the number of ASVs recorded in each sample.

Figure 7: Principal coordinate analysis of the soil nematode community comparing cavalcade-treated plot (C) and untreated control (F) at three different soil sampling periods: before planting (C1 and F1), three weeks after the legume was incorporated into the soil (C2 and F2), and seven days after rice harvest (C3 and F3) based on Bray–Curtis distances.

3.3 *Soil properties*

Properties of the soil amended with cavalcade legume and the untreated control soil were compared at the beginning and termination of the experiment to determine the effects of the cavalcade legume treatment. No significant differences were noted for pH, soil organic matter content (SOM), exchangeable K, and Mg between the beginning stage and termination of the treatments. However, available P was significantly higher in the cavalcade treatment, whereas no difference was observed in the control plot, and exchangeable Ca significantly increased in both treatments. For SOM, although there was no significant difference, the cavalcade treatment showed some enhancement, while the control showed a reduction (Table 1). The data showing increases in certain soil properties (available P and SOM) indicates that using cavalcade as a soil amendment in the RD41 rice fields is potentially beneficial.

Values are means \pm SE of three replications. Means were compared using the Student's paired-plot design test at the 0.05 significance level.

Values are means \pm SE of three replications for actual yield and eighteen replications for other parameters. Means were compared using the Student's paired-plot design test at the 0.05 significance level.

3.4 *Rice yield components*

Soil amended with cavalcade benefited RD41 rice plants by increasing plant height and the number of tillers. However, there were no significant differences in panicle weight, 1000 seed weight, percentage of filled grain, or actual yield between the treatments (Table 2).

4 Discussion

Plant parasitic nematodes (PPNs) are a significant biotic limitation, which harms plant growth and causes substantial losses to rice production in Asian countries [7]. In this study, the influence of soil amended with cavalcade legume on the soil nematode community, soil properties, and rice yield components was investigated and compared with untreated control plots. The data revealed that the population of beneficial

and free-living nematodes in the treated soil increased abundantly, whereas the number of PPNs recovered from soil and root samples declined significantly throughout all soil sampling periods. Moreover, adding cavalcade legumes seemed to have a positive effect on soil properties (SOM and available P) and yield benefits (plant height and the number of tillers). These results are consistent with previous experiments conducted in Cambodian lowland rice fields managed under a conservation agriculture system, with notillage and a cover crop of *Stylosanthes guianensis* (cv. Nina), as reported by Beesa *et. al*.*,* [18] and Masson *et. al*.*,* [21]. The current findings similarly showed that soil amended with the legume provided significant benefits, notably reducing the number of PPNs (*H. mucronata* and *M. graminicola*) and somewhat elevating soil quality (soil organic carbon, available P, and exchangeable K) as well as increasing rice growth.

The predominant PPN found in the studied rice fields was *Hirschmanniella* sp., identified by its morphological and molecular characteristics. This nematode is notoriously widespread in paddy fields in Thailand's central plain region [37]. The dynamics of the nematode population were studied, revealing a much smaller population of *Hirschmanniella* sp. in rice fields treated with cavalcade legume at all sampling periods, compared to the untreated control plots. It is most likely that incorporating cavalcade legume achieved this beneficial outcome by reducing the *Hirschmanniella* sp. population in the soil before the rice was transplanted, thereby resulting in a lower infection rate in the rice roots. Other studies have similarly demonstrated that using leguminous crops as a soil amendment or crop rotation reduces PPN populations. Studies of various legumes, such as *Canavalia ensiformis*, *Crotalaria retusa*, *Indigofera hirsuta*, *I. nummularifolia*, *I. spicata*, *I. suffruticosa*, *I. tinctoria*, and *Tephrosia adunca*, were found to reduce root-knot nematode populations in infested tomato fields [15]; crops rotated with *Mucuna pruriens*, *Crotalaria spectabilis*, and *C. retusa* effectively decreased mixed field populations of *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* [16]; soil amended with cavalcade leaves showed significantly lower numbers of galls and egg masses on okra infected by *M. javanica*, compared to nematode-inoculated control plants [38]. However, using cavalcade to manage PPNs in Thai rice fields had not been previously studied. Indeed, this study is the first to report the potential efficacy of using cavalcade to decrease PPN populations, particularly *Hirschmanniella* sp.,

which has been determined to cause such severe damage in Thailand's rice fields.

There are several plausible mechanisms explaining the role cavalcade legume has in reducing PPN populations: 1) Beesa *et al*., [24] determined that secondary metabolites in this legume (particularly, quercetin, kaempferol, and rutin) kill, paralyze, and repel *H. mucronata* and *M. graminicola*. 2) The soil amended with this legume enhances the SOM, which simultaneously increases the number of predatory nematodes and decrease the PPN population. This hypothesis is supported by our findings and other studies that showed similar results when cavalcade was applied as a soil amendment in maize [23], [39] and rice fields [18]. 3) Root exudates released from certain leguminous species contain carbon-rich compounds (amino acids, organic acids, sugars, phenolics, secondary metabolites, and proteins), which attract soil microbial communities and fauna [40], resulting in increased microbial abundance and diversity [41], [42]. The increase of some soil antagonistic fungi and bacteria, such as *Trichoderma* spp., *Streptomyces* spp., and *Bacillus* spp., negatively affects the survival of PPNs [43]–[45]. Additionally, these microorganisms have been reported to promote plant growth and induce plant resistance, thereby suppressing a wide range of phytonematodes such as *Meloidogyne* spp., and *Heterodera glycines* [46]–[48].

Besides reducing population densities of PPNs, the application of this legume showed some potential for promoting plant growth, specifically plant height and the number of tillers, although there were no significant changes in actual yields. The improvement in these growth parameters is likely linked to a decrease in PPN infections in the rice roots and the elevation of available phosphorus and soil organic matter (SOM) levels in the plots treated with cavalcade. Phosphorus is a key component of the ATP molecule, which supplies energy to plants for various processes, including photosynthesis, protein synthesis, nutrient translocation, respiration, and enhanced root development [49]. Several studies indicated that phosphorus deficiency in rice limits rice tillers and stunts plant growth and development, resulting in decreased grain yield productivity [50], [51]. Yoseftabar [52] found that more phosphorus application improves rice growth parameters such as a higher number of tillers, more fertile tillers, and greater grain yield. Currently, there is no substantiated evidence suggesting that elevated levels of soil organic carbon improve the growth and development

of rice. However, several reported studies have shown that rice yields significantly increased in fields with higher soil organic carbon content [53], [54].

5 Conclusions

This study provides strong evidence of specific benefits provided to RD41 rice fields by amending the soil with cavalcade legume: The application of this legume reduced significantly the population of plantparasitic nematodes and abundantly increased the population of beneficial and free-living nematodes. Soil properties were improved—plots treated with cavalcade exhibited a significant increase in available phosphorus (P) and a slight improvement in soil organic matter (SOM), compared to the untreated control plots. And, important rice growth parameters improved, specifically plant height and the number of tillers. Based on our findings, this strategy can be adopted by farmers to manage PPNs in RD41 rice fields. The suggested application rate for the amendment is 5 kg per 0.16 hectare, with a three-week incubation period (following the incorporation of a six-week-old cavalcade into the soil) before transplanting rice to prevent phytotoxicity. The use of this legume not only reduces PPNs but also promotes soil fertility and yield benefits, enabling farmers to achieve higher profits. Although cost-effectiveness was higher in cavalcade-treated plots compared to the untreated control (~250 THB/0.16 ha), the actual rice yield was approximately 23 kg/0.16 ha higher than that of the control. Additionally, in the long term, it can enhance soil fertility, leading to reduced chemical fertilizer and pesticide inputs, thereby lowering costs. However, this result requires further investigation, particularly regarding the utilization of this legume in agriculture or large-scale industry, which needs to be clarified.

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Author Contributions

N.B.: conceptualization, investigation, methodology, investigation, writing an original draft, data curation, data analysis; P.M.: investigation, data analysis, N.K.: investigation, research design, writing—reviewing and editing; T.D.: reviewing and editing; K.J.: investigation, conceptualization, reviewing and editing; A.S.: reviewing and editing; B.C.: conceptualization, research design, investigation, writing—reviewing and editing, funding acquisition, project administration. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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