

## Article

# Impacts of Fallow Conditions, Compost and Silicate Fertilizer on Soil Nematode Community in Salt-Affected Paddy Rice Fields in Acid Sulfate and Alluvial Soils in the Mekong Delta, Vietnam

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**Abstract:** Avoidance of intensive rice cultivation (IRC) and soil amendments are potential practices to enhance soil properties. There is only limited information on the effects of reduced IRC and its mixture with compost or silicate fertilizer (Si) on the soil nematode community in salt-affected soils. This study aimed to assess the shifts of soil nematode community by reducing a rice crop from triple rice system (RRR) to a double rice system and mixed with compost or Si in paddy fields in acid sulfate soil (ASS) and alluvial soil (AL) in the Mekong Delta, Vietnam. Field experiments were designed with four treatments in four replicates, including RRR and a proposed system of double-rice followed by a fallow (FRR) and with 3 Mg ha<sup>-1</sup> crop<sup>-1</sup> compost or 100 kg ha<sup>-1</sup> crop<sup>-1</sup> Si. Soils were collected at harvest after the 2 year experiment, reflecting the fifth and third consecutive rice crop in RRR and FRR system, respectively. Results showed that reduced IRC gave a significant reduction in abundance of plant-parasitic nematodes (PPN), dominated by *Hirschmanniella* and increased abundance bacterivorous nematodes when mixed to compost and silicate fertilizer in ASS. In addition, reduced IRC increased nematode biodiversity Hill's indices and reduced herbivorous footprint in ASS. Proposed system having compost or Si had strongly increased in bacterivorous and omnivorous footprints. Particularly, reduced IRC mixture with Si increased abundance of *Rhabdolaimus*, *Mesodorylaimus* and *Aquatides*, metabolic footprints (structure footprint, bacterivorous, omnivorous and predator) and diversity Hill's N1 index in ASS. Our results highlighted that reduced IRC was a beneficial practice for decreasing abundance of PPN in salt-affected soils and increasing abundance of FLN in ASS. IRC mixture with compost or Si had potential in structuring the nematode communities with increasing biodiversity, trophic structure, and metabolic footprints.

**Keywords:** metabolic footprints; omnivorous; saline soil; soil amendment; sustainability; trophic structure

## 1. Introduction

The Vietnamese Mekong Delta (VMD) region predominantly grows rice and has a long history of extremely intensive cultivation of rice in irrigated paddy fields. The consecutive growth of three rice crops in the same field per year (called triple-rice cropping system) is a usual cropping system in the VMD, although it is not popular in the rest of the world. In this system, the soil is irrigated and flooded frequently during rice cultivation periods, and this process has been repeated every crop and year. Moreover, a previous study reported that growing rice in this system relies on a high amount of chemical fertilizers and pesticides [1], which may cause serious environmental problems, e.g., water pollution [2], increase in pests and diseases [3], and declined soil quality [4]. In the VMD, acid sulfate soil occupies 40% of total rice cultivation land in the VMD and the acid sulfate soil has two common forms, called the potential and actual acid sulfate soil [5,6]. In the potential acid sulfate soil, an acidic material, pyrite, has not been exposed to air, and the soil is not strong acidic and there are no direct effects on soil quality and crop. In contrast, the actual acid sulfate soil occurs when the pyrite has reacted with oxygen to form sulfuric acid, which has potential effects on soil quality and crop. In recent years, salinity water intrusion into agricultural lands is becoming a severe problem in this area due to climate changes, particularly in the dry season from February to May [7]. This problem occurs annually, causing many difficulties in growing rice and damage to farmers' income [1]. According to Wassmann et al. [8], the paddy fields in Kien Giang (KG) and Ben Tre (BT) sites have been affected by saltwater intrusion and it becomes more serious in recent years. For instance, salinity water exceeding 4 g L<sup>-1</sup> salinity concentration intruded up to 30–40 km into upstream parts of rivers and surrounding agricultural areas, particularly in BT site, in which the salinity concentration in irrigated water reached up to 30–35 g L<sup>-1</sup> and reduced not only total rice cultivated areas but caused loss of 240,000 t of rice across the VMD in the dry season [1,7,9]. Thus, the triple-rice cultivation system will not be a sustainable strategy for the future and it is now a priority to find a suitable cropping system, including soil amendments, for sustainable rice cultivation farming. Therefore, reduced-intensive rice crop in triple rice cropping system has been considered to be a stable cropping system that can adapt to climate changes and might improve soil physicochemical and biological properties by reducing the extremely intensification of crop. Soil nematodes are tiny and diverse organisms, and are widely distributed. Free-living nematodes (FLN) play an important role in the decomposition of organic matter in soil habitats, and directly and indirectly contribute to nutrient cycling, resulting in improving soil fertility [10,11]. Also, soil nematodes are sensitive to environmental conditions such as different management techniques like crop rotation, tillage and fertilization regimes [12–16]. However, most studies have been conducted in natural soils and paddy fields in non-salt affected areas. Our recent study focused on the effect of saltwater intrusion on nematode community in paddy rice fields and found that saltwater intrusion reduced total abundance and changed the trophic structure and diversity [17]. However, how nematodes respond to changes in cropping system and soil amendment in salt-affected soils has not been reported. In the VMD, the triple rice cropping system has been adopted since the 1980s [18]. Long-term rice intensive cultivation increases soil-borne plant pathogens [19] and plant-parasitic nematodes (PPN) [20]. Many PPN have been identified that cause yield losses, e.g., *Meloidogyne* and *Hirschmanniella* are two predominant genera reported worldwide, particularly in irrigated paddy fields [20]. The abundance of *Hirschmanniella* generally reaches a maximum at tiller and flowering stages of rice plants and abundance has increased with the addition of high amount nitrogen fertilizer during the cultivation period [21]. Several agricultural techniques have been proposed to suppress the numbers of PPN in various crops, particularly in upland conditions. Of these, crop rotation is a potential practice that suppresses the numbers of PPN and stimulates beneficial nematodes in paddy rice fields [22]. Soriano and Reversal [23] recommended that rotating one or two

consecutive crops of cowpea in rice field or including a fallow period before a rice crop reduced PPN populations and improved rice yield by 30–80%.

The utilization of biomass residues like compost [24], biochar [25] and green manure [26] in agriculture showed many benefits that not only can improve soil physicochemical and biological properties and crop productivity but also be a potential technique in suppressing plant pathogens including PPN like *Meloidogyne* and *Hirschmanniella* [27,28]. For example, the application of organic manure combined with less chemical fertilizer increased total abundance of FLN in rice fields, particularly in abundance of omnivorous, predator nematodes, compared to that when chemical fertilizer only was used [29]. Also, composted materials may indirectly affect the nematode community by increasing the food resources, e.g., predators controlled the number of PPN [30–32].

Silicate is the second most abundant element after oxygen in the earth's crust and has frequently been reported as a beneficial mineral element for plant growth [33,34]. Generally, Si increases plant resistance to different abiotic and biotic stresses [35], such as salt and drought [36], extreme temperature [37], nutrient deficiency [38], aluminum toxicity [39] and disease [40]. Moreover, Si enhances multiple adaptive responses during stress such as hormonal regulation, antioxidant activity activation, minerals uptake and organic acid anions, and phenolic compounds exudation [39,41,42]. Several studies have demonstrated that soil-applied Si can induce systemic defenses against different pathogens in various plants. For example, the applications of Si to paddy fields resulted in increased control of several economically important diseases such as blast (*Magnaporthe grisea*) [43], brown spot (*Bipolaris oryzae*) [44], and sheath blight (*Rhizoctonia solani*) [45] with improved yield and quality. In recent years, there have been studies on Si and PPN interaction but they are still based on laboratory conditions. For example, the effect of root-applied Si on the enhancement of induced resistance to the root-knot nematode *Meloidogyne graminicola* in rice has been reported [46], indicating that Si application induced phenolic compounds and hydrogen peroxide in rice plants against the nematode. Besides *M. graminicola*, *Hirschmanniella* is the most dominant genus in the irrigated paddy field, particularly in the extremely intensive rice system in the VMD [14]. However, the impacts of Si fertilizer on soil nematode community in the paddy rice field are unknown especially its potential suppression of *Hirschmanniella* in field conditions.

To cope with increasing saline water intrusion and drought condition, alternative agricultural systems, that is reducing the intensive growth of rice from a triple-rice cropping to a double-rice cropping system and adding different amendments to improve soil properties, are proposed to enhance soil biological properties for sustainable agriculture practices. The objective of this study was to evaluate the effects of reduced intensive rice crops and compost and Si application on the soil nematode communities, with the focus on composition, trophic structure, metabolic footprint, diversity, and particularly the PPN community, in paddy fields.

## 2. Materials and Methods

### 2.1. Study location and Experiment Design

Two fields used for experiments in this study were the same as described in our previous study [47]. One was located in an acid sulfate soil in Ben Tre province (9°58'22.51" N, 106°28'51.22" E) and the other in an alluvial soil in Kien Giang province (9°43'34.43" N, 105°10'55.06" E). These rice fields are intensively monocultured, i.e., consecutive cultivation of three rice crops annually. Overall, VMD is in the tropical monsoon area where the dry and rainy seasons are separated by the different precipitation amounts and temperature. Generally, the rainy season starts from May until October, and the dry season starts from November until April of the following year. Climate conditions are suitable for rice growth throughout the year in this area, and rice is usually grown in the spring–summer (SS) season (February to May), the summer–autumn (SA) season (June–September), and the winter–spring (WS) season (October–January). The research

areas were in a typical tropical moist climate with an average annual temperature of 27°C. The average rainfall for each crop in the VMD accounts for around 15%, 60% and 25% of the total annual rainfall for SS, SA and WS crops, respectively [9]. These fields have a long history of use for paddy rice cultivation (more than 10 years). The soils are classified as Sali–Thionic Fluvisols at BT and Sali–Gleyic Fluvisols at KG according to the International Union of Soil Sciences (IUSS) Working Group World Reference Base for Soil Resources (WRB) (2015). The initial soil physicochemical properties of the acid sulfate soil were pH 4.64, EC 1.24 mS cm<sup>-1</sup>, total C 15.7 g kg<sup>-1</sup>, total N 1.55 g kg<sup>-1</sup>, available P 6.91 mg kg<sup>-1</sup> and soil texture of silty clay (1.9% sand, 56.2% silt, 41.8% clay), and those of the alluvial soil were pH 4.65, EC 1.16 mS cm<sup>-1</sup>, total C 15.0 g kg<sup>-1</sup>, total N 1.32 g kg<sup>-1</sup>, available P 20.9 mg kg<sup>-1</sup> and soil texture of silty clay (0.8% sand, 42.0% silt, 57.2% clay). The annual rainfall in the VMD varied by years and regions from 1984 to 2015 [9] both experiment sites, with an average of 1505 mm in Ben Tre site and 2154 mm in Kien Giang site. Soil physicochemical properties of RRR treatment among locations at harvest were analyzed (Table 1).

**Table 1.** Soil characteristics at harvest in two soils with triple rice system, the acid sulfate soil in Ben Tre province and the alluvial soil in Kien Giang province, Vietnam.

Soil	SM	pH <sub>1:2.5</sub>	EC <sub>1:2.5</sub>	Total C	Total N	C/N	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	ESP
Acid sulfate soil	29 ± 1.5 <sup>b</sup>	5.0 ± 0.1	1.18 ± 0.04 <sup>a</sup>	18.3 ± 0.5 <sup>b</sup>	1.7 ± 0.02 <sup>b</sup>	10.6 ± 0.2	681 ± 26 <sup>a</sup>	38.3 ± 1.4 <sup>a</sup>	172 ± 0.4 <sup>a</sup>	19.2
Alluvial soil	36 ± 0.6 <sup>a</sup>	4.9 ± 0.1	0.56 ± 0.04 <sup>b</sup>	22.8 ± 1.1 <sup>a</sup>	2.0 ± 0.04 <sup>a</sup>	11.4 ± 0.3	294 ± 25 <sup>b</sup>	31.2 ± 1.5 <sup>b</sup>	10.9 ± 2.3 <sup>b</sup>	8.8
<i>p-value</i>	**	ns	***	**	**	ns	***	*	***	

Value is mean and standard error ( $n = 4$ ). Different letters indicate significant differences among soils at \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns-not significant. SM, soil moisture (%); EC, electric conductivity (mS cm<sup>-1</sup>); Total C, total carbon (g kg<sup>-1</sup>); Total N, total nitrogen (g kg<sup>-1</sup>); Na<sup>+</sup>, soluble sodium (mg kg<sup>-1</sup>); K<sup>+</sup>, soluble potassium (mg kg<sup>-1</sup>); Ca<sup>2+</sup>, soluble calcium (mg kg<sup>-1</sup>); ESP, exchangeable sodium percentage (%).

A total of four treatments and four replicates were designed in randomized blocks in two fields with different soils, that is acid sulfate soil in the Ben Tre site and alluvial soil in the Kien Giang site (Table 2). The experiment was done with six consecutive crops in total from February 2018 and repeated in 2019. Compost and silicate fertilizer were basal applied to every crop from the start of SS season in 2018 until the SA season in 2019, except in fallow plots in SS season. The compost used was a commercial product made from sugarcane filter cake (Phan Huu Co Sinh Hoc Nha Nong PPE; PPE Co., Ltd., Can Tho, Vietnam), which comprises 30% moisture, 8.7 pH, 17.1 mS cm<sup>-1</sup> EC, 154 g total C kg<sup>-1</sup>, 26 g total N kg<sup>-1</sup> and 3.6 g available P kg<sup>-1</sup>. Silicate fertilizer used was a commercial product (Super Silic + TE; SITTO Vietnam Co., Ltd., Dong Nai, Vietnam) containing 650 g kg<sup>-1</sup> SiO<sub>2</sub> and macronutrients of 20 g kg<sup>-1</sup> K<sub>2</sub>O, 10 g kg<sup>-1</sup> magnesium and microelements 0.2 g kg<sup>-1</sup> manganese and 0.1 g kg<sup>-1</sup> zinc. Both compost and silicate fertilizer were base applied in the soil surface 3 days before sowing each crop with rates of 3 Mg ha<sup>-1</sup> and 100 kg ha<sup>-1</sup>, respectively. All treatments received the same amount of NPK (inorganic) fertilizers, e.g., urea, superphosphate, and potassium chloride at rates of 100 kg N, 60 kg P<sub>2</sub>O<sub>5</sub>, and 30 kg K<sub>2</sub>O ha<sup>-1</sup> for the WS crop (WS18–19) and 80 kg N, 60 kg P<sub>2</sub>O<sub>5</sub>, and 30 kg K<sub>2</sub>O ha<sup>-1</sup> for SS18, SA18, SS19, and SA19. The total dose of P was applied as basal in each crop. For urea, 20% of total N was applied at 7 days after sowing or transplanting (depending on season), 40% of total N and 50% of total K were applied at 20 days after sowing or transplanting, and the remaining N and K were applied at 40 days after sowing or transplanting. In rice crops, soils are kept in flooded conditions during the cultivation period, in which the water was kept above the soil surface at 5–10 cm height, depending on the growing stages. In general, the water level was maintained in rice plots from 7 days after sowing until 10 days before harvest. All data related to nematodes were already reported in our previous study [17], in which average data in each location were shown, while this study focused variations within a single location, which were affected by cropping pattern and amendment, de-

scribed above. Some results in the physicochemical properties are also shown in our previous study [17], in which the effects of cropping pattern and amendment were not evaluated.

**Table 2.** The schema of cropping system and soil amendment in field experiment.

Treatments	Cropping Sequence	Amendment (Amount Crop <sup>-1</sup> )	Cultivation Seasons				
			2018			2019	
			SS (Feb–May)	SA (Jun–Sep)	WS (Oct–Jan)	SS (Feb–May)	SA (Jun–Sep)
RRR	R–R–R–R–R	No	R–	R–	R–	R–	R–
FRR	F–R–R–F–R–R	No	F	R–	R–	F	R–
FRR + Compost	F–R–R–F–R–R	Compost (3 Mg ha <sup>-1</sup> )	F	R+	R+	F	R+
FRR + Si	F–R–R–F–R–R	Silicate fertilizer (100 kg ha <sup>-1</sup> )	F	R+	R+	F	R+

RRR, triple–rice cropping system; FRR, a fallow and double–rice cropping system. SS, spring–summer; SA, summer–autumn; WS, winter–spring in 2018 and repeated in 2019. R–, rice crop without amendments; R+, rice crop with amendments; F, fallow without amendments.

## 2.2. Soil Sampling and Analyses

### 2.2.1. Soil Sampling

Soil samples were collected at harvest in the SA season in September 2019. This crop was a rice crop after the fallow in the double rice cropping system. A total of five crops of rice were grown in the conventional triple–rice cultivation system, and it was the third crop of rice in the double rice system where soil amendments (compost and silicate fertilizer) had been applied three times during the experiment period. The topsoil (0–10 cm depths) was collected at five random positions on each plot using an auger of 20 cm length and 3.5 cm diameter. All of the plant residues, roots, gravel were removed from the soil, then evenly mixed to obtain the homogeneous sample from each plot. The soil sample was divided into two parts, one for soil physicochemical analyses, and the other for nematode extraction.

### 2.2.2. Nematode Processing, Identification and Community Characteristics

A 100 g of non–sieved moist soil from each plot was used to extract nematodes by using the decanting and sieving method [48] and sucrose centrifugation [49]. A few drops of 1% rose Bengal solution were added to dye the organs of nematodes to facilitate observations in counting and identification. The total numbers of individual nematodes were counted under a microscope and converted to the abundance per 100 g of dry soil. A total of 100 nematode individuals from each sample were picked out at random for fixation and identification. Nematodes were fixed [50] and mounted in a drop of glycerin on a glass slide and sealed with a paraffin ring for nematode identification. Extracted nematodes were transferred into a staining block with the solution I (4% formalin solution and glycerin = 99:1 (v/v)), then put into a desiccator saturated with ethanol at the bottom. The desiccator was kept in an oven at 40°C for 24 h; the next day the staining block was taken out of the desiccator, and  $\frac{3}{4}$  partially covered by a glass piece on top to allow slow evaporation of the ethanol, and placed into the oven. Solution II (98% ethanol and glycerin = 95:5 (v/v)) was added every 2 h for a total of 4–5 times, then solution III (96% ethanol and glycerin = 50:50 (v/v)) was added. The day after it was checked if the nematodes were in pure glycerin (no whirling should be noticed when pure glycerin was added). Finally, the nematodes were mounted into a drop of glycerin on a glass slide and sealed with a paraf-

fin ring. These slides were used during the identification process for nematodes. Nematodes were classified into five trophic groups, i.e., bacterivorous, fungivorous, herbivorous, omnivorous, and predators [51]. The biological diversity Hill's indices (N1 and N2) were calculated using a PRIMER package version 6 [52]. Metabolic footprints, community indices such as total maturity index ( $\Sigma$ MI) and plant-parasitic index (PPI) were calculated using the NINJA online program at <https://sieriebriennikov.shinyapps.io/ninja/> [53,54]. Nematode metabolic footprints quantify carbon utilization by different food web components and provide information of energy flow through various trophic groups, which gives additional descriptive information on food web form and soil functions [11,54–56]. For example, the enrichment footprint is the metabolic footprint of nematodes, which rapidly responds to the resource enrichment. The structure footprint is the metabolic footprint of higher trophic levels, which may have a regulatory function in the food web and which are indicative of the abundance of organisms of similar functions in non-nematode taxa. The herbivorous, bacterivorous, omnivorous, fungivorous and predator footprints are based on the nematode indicators of carbon and energy entering the soil food web through their respective channels.

### 2.2.3. Rice Yield and Straw Assessment

At harvest, rice plants were collected from a 5 m<sup>2</sup> area randomly selected in duplicate in each plot. All rice plants aboveground parts were cut and measured for fresh straw biomass and fresh yield. The straw biomass was then oven-dried at 105°C for 3 days. Rice yields were obtained after air-drying. Then, rice straw and actual yield were converted to the total dry biomass of rice straw and yield per hectare.

### 2.3. Data Analyses

Data were analyzed by using one-way ANOVA to determine the difference of nematode community composition or agronomy characteristics, straw biomass and yield of rice plant among treatments when the test for homogeneity of variances was fulfilled by the assumption obtained from Levene's test ( $p > 0.05$ ), then the significant differences were compared from univariate results if  $p < 0.05$ . Then, the Post hoc Fisher' LSD test was used to compare the significant differences among treatments. In contrast, when the p-value obtaining from Levene's test was lower than 0.05, nematode abundance, diversity and community indices, metabolic footprint and agronomy data was transformed to  $\log_{10}(X)$ , and homogeneity of variances was assessed by Levene's test again. In case the Levene's test was not fulfilled for homogeneity of variances after  $\log_{10}(X)$  transformation, the non-parametric test was used and the comparison by Kruskal-Wallis was performed. To find out the main corresponding variables of soil physicochemical properties and nematodes composition among locations, the principal component analysis (PCA) was performed based on taxonomic nematodes abundance, and soil physicochemical properties among locations. PCA was run on a full set (normalized data) of nematodes abundance or soil physicochemical properties to get the same metric for all variables. All statistical analyses were performed by using the statistical package STATISTICA version 7.

Further, to demonstrate the distribution pattern of taxonomic nematodes communities among locations, non-metric multidimensional scaling (nMDS) was performed based on composition of soil nematodes' taxonomic abundance to illustrate the distribution patterns of the overall entire nematode communities or individual dominant nematode taxa among locations. nMDS was run on  $\log(X + 1)$  transformed data set and a resemblance measure by S17 Bray-Curtis similarity among samples across all locations. The 'stress' value from nMDS should be small, at least less than 0.20 and ideally less than 0.10, showing that the reduction to two dimensions implies very little loss of information [57]. The difference of entire nematode community composition among locations was assessed by permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarities. Significant differences between groups in nematode composition datasets

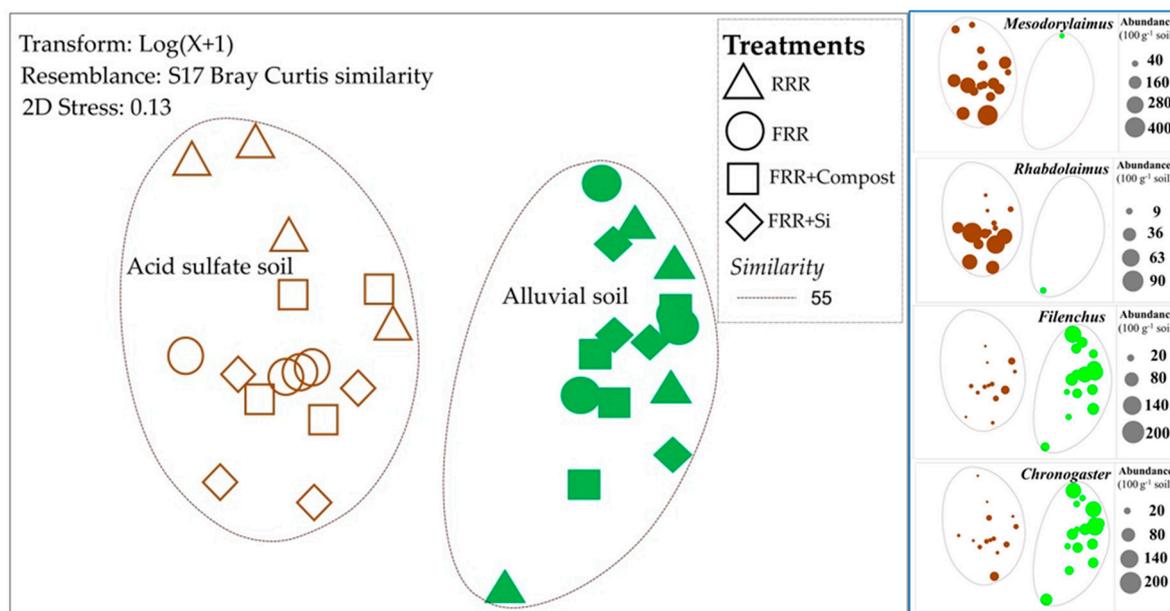
were tested with PERMANOVA. Significant values were considered when  $p < 0.05$ . Data were square root transformed before analysis to downsize the effects of dominant nematodes genera [58]. The SIMPER (Similarity percentages - species contributions) analysis was used to calculate the similarity within treatments, and the dissimilarity between treatments based on nematodes communities. Those analyses were supported by using PRIMER version 6 with the addition of PERMANOVA+ [59].

### 3. Results

#### 3.1. Impacts of Reduced Rice Cropping, Reduced Rice Cropping Mixed with Compost or Silicate Fertilizer on Nematode Abundance and Composition

Overall, non-metric dimension scale analysis based on the composition including the entire nematode community in different treatments for both acid sulfate soil (ASS) and alluvial soil (AL) was conducted. Results showed a clear distribution pattern between soil types, illustrated by the differences in abundance of some dominant genera, e.g., *Mesodorylaimus* and *Rhabdolaimus* in ASS, and *Filenchus* and *Chronogaster* in AL (Figure 1). PERMANOVA of the total nematode abundance showed highly significant differences between locations ( $df = 1$ ,  $p = 0.001$ ). Therefore, we assessed independently the impacts of reduced rice cropping and reduced rice cropping mixed with compost or silicate fertilizer use on the nematode community of each soil type.

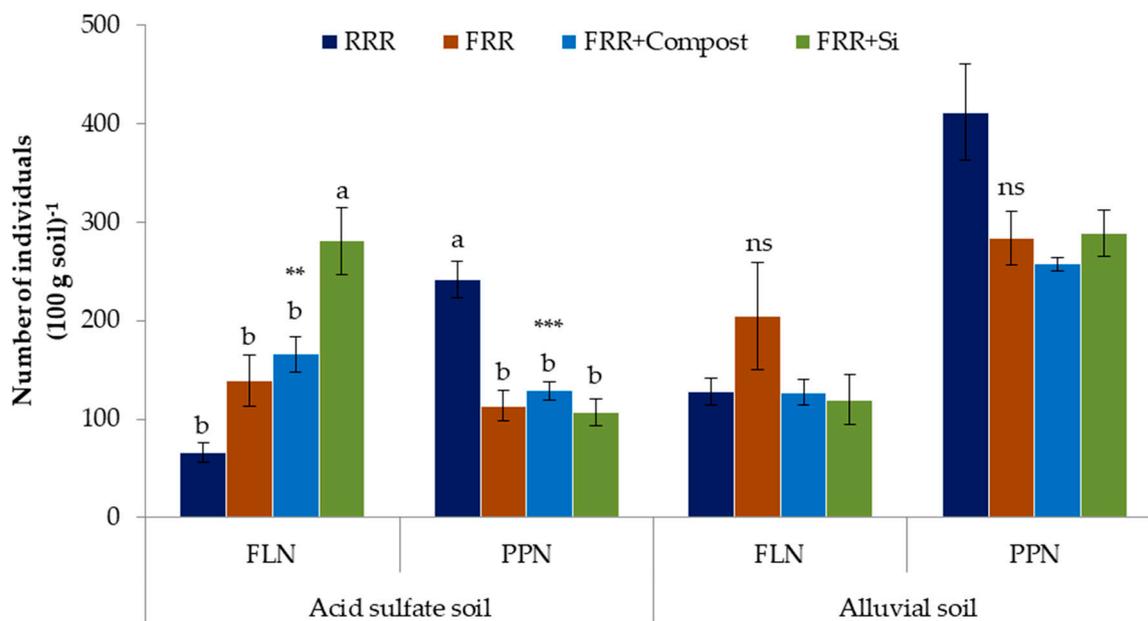
Twenty nematode genera were identified in this study, including 15 and 20 genera in ASS and AL, respectively (Table S1 and Table S2). The PPN genus *Hirschmanniella* was the most dominant across the soil types and accounted for 78.2% and 76.2% of total abundance in the nematode community in triple rice cropping system in ASS and AL, respectively. The subdominant genera were different between soil types. An omnivorous genus, *Mesodorylaimus* (16.2%–49.9%), was dominant in ASS, whilst a fungivorous genus, *Filenchus* (6.7%–14%), and bacterivorous genus, *Chronogaster* (7.6%–12.8%), were the subdominant taxa in AL.



**Figure 1.** Non-metric dimension scale (nMDS) analysis of entire nematode composition among two different soil types, the acid sulfate soil in Ben Tre province and the alluvial soil in Kien Giang province, Vietnam. This study was a part in our previous study [17]. Circle lines indicate the similarity of nematode composition within a soil type by nMDS analysis, representing a 55% similarity. The distribution patterns of dominant genera have also been demonstrated by nMDS analysis, indicating the most common genera (excluding *Hirschmanniella*) in different soil types (the size of the circle indicates the abundance 100 g<sup>-1</sup> dry soil). *Mesodorylaimus* and *Rhabdolaimus* (brown circle) are present in acid sulfate soil and *Filenchus* and *Chronogaster* (green circles) are present in alluvial soil.

The abundances of FLN and PPN showed that these communities in different soil types had different responses to the cropping system and soil amendment (Figure 2). In ASS, all treatments in the reduced intensive rice cropping system had a greater abundance of FLN, particularly in the FRR + Si treatment, which had the significantly highest ( $p < 0.01$ ) abundance of FLN. In contrast to FLN, reduced intensive cropping system with or without compost and silicate fertilizer significantly reduced the abundance of PPN compared to that in RRR treatment in ASS. In AL, fallow condition with compost and silicate fertilizer had a lower abundance of PPN compared to that in RRR treatment, but it had no statically different ( $p = 0.09$ ). There was no difference in the abundance of FLN among treatments in AL.

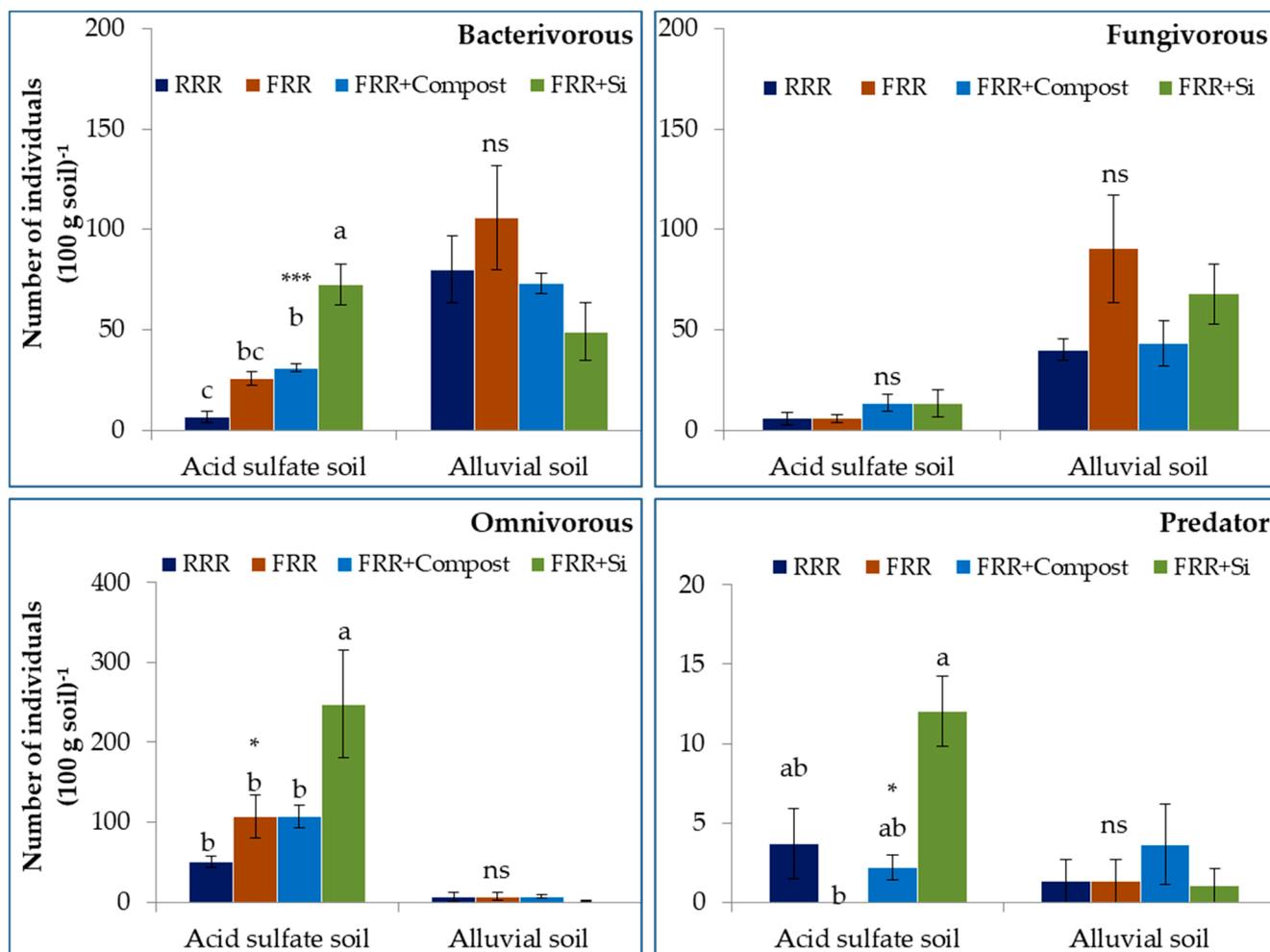
The abundance of *Hirschmanniella* was significantly greater in RRR treatment than in FRR, FRR + Compost and FRR + Si treatments ( $p < 0.001$ ) in ASS and it tended to decrease a 39% and 33% in FRR + Compost and FRR + Si treatments ( $p = 0.09$ ) compared to RRR in AL (Supplementary Material Table S1 and Table S2). In ASS, the abundance of subdominant genera *Mesodorylaimus* ( $p < 0.01$ ) and *Rhabdolaimus* ( $p < 0.051$ ) was significantly greater in FRR + Si treatment than in other treatments. In AL, there was no difference in the abundance of subdominant genera like *Filenchus* and *Chronogaster* between treatments.



**Figure 2.** Abundance (mean  $\pm$  standard error,  $n = 4$ ) of free-living nematode (FLN) and plant-parasitic nematode (PPN) among treatments in different soil types, the acid sulfate soil in Ben Tre province and the alluvial soil in Kien Giang province, Vietnam. Different letters indicate a significant difference in abundance of FLN and PPN among treatments at \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns, not significant. RRR, triple-rice system; FRR, fallow and double-rice; FRR + Compost, fallow and double-rice with 3 Mg ha<sup>-1</sup> sugarcane filter cake compost; FRR + Si, fallow and double-rice with 100 kg ha<sup>-1</sup> silicate fertilizer.

### 3.2. Impacts of Reduced Rice Cropping, Reduced Rice Cropping Mixed with Compost or Silicate Fertilizer on Trophic Structure of Nematode in Soils

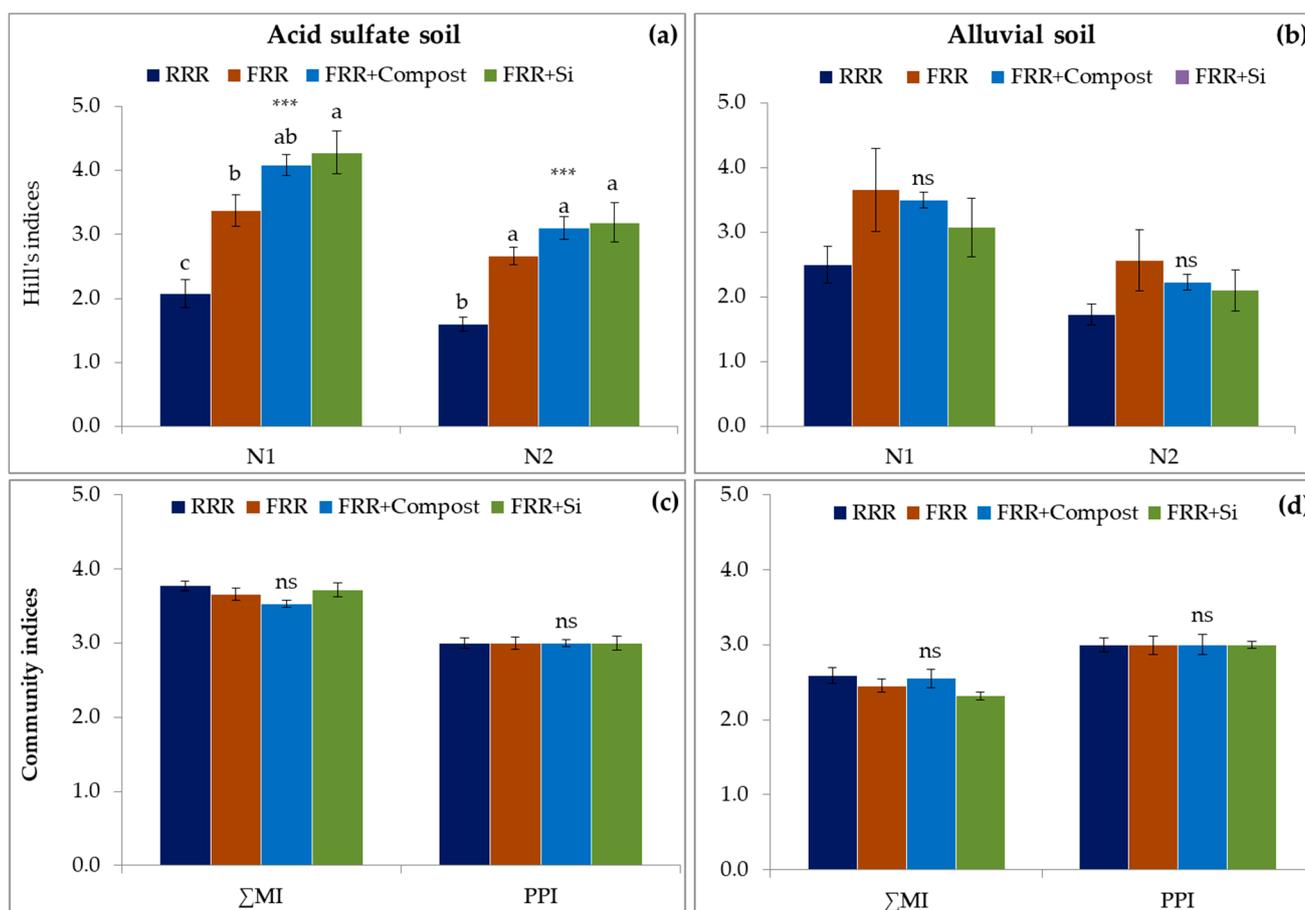
In ASS, the abundance of bacterivorous nematodes was significantly ( $p < 0.001$ ) greater in FRR, FRR + Compost and FRR + Si treatments than in RRR treatment and it was significantly greatest in FRR + Si treatment to other treatments (Figure 3). The abundance of omnivorous nematodes was significantly ( $p < 0.05$ ) greatest in FRR + Si treatment in ASS. The abundance of predator nematodes was significantly ( $p < 0.05$ ) greater in FRR + Si than in FRR treatment in ASS. In AL, the abundance of bacterivorous, fungivorous, omnivorous and predator did not differ.



**Figure 3.** The abundance (mean  $\pm$  standard error,  $n = 4$ ) of the trophic structure of the free-living nematode community among treatments in the acid sulfate soil in Ben Tre province and the alluvial soil in Kien Giang province, Vietnam. The different letters indicate a significant difference among treatments at \*  $p < 0.05$ ; \*\*\*  $p < 0.001$ ; ns, not significant. RRR, triple-rice system; FRR, fallow and double-rice; FRR + Compost, fallow and double-rice with 3 Mg ha<sup>-1</sup> sugarcane filter cake compost; FRR + Si, fallow and double-rice with 100 kg ha<sup>-1</sup> silicate fertilizer.

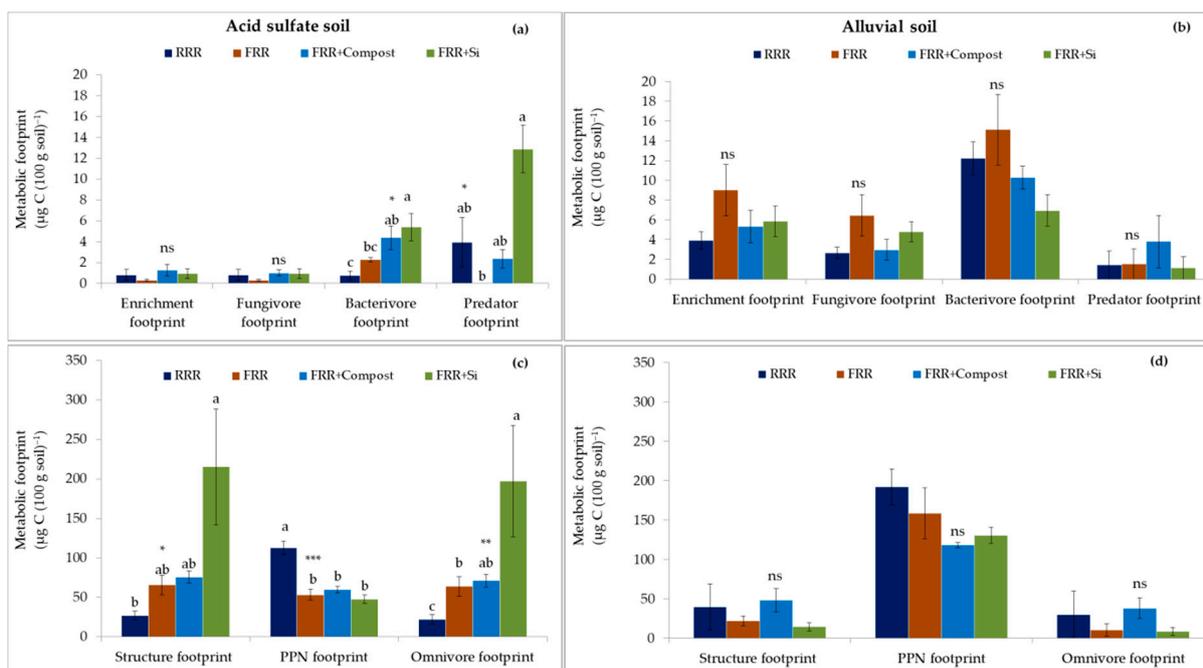
### 3.3. Impact of Reduced Rice Cropping, Reduced Rice Cropping Mixed with Compost or Silicate Fertilizer on Diversity, Community Indices and Metabolic Footprints of Nematode in Soils

In ASS, Hill's indices (N1 and N2) were significantly greater in FRR, FRR + Compost and FRR + Si than in RRR treatment ( $p < 0.001$ ). particularly Hill's N1 index was significantly greater in FRR+Si than in FRR treatment. (Figure 4a). In AL, there were no differences in the diversity Hill's indices between treatments (Figure 4b). The community indices of nematode such as  $\Sigma$ MI and PPI were not different among treatments both in ASS and AL (Figure 4c,d).



**Figure 4.** The diversity indices (Hill's indices) and community indices (total maturity indices  $\Sigma$ MI and plant-parasitic index (PPI) (mean  $\pm$  standard error,  $n = 4$ ) of nematode community among treatments in acid sulfate soil (a,c) and alluvial soil (b,d) in Ben Tre province and Kien Giang province, Vietnam, respectively. The different letters indicate the significant differences among treatments at \*\*\*  $p < 0.001$ ; ns, not significant. RRR, triple-rice system; FRR, fallow and double-rice; FRR + Compost, fallow and double-rice with 3 Mg ha<sup>-1</sup> sugarcane filter cake compost; FRR + Si, fallow and double-rice with 100 kg ha<sup>-1</sup> silicate fertilizer.

The metabolic footprints of the nematode community were different between treatments in ASS but not in AL (Figure 5). In ASS, the bacterivorous footprint was significantly greater ( $p < 0.05$ ) in FRR + Compost and FRR + Si than in RRR treatment, and it was greater in FRR + Si than in FRR treatment (Figure 5a). The predator footprint was significantly greater ( $p < 0.05$ ) in FRR + Si than in FRR. The structure footprint in FRR + Si was significantly greater ( $p < 0.05$ ) than in FRR (Figure 5c). The omnivorous footprint was significantly greater ( $p < 0.05$ ) in FRR, FRR + Compost and FRR + Si treatments than in RRR treatments, and it was greater in FRR + Si than in FRR. By contrast, the herbivorous footprint was significantly ( $p < 0.001$ ) greater in RRR treatment.

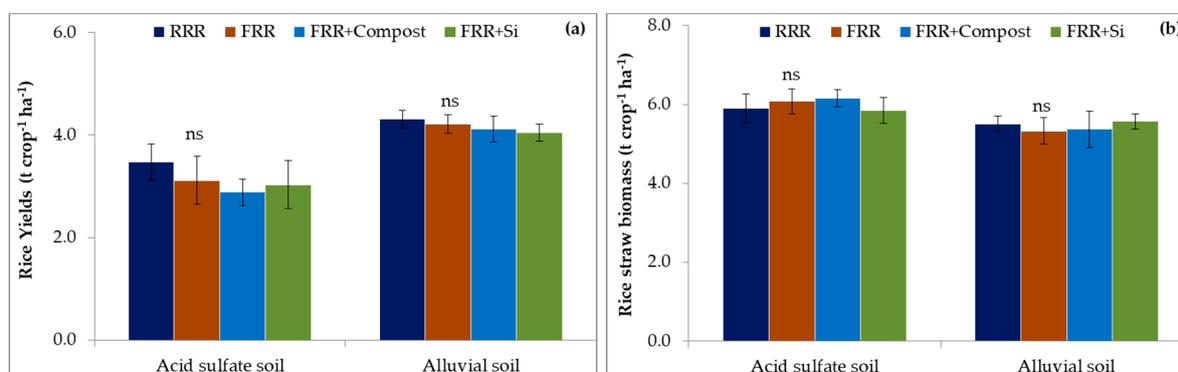


**Figure 5.** The metabolic footprints (µg C (100 g soil)<sup>-1</sup>) of the nematode community among treatments in acid sulfate soil (a,c) and alluvial soil (b,d). Value is mean and standard error (n = 4). Different letters indicate the significant differences among treatments at \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001; ns, not significant. RRR, triple–rice system; FRR, fallow and double–rice; FRR + Compost, fallow and double–rice with 3 Mg ha<sup>-1</sup> sugarcane filter cake compost; FRR + Si, fallow and double–rice with 100 kg ha<sup>-1</sup> silicate fertilizer.

### 3.4. Impact of Reduced Rice Cropping, Reduced Rice Cropping Mixed with Compost or Silicate Fertilizer on Rice Yield and Straw Biomass and Its Relationship with Abundance of *Hirschmanniella* in Soils

Rice yields varied from 2.9 ± 0.3 to 3.5 ± 0.4 t crop<sup>-1</sup> ha<sup>-1</sup> and from 4.0 ± 0.2 to 4.3 ± 0.2 t crop<sup>-1</sup> ha<sup>-1</sup> in ASS and AL, respectively (Figure 6a,b). Rice straw biomass ranged from 5.8 ± 0.3 to 6.2 ± 0.2 t crop<sup>-1</sup> ha<sup>-1</sup> in ASS and 5.3 ± 0.3 to 5.6 ± 0.2 t crop<sup>-1</sup> ha<sup>-1</sup> in AL. There were no differences in the rice yield and straw biomass among treatments in both soil types.

Our result showed that the abundance of *Hirschmanniella* in soil had no relationship to rice yield (R<sup>2</sup> = 0.12, p = 0.19 in ASS; R<sup>2</sup> = 0.0023, p = 0.86 in AL) and straw biomass (R<sup>2</sup> = 0.0009, p = 0.91 in ASS and R<sup>2</sup> = 0.21, p = 0.08 in AL) in both ASS and AL (Figure S1).



**Figure 6.** Rice yields and straw biomass (mean ± standard error, n = 4) among treatments in acid sulfate soil (a) and alluvial soil (b) in Ben Tre province and Kien Giang province, Vietnam, respectively. There were no differences in rice yield and straw biomass between treatments in both soil types. ns, not significant. Rice yield and straw biomass in acid sulfate soil have been reported to RRR, FRR and FRR + Compost treatments in study by Phuong et al. [47]. RRR, triple–rice system; FRR, fallow and double–rice; FRR + Compost, fallow and double–rice with 3 Mg ha<sup>-1</sup> sugarcane filter cake compost; FRR + Si, fallow and double–rice with 100 kg ha<sup>-1</sup> silicate fertilizer.

#### 4. Discussion

##### 4.1. Impacts of Reduced Rice Cropping, Reduced Rice Cropping Mixed with Compost or Silicate Fertilizer on Nematode Abundance and Composition

In this study, *Hirschmanniella* was the predominant PPN taxon in the triple rice cropping system in both the salt-affected ASS and AL in the VMD. SIMPER analysis showed *Hirschmanniella*, *Mesodorylaimus*, *Rhabdolaimus*, *Chronogaster* and *Filenchus* were dominant taxon that most contributed to the similarity within treatment and dissimilarity between treatments and varied by reduced intensive rice cropping systems and mixed with compost or silicate fertilizer (Table 3). This result agrees with our previous study in which *Hirschmanniella* was also the most dominant taxon in non-salt-affected soil in the triple rice cropping system in the VMD [14]. Generally, in a triple-rice cropping system, soil is often in prolonged flooding conditions during the crop cultivation, resulted in more anaerobic conditions than in a double-rice cropping system with a fallow. *Hirschmanniella oryzae*, a major plant-parasitic species in paddy rice fields, can adapt to anaerobic soils conditions [60], while the FLN taxagenerally prefers aerobic soils [12,61]. Our result agrees with the reports by Okada et al. [12] and Liu et al. [61], who found that the abundance of *Hirschmanniella* was relatively decreased in soil with decreased water contents. Soriano and Reversal [23] revealed that keeping a fallow season (120 days) in a rice field before a rice crop reduced the abundance of *Meloidogyne graminicola*, a major PPN on rice. Therefore, our study suggests that keeping fallow conditions during dry season in a double rice cropping system may reduce the number of PPN in soil of subsequent crops.

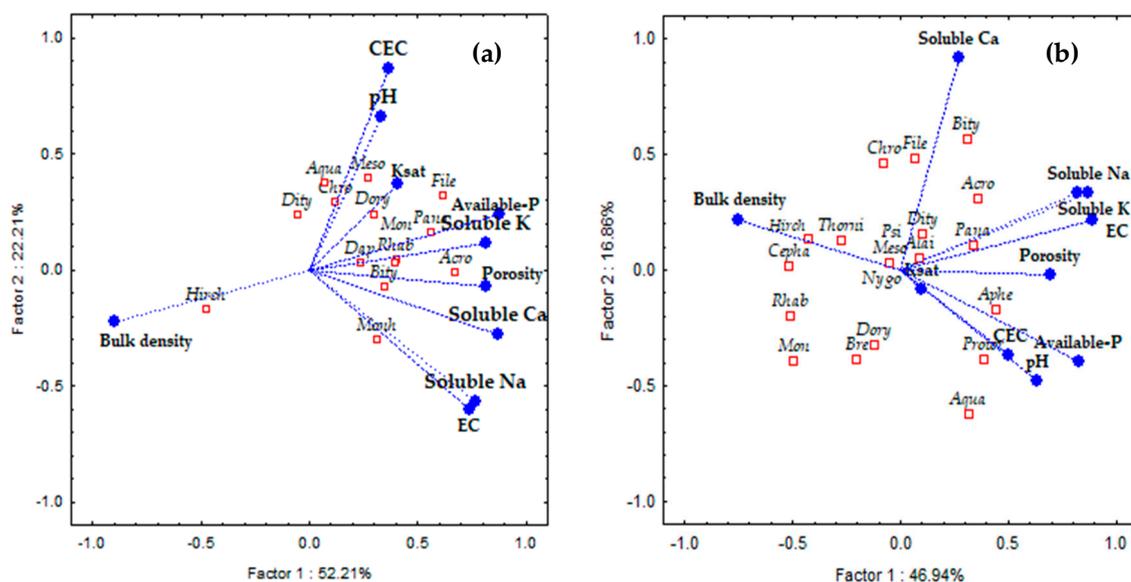
**Table 3.** SIMPER analysis based on nematode abundance for analyzing the similarity within treatment and dissimilarity between treatments in acid sulfate soil and alluvial soil.

	RRR	FRR	FRR+Compost	FRR+Si
<b>Acid sulfate soil</b>				
RRR	<i>Hirschmanniella</i> (82.9%)			
	<i>Mesodorylaimus</i> (15.7%)			
FRR	<i>Hirschmanniella</i> (58.8%)			
	<i>Mesodorylaimus</i> (22.7%)		<i>Hirschmanniella</i> (51.2%)	
	<i>Rhabdolaimus</i> (6.4%)		<i>Mesodorylaimus</i> (36.2%)	
FRR+Compost	<i>Hirschmanniella</i> (50.7%)		<i>Mesodorylaimus</i> (36.6%)	
	<i>Mesodorylaimus</i> (21.5%)		<i>Hirschmanniella</i> (23.2%)	
	<i>Rhabdolaimus</i> (10.6%)		<i>Rhabdolaimus</i> (16.3%)	
			<i>Filenchus</i> (5.7%)	
FRR+Si	<i>Mesodorylaimus</i> (38.0%)		<i>Mesodorylaimus</i> (47.8%)	
	<i>Hirschmanniella</i> (33.8%)		<i>Mesodorylaimus</i> (45.5%)	
	<i>Rhabdolaimus</i> (12.6%)		<i>Rhabdolaimus</i> (14.9%)	
			<i>Hirschmanniella</i> (12.1%)	
			<i>Dorylaims</i> (7.0%)	
			<i>Dorylaims</i> (7.2%)	
			<i>Hirschmanniella</i> (48.2%)	
			<i>Mesodorylaimus</i> (28.2%)	
			<i>Rhabdolaimus</i> (14.2%)	
<b>Alluvial soil</b>				
RRR	<i>Hirschmanniella</i> (82.7%)			
	<i>Chronogaster</i> (8.0%)			
FRR	<i>Hirschmanniella</i> (44.2%)		<i>Hirschmanniella</i> (67.7%)	
	<i>Filenchus</i> (16.1%)		<i>Filenchus</i> (12.7%)	

	Chronogaster (12.8%)	Chronogaster (10.3%)	
	Acrobeloides (5.7%)		
<b>FRR+Compost</b>	Hirschmanniella (58.6%)	Hirschmanniella (33.4%)	Hirschmanniella (73.1%)
	Chronogaster (10.2%)	Filenchus (21.2%)	Chronogaster (11.4%)
	Filenchus (5.4%)	Chronogaster (16.2%)	Filenchus (7.6%)
		Acrobeloides (6.6%)	
<b>FRR+Si</b>	Hirschmanniella (53.4%)	Hirschmanniella (34.8%)	Hirschmanniella (29.0%)
	Chronogaster (12.9%)	Filenchus (18.9%)	Filenchus (17.5%)
	Filenchus (8.4%)	Chronogaster (18.3%)	Chronogaster (12.7%)
		Acrobeloides (6.6%)	Bitylenchus (7.9%)
		Bitylenchus (5.8%)	Acrobeloides (5.6%)
			Ditylenchus (5.3%)
			Hirschmanniella (77.2%)
			Filenchus (12.0%)
			Chronogaster (5.9%)

Pink boxes comparison of stations within treatment: average of similarity and genera that contributed most. Blue box indicates percentage of dissimilarity between treatments and genera that contributed most (cut-off percentage: 90%).

The results of principal components analysis (PCA) showed that *Hirschmanniella* was associated with bulk density in both soils (Figure 7a,b). Besides, our previous study reported that the reduced intensive rice cultivation and application of compost or biochar reduced bulk density of soil. Moreover, the reduced intensive rice cultivation by rotating with upland crops like mung bean and maize significantly reduced bulk density and improved total porosity, aggregate stability index in paddy rice field [62]. We suggest that the reduction of intensive rice cultivation may reduce the population of *Hirschmanniella* in soil by improving some soil properties such as bulk density and other characteristics.



**Figure 7.** The principal components analysis (PCA) based on abundance of nematode community and soil physicochemical properties in acid sulfate soil (a) and alluvial soil (b). The physicochemical properties have been reported by Phuong et al. [47]. The abbreviation names of nematode taxa have been mentioned in the supplementary material.

In this study, the amount of compost used, 3 Mg ha<sup>-1</sup> crop<sup>-1</sup>, is less than other studies [63,64]. Pan et al. [63] reported that there was no difference in PPN abundance among organic amendment amounts, although the abundance of PPN decreased at higher application rate (manure at 7.5 Mg ha<sup>-1</sup> and 22.5 Mg ha<sup>-1</sup>). The application of 50 m<sup>3</sup> ha<sup>-1</sup> farm

compost did not affect the abundance of PPN [64]. Renco et al. [65] reported that application of five types of compost caused a significant reduction of PPN abundance in the soil; the highest amount of  $100 \text{ g (kg soil)}^{-1}$  was effective in suppressing PPN abundance in soil. Therefore, we suggest that the amount of compost needed to alleviate salinity in soil may have the additional effect of changing abundance of soil microorganisms, including nematode communities.

Reduced intensive rice cropping mixed with Si fertilization suppressed *Hirschmanniella* in AL and resulted in the lowest relative abundance of *Hirschmanniella* in both soils. It is well known that Si fertilization generally increases plant resistance to different abiotic and biotic stresses and induces resistant mechanisms against insects and other pathogens [35]. In addition, the use of Si fertilizers to suppress nematode infestation has been reported. Si fertilization induced resistance in rice to *M. graminicola* by enhancing  $\text{H}_2\text{O}_2$  accumulation and phenolic compounds, and changing ethylene signaling pathway [46]. Besides, Si fertilization stimulates the activities of catalase and superoxide dismutase against the infestation of PPN in rice [66]. Another mechanism is that Si fertilization inhibits the attack of root-feeding insects by increasing the thickness of root cell walls [67]. Therefore, it is possible that Si fertilization has the same mechanism against *Hirschmanniella* infestation in rice root in this study as it has against *Meloidogyne* infection.

In contrast to PPN, the abundance of FLN increased in FRR in ASS, but it was not the case in AL. The abundance of FLN did not change in AL, which may relate to soil texture as the clay contents of ASS and AL were 42% and 57%, respectively [47], and clay content is a factor that affects nematode community more strongly than soil management [68]. In reduced intensive cropping in ASS with a low clay content more aerobic conditions may be induced, which is suitable for FLN in general (e.g., *Chronogaster*, *Rhabdolaimus*, *Mesodorylaimus*, *Filenchus*, *Acrobeloides*, *Panagrolaimus*) [22]. PCA results showed that the nematode taxon *Panagrolaimus*, *Acrobeloides*, *Aphelenchoides* and *Filenchus* were associated with porosity, available phosphorus and soluble potassium. Taxa with higher functional guilds like *Dorylaimus*, *Mesodorylaimus* and *Nygolaimus* were associated with Ksat, while *Aquatides* showed a trend in association with soil pH. The changes in cropping system and amendment affected microbes and nematode communities belowground. For examples, the application of organic amendment increased the extent of the bacterivorous, omnivorous and bacterivorous footprint [55,63]. In this study, the reduced intensive crop reduced bulk density and increased porosity in the topsoil (0–15 cm depth) [47], resulting in increased abundance of the FLN community in soil under more aerobic conditions. Our study is supported by Okada [12], who reported that *Acrobeloides* and *Filenchus* were dominant in aerobic soil compared to flooded soil conditions. Therefore, reducing the intensive triple rice cropping system might be necessary to increase total abundance of the beneficial FLN in soil.

In this study, *Mesodorylaimus* and *Rhabdolaimus* were subdominant genera in a paddy rice field in ASS, while the subdominant genera in AL were *Filenchus* and *Chronogaster*. Interestingly, the application of Si in ASS induced the greatest abundance of FLN, particularly *Mesodorylaimus* and *Rhabdolaimus*. Silicate fertilizer may change natural food resources, resulting in the increase of FLN. Several studies have reported that silicate fertilization provides an essential nutrient for growth of Gramineae plants, suppresses pathogens and stimulates the growth of algae and diatoms [67,69,70]; silicate is an essential element for diatoms because they utilize silicic acid to construct their cell walls [71,72]. In paddy fields, algae and diatoms generally exist in the water and they are present on the soil surface during flooding [73,74] and have greater biomass and a positive correlation with salinity water, particularly in the estuarine environment with a range of water salinity from 4.9–8.6  $\text{g L}^{-1}$  [75,76]. A similar level of water salinity concentration was observed at the Ben Tre site, which had of 5.67  $\text{g L}^{-1}$  [77], whilst the level in Kien Giang was generally lower [7,78].

The greatest variables among soils are cations like  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$ , and EC, which were greater in ASS compared to AL (Table 1). The site in Ben Tre is also the area most

affected by saltwater intrusion due to its close location to the sea and natural geology [7]. These conditions could increase abundance of algae and diatoms in river water, which would then pass into rice fields by irrigation, particularly in the dry season. Moreover, both algae and diatoms are preferred food for *Mesodorylaimus* and *Rhabdolaimus* [12,51,61]. Therefore, it is probable that the application of silicate in acid sulfate paddy field soil stimulates the growth of algae and diatoms in the water column and soil surface, which could indirectly increase the abundance of *Mesodorylaimus* and *Rhabdolaimus*. The application of silicate fertilizer was efficient in ASS only, a more severely salt-affected soil. Therefore, further studies on the relationship between a gradient range concentration of salinity and silicate with the presence of specific diatoms or algae communities, and *Mesodorylaimus* or *Rhabdolaimus* will be required to clarify their interactions in the natural food web structure.

#### 4.2. Impact of Reduced Rice Cropping Mixed with Compost or Silicate Fertilizer on Trophic Structure of Free-Living Nematode in Soils

Reducing intensive rice cropping system is primary factor that affects trophic structure of nematode in soils rather than amendment, particularly in ASS in this study. Our results showed that the changes of trophic structure of FLN were observed only in ASS but not in AL that we have discussed in the first previous session on the FLN. Particularly, the abundance of bacterivorous increase in FRR and FRR + Si treatments, while compost did not affect. Because reducing rice intensive cropping changes soil environment conditions among aerobic of fallow in FRR and anaerobic conditions of flooded paddy field in RRR which changes soil physicochemical properties [62] and may induce changes in the belowground nematode community. For instance, Liu et al. [61] reported that the greater abundance of bacterivorous and fungivorous nematodes was recorded in aerobic conditions in upland soils. Also, this observation agrees with Sohlenius [79], who reported that a high density of fungivorous nematodes was observed in dry conditions or upland fields. A recent study in non-salt-affected alluvial soil concluded that rotating upland crop in the paddy rice field increased abundance of bacterivorous nematodes in the soils [22]. Particularly, the application of silicate fertilizer into reduced intensive rice cropping system stimulated the abundance of bacterivorous, omnivorous and predator nematodes in ASS. This result can be explained that the application of silicate fertilizer might increase total amount of microorganisms because it increased available nitrogen (N) and phosphorus (P) [70]. Microorganisms are an essential food for nematode communities, particularly bacterivorous and other omnivorous-predator nematodes [80]. Besides, in previous session, we have explained the stimulation of *Mesodorylaimus* and *Rhabdolaimus* by silicate fertilization. These were the two most dominant taxon of omnivorous and bacterivorous, respectively, in ASS. Therefore, this result caused the greater abundance of bacterivorous and omnivorous nematodes that were observed in FRR + Si in this study.

#### 4.3. Impact of Reduced Rice Cropping, Reduced Rice Cropping Mixed with Compost or Silicate Fertilizer on Diversity, Community Indices, and Metabolic Footprints of Nematode in Soils

The diversity of nematode community is strongly related with the agricultural practices, particularly the conversion in land use from an intensive to extensive cropping system [81]. Reduced rice intensive cropping with or without amendment increased diversity Hill's indices in ASS, particularly soil amended with silicate fertilizer. In a previous study, a rotation system of rice and upland crops in a paddy field increased diversity indices [22]. The application of silicate fertilizer strongly increased the microbial food resources for nematodes in soils [70,80]. Moreover, the application of silicate fertilizer in rice cultivation increased abundance of bacteria of betaproteobacteria and cyanobacteria and fungi of ascomycota, basidiomycota, and increased abundance of functional genes involved in labile C degradation, C and N fixation, phosphorus utilization [82]. Besides, the application of silicate fertilizer can stimulate food resources of aboveground as algae and diatom

in the rice field [83]. Therefore, the application of silicate fertilizer may stimulate the diversity indices of nematodes belowground in paddy rice condition due to their diverse food resources and ecological service. In this study, reduced intensive rice cropping, applied compost, and silicate fertilizer use showed a potential for improving the diversity of nematode communities in ASS. This result agrees with several studies [84–86] reporting that the intensification of crop induces diversity losses. In the triple rice cropping system, the continuous application of chemical fertilizer may reduce the diversity of nematode communities. Luo et al. [84] reported that less chemical fertilizer use could restore the biodiversity of soil organisms belowground such as arthropods in the paddy field, and achieve ecological sustainability in the rice cropping system. In addition, the diversity of soil organism communities, including nematode communities, was strongly related to soil physicochemical properties. For instance, soil pH is one of the key factors that enhance soil biodiversity [87–89]. In our previous study, the application of compost showed an increase in soil pH in ASS [47], which might cause higher diversity of nematodes in the soil habitat. Therefore, this result suggests that the reduced intensive rice growing could provide suitable conditions for belowground biota diversity, including nematode community indices.

The metabolic footprints of bacterivorous, omnivorous and predator nematodes were greatest in FRR + Si compared to RRR. This result can be explained by the result that the reduced intensive rice cultivation in triple rice cropping system enhanced microbial activity and other soil physicochemical properties [22,62], therefore, it may produce higher food resources for nematode in different trophic structures in soil habitat. Besides, as our explanation in the previous part that silicate fertilizer can stimulate much higher food resource above-and belowground [70,80,82,83], it may provide foods for nematodes with higher trophic functional guilds as omnivores and predator due to prey-predator interaction in the soil food web [54]. Particularly, an increase in bacterivorous footprint was only observed in ASS when compost was added into the proposed system compared to the conventional cropping system. This result may relate to decreased soil bulk density when compost added to the proposed system (a fallow and double rice cropping) in ASS where clay content was lower than in AL [47]. Therefore, it may induce more aerobic conditions that are suitable for FLN and bacteria activity. The application of compost enhances the abundance of bacterivorous nematodes in several crops [90]. Composts may indirectly affect the nematode community by driving the food resources, which results in changes in soil microbes or nematode communities. Zhong and Zeng [91] also reported that the application of organic amendment increased the biomass and activity of bacterial communities in soil. Thoden et al. [90] reported that the numbers of FLN, especially bacterivorous and fungivorous nematodes, usually increase markedly after the addition of any form of organic soil amendment.

#### *4.4. Effects of Reduced Rice Cropping, Reduced Rice Cropping Mixed with Compost or Silicate Fertilizer on Rice Straw Biomass and Yield*

Rice yields in RRR, FRR and FRR + Compost have been reported and compared [47], except in FRR + Si treatment. In this study, there were no differences in straw biomass and rice yield among treatments both in ASS and AL. This result has been reported and explained in our previous study for reduced intensive rice cropping and compost amendment [47], except for the result in Si fertilizer treatment. This result can be explained that saline water intrusion and drought are major problems in recent years in the VMD, particularly in ASS and AL sites [7,78]. Exchangeable sodium percentage in soil in this study was 8.8 and 19.2%. According to the report by Sys et al. [92], ESP 10% is the critical value suppressing rice growth. These results may indicate that sodium toxicity in soils is the primary factor inhibiting rice growth and masked effects by other factors, such as plant-parasitic nematodes and compost application. As mention by Phuong et al. [47], compost is expected to enhance the capacity for washing of salinity concentration in the soils, however, in our study area, the clay particles in soil were as high as 42% and 57% in ASS and

AL, respectively, therefore the amount of 3 Mg crop ha<sup>-1</sup> might not be enough in the short-term experiment. The authors recommended that a higher dose of application is necessary in this area. In general, the amount of compost and timing of application are more important to enhance rice productivity. For instance, long-term (10 years) application of 6 Mg ha<sup>-1</sup> crop<sup>-1</sup> of rice straw compost in a double rice system increased rice yield in non-salt-affected soil and enhanced the available silicate in soil [93].

In our study, the abundance of *Hirschmanniella* in ASS reduced by 53% in FRR compared to that in RRR, and its reduced by 19%, 39% and 33% in FRR, FRR + compost and FRR + Si in AL, respectively, however, this reduction did not increase the rice yield and straw biomass among treatments in both ASS and AL. This can be explained by the result that the abundance of *Hirschmanniella* in RRR, which was average 241 and 412 individuals 100 g dry soil<sup>-1</sup> in ASS and AL, respectively, might be less than the threshold causing damage to rice yield. Another reason may alter the soil and environment conditions. Likely, the high amount of soluble salinity remained in soil [47], it might affect the rice growth during the cultivation period. Also, the occurrence of drought and salinity water intrusion may affect the soil condition during the dry period. The present study suggested that the reduced-intensive rice crop and apply compost and Si fertilizer may have potential suppress *Hirschmanniella* in soil, but we need to consider soil conditions to enhance crop productivity. Keeping a fallow season (120 days) in a rice field suppressed abundance of *Meloidogyne graminicola* and improved rice yield by 41% [23]. Therefore, our study suggests that keeping fallow conditions during dry season in a double rice cropping system may reduce the number of PPN in soil of subsequent crops. Therefore, we suggest that the reduced-intensive rice crop in the extremely intensive cropping system and addition of compost and Si fertilizer in salt-affected soils should be considered in order to alleviate the effects of salinity in the soil, enhance soil physicochemical and biological properties and suppress the population of *Hirschmanniella* in soil.

## 5. Conclusions

The use of reduced intensive rice cropping alone resulted in a significant reduction in the abundance of PPN, dominated by *Hirschmanniella*, in salt-affected soils, and increased the abundance of FLN, in particular bacterivorous (*Rhabdolaimus*) nematodes, in ASS. In ASS, this proposed system increased diversity Hill's indices and reduced herbivorous footprint. Reduced intensive rice cropping with compost amendment reduced the abundance of *Hirschmanniella* in AL compared to a conventional cropping system of triple rice cultivation. Reduced intensive rice cropping mixed with Si fertilizer amendment further increased the total abundance of FLN, including bacterivorous (*Rhabdolaimus*), omnivorous (*Mesodorylaimus*) and predatory nematodes, and resulted in high diversity in ASS, as reflected by Hill's N1 index. Moreover, reduced intensive rice cropping mixed with Si fertilization increased structure footprint, bacterivorous, omnivorous and predatory nematode footprints in ASS. We suggest that reduced intensive rice cropping and compost or Si applications can be utilized in paddy rice fields in salt-affected soils, especially in region at high risk of saltwater intrusion, to provide benefits in controlling PPN and enhancing FLN abundance, which will mitigate long-term crop losses.

Further studies on the efficacy of Si fertilizer or the combination of Si and compost to suppress the most dominant taxon, the rice-root nematode *Hirschmanniella*, should be assessed to determine the mode of action in rice. Also, a long-term study should be conducted to see further effects on the soil physicochemical properties, crop productivity and their relationship to nematode communities in soil.

**Supplementary Materials:** The following are available online at [www.mdpi.com/2073-4395/11/3/425/s1](http://www.mdpi.com/2073-4395/11/3/425/s1), Figure S1: The relationship of abundance of *Hirschmanniella* in soil with rice yield and straw biomass in acid sulfate soil (a,b) and alluvial soil (c,d), respectively title, Table S1: The abundance (means  $\pm$  SE) of nematode community in acid sulfate soil among treatments, Table S2: The abundance (means  $\pm$  SE) of nematode community in alluvial soil among treatments, Table S3: The factor coordinates based on correlations of PCA for acid sulfate soil, Table S4: The factor coordinates based on correlations of PCA for alluvial soil.

**Author Contributions:** N.V.S., C.M.K. and K.T. conceptualized the study; N.T.K.P., T.B.L. and D.D.M. conducted the experiments and collected samples, performed sample analysis; N.V.S. data curation; N.V.S. and K.T. validated data; N.V.S. wrote the paper—original draft; N.V.S., C.M.K., N.T.K.P., R.N.P. and K.T. reviewed, edited, and finalized the paper. All authors have read and agreed to the published version of the manuscript.

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