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**MASTER OF SCIENCE THESIS**

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**FOOD WEB STRUCTURE OF NEMATODE  
COMMUNITIES ASSOCIATED WITH RICE IN MWEA,  
KENYA**

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FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT  
ECOLOGY IN THE UNIVERSITY OF EMBU**

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**DECLARATION**

This thesis is my original work and has not been presented for a degree in any other University.

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## **DEDICATION**

I dedicate this work to my parents, Obadiah Mokuah and Zipporah Mose who were committed and supportive of the completion of my studies.

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## TABLE OF CONTENTS

<b>DECLARATION</b> .....	<b>i</b>
<b>DEDICATION</b> .....	<b>ii</b>
<b>ACKNOWLEDGEMENT</b> .....	<b>iii</b>
<b>LIST OF TABLES</b> .....	<b>i</b>
<b>LIST OF FIGURES</b> .....	<b>ii</b>
<b>LIST OF ABBREVIATIONS AND ACRONYMS</b> .....	<b>iv</b>
<b>GENERAL ABSTRACT</b> .....	<b>vi</b>
<b>CHAPTER ONE</b> .....	<b>1</b>
<b>GENERAL INTRODUCTION TO THESIS</b> .....	<b>1</b>
1.1 Background information.....	1
1.2 Statement of the problem .....	<b>Error! Bookmark not defined.</b>
1.3 Justification .....	3
1.4 Objectives.....	4
1.4.1 General objectives.....	4
1.4.2 Specific objectives .....	4
1.5 Null hypotheses .....	4
<b>CHAPTER TWO</b> .....	<b>5</b>
<b>LITERATURE REVIEW</b> .....	<b>5</b>
2.1 Production and importance of rice in Kenya.....	5
2.2 Rice cropping practices .....	6
2.3 Plant-parasitic nematodes in rice.....	7
2.3.1 Incidence of <i>Aphelenchoides</i> species in dry rice grains .....	8
2.4 Additive diversity partitioning of nematodes .....	10
<b>CHAPTER THREE</b> .....	<b>12</b>
<b>Determination of the prevalence and abundance of rice nematodes in Mwea</b> .....	<b>12</b>
3.0 ABSTRACT .....	12
3.1 INTRODUCTION.....	13
3.2 Materials and methods.....	14
3.2.1 Site description.....	14
3.2.2 Soil sampling, nematode extraction and identification.....	14
3.2.8 Data analysis .....	15

3.3 RESULTS.....	16
3.4 DISCUSSION .....	24
3.5 CONCLUSION .....	25
<b>CHAPTER FOUR.....</b>	<b>26</b>
<b>Evaluation of the nematode-based soil food web in rice fields in Mwea.....</b>	<b>26</b>
4.0 ABSTRACT .....	26
4.1 INTRODUCTION.....	27
4.2 Materials and methods.....	28
4.2.1 Site description and soil sampling .....	28
4.2.2 Nematode analyses .....	29
4.3 RESULTS.....	31
4.4 DISCUSSION .....	36
<b>CHAPTER FIVE.....</b>	<b>39</b>
<b>Assessment of alpha and beta diversity of nematodes in Mwea rice fields based on additive diversity partitioning.....</b>	<b>39</b>
5.0 ABSTRACT .....	39
5.1 INTRODUCTION.....	39
5.2 Materials and methods.....	41
5.2.1 Site description .....	41
5.2.2 Nematodes extraction and identification .....	41
5.2.3 Data analysis.....	41
5.3 RESULTS.....	42
5.4 DISCUSSION .....	47
CONCLUSION .....	48
<b>CHAPTER SIX .....</b>	<b>49</b>
<b>GENERAL OVERVIEW (SYNTHESIS).....</b>	<b>49</b>
6.1 INTRODUCTION.....	49
6.2 SUMMARY OF THE MAJOR FINDINGS .....	49
6.2.1 Determination of the prevalence and abundance of rice nematodes in Mwea	49
6.2.2 Evaluation of the nematode-based soil food web in rice fields in Mwea.....	50
6.2.3 Assessment of alpha and beta diversity of nematodes in Mwea rice fields based on additive diversity partitioning.....	50

6.3 RECOMMENDATIONS, IMPLICATIONS AND WAY FORWARD.....	51
REFERENCES.....	52

## LIST OF TABLES

Table 3.1. Mean number ( $\pm$ standard error) of nematode genera in 250g of soil from Nyangati and Tebere, Mwea. ....	17
Table 3.2: Soil physico-chemical properties of Nyangati and Tebere rice fields, Mwea. ....	20
Table 4.1: Soil food web indices and metabolic footprints (log-transformed) in Nyangati and Tebere rice fields.....	34
Table 5.1: Nestedness and turnover components of beta diversity in Nyangati and Tebere rice fields, Mwea.....	45
Table 5.2: Nestedness and turnover components of beta diversity within Nyangati and Tebere rice fields Mwea.....	45

## LIST OF FIGURES

Figure 2.1: Rice cropping systems along an upland (rainfed) and lowland (irrigated) continuum (Tantiwi 2004).....	6
Figure 2.2: <i>Meloidogyne graminicola</i> infection in rice (Source EPPO Global Database, 2019) .....	8
Figure 2.3 ; White tip disease on four rice varieties (a-d). Source, Hui et al., 2014) .....	9
Figure 2.4: Linkage between biodiversity effects and additively partitioned beta diversity (Source, Mori et al., 2018). .....	10
Figure 3.1: Non-metric multidimensional scaling ordination of nematode communities in rice fields in Nyangati and Tebere, Mwea (PERMANOVA: P = 0.02; NMDS Stress = 0.2) .....	19
Figure 3.2: Canonical correspondence analysis of nematodes in Nyangati and Tebere rice fields, Mwea. First and second axis eigenvalues are 0.21 and 0.20, respectively. ...	21
Figure 3.3: Proportion of nematode trophic groups in Nyangati and Tebere rice growing regions. Herbt-Herbivores, % of total number of nematodes, Fungt-Fungivores, % of total number of nematodes, Fungf-Fungivores, % of free-living , Bactt-Bacterivores, % of total number of nematodes, Bactf-Bacterivores, % of free-living, Predt-Predators, % of total number of nematodes, Predf-Predators, % of free-living, Omnt-Omnivores, % of total number of nematodes, Omnf-Omnivores, % of free-living, Sedh-Sedentary parasites, % of herbivores, Migh-Migratory endoparasites, % of herbivores, Semih-Semi-endoparasites, % of herbivores, Ectoh-Ectoparasites, % of herbivores .....	22
Figure 3.4: Proportion of nematode Cp groups in Nyangati and Tebere rice growing regions, Mwea. Cp1f-Cp1 % of free-living, Cp2f-Cp2 % of free-living, Cp3f-Cp3 % of free-living, Cp4f-Cp4 % of free-living, Cp5f-Cp5 % of free-living, Pp2h-Pp2 % of herbivores, Pp3h-Pp3 % of herbivores, Pp4h-Pp4 % of herbivores, Pp5h-Pp5 % of herbivores.....	23
Figure 4.1: Study areas at Nyangati and Tebere, in Mwea, Kirinyaga County, Kenya...	29
Figure 4.2: Maturity index (MI) in rice fields in Nyangati and Tebere regions in Mwea. ....	31
Figure 4.3: Maturity index 2-5 (MI 2-5) in rice fields in Nyangati and Tebere regions in Mwea.....	32
Figure 4.4: Soil food web condition in rice fields in Nyangati and Tebere in Mwea.....	32
Figure 4.5: Nematode groups (colonizer-persister 1-5) in rice fields in Nyangati and Tebere in Mwea.....	33
Figure 4.6: Soil food web condition in Nyangati and Tebere rice fields based on the enrichment and structure indices.....	35
Figure 5.1: Heatmap of nematode communities in Nyangati and Tebere rice fields, Mwea.....	43
Figure 5.2: Renyi diversity profiles of nematode communities in Nyangati and Tebere rice fields, Mwea. Scale parameter values are 0- species richness, 1- Shannon index, 2- logarithm of the reciprocal Simpson index, and infinity- Berger–Parker index.....	43

Figure 5.3: Additive  $\gamma$  diversity partitioning (alpha,  $\alpha$  and beta,  $\beta$ ) components) of A) nematode genus richness B) Shannon diversity and C) Simpson diversity indices in Nyangati and Tebere rice fields, Mwea. ....44

Figure 5.4: Nestedness and turnover components of beta diversity in Nyangati and Tebere rice fields, Mwea. CNT-Nyangati and Tebere rice fields, NYR- Nyangati rice fields, TBR- Tebere rice fields.....46

## LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
BI	Basal Index
CCA	Canonical correspondence analysis
CI	Channel Index
CNT	Nyangati and Tebere rice fields
Cp	Colonizer-Persister
EC	Electrical conductivity
EI	Enrichment index
FLN	Free-living nematodes
KNRDS	Kenya national rice development strategy
MI	Maturity Indices
MI2-5	Maturity Indices
MOA	Ministry of agriculture
NMDS	Non-metric multidimensional scaling ordination
NYR	Nyangati rice fields
p.a.	Per Annum
PPI	Plant Parasitic Index
PPN	Plant-parasitic nematodes
SI	Structure Index
SRI	System of rice intensification
TBR	Tebere rice fields
USA	United States of America
$\gamma$	Gamma diversity
$\alpha$	Alpha diversity
$\beta$	Beta diversity
$\beta_{sne}$	Nestedness

$\beta_{\text{sim}}$

Simpson diversity

$\beta_{\text{sor}}$

Sorensen dissimilarity

## GENERAL ABSTRACT

In Kenya, rice is an important staple crop after wheat and maize and the rate of consumption surpasses its production. Apart from consumption, rice is a cash crop for smallholder farmers including those in Kirinyaga County. Despite its usefulness in contributing to food security, rice production has remained low due to various constraints. Among these are plant-parasitic nematodes (PPN) that account for up to 20% of yield losses. Other than the deleterious effect of PPN, rice agroecosystems contain free living nematodes (FLN) that play important ecological roles such as decomposition of organic matter and suppression of destructive PPN. However, through various cropping practices, the diversity and structure of the nematode communities is altered which affects crop productivity. This study examined the abundance and additively partitioned diversity of nematode communities in rice in Mwea. Soil samples were collected from 30 rice fields in Nyangati (15) and Tebere (15) in Mwea, Kirinyaga county. Nematode extraction was done using the modified Baermann technique before enumeration and identification to the genus level. To determine the differences in the abundance of nematode genera, one-way analysis of variance (ANOVA) was used. Spatial distribution of nematode communities in Nyangati and Tebere was examined using Jaccard index-based non-metric multidimensional scaling. One-way ANOVA was used to evaluate the differences in the indices and metabolic footprints in Nyangati and Tebere. Gamma diversity was additively partitioned into  $\alpha$  and  $\beta$  components in the R package *vegan*. Turnover and nestedness components of beta diversity were computed using the *betapart* package in R software. The relationship between soil properties and nematode genera was evaluated using canonical correspondence analysis. There were 17 nematode genera in both regions with *Aphelenchoides* and *Longidorus* occurring in greater proportions in Tebere. The population of *Helicotylenchus* was high in Nyangati and *Prodorylaimus* occurred in high numbers in both regions. *Aphelenchoides* was positively correlated to electrical conductivity while *Helicotylenchus* was negatively correlated. The two regions could not be unequivocally ordered due to crossing of the Renyi diversity profiles along the scale parameter. Genus richness partitioning, showed that  $\alpha$  and  $\beta$  components contributed 33.7% and 66.3% of the  $\gamma$  diversity, respectively. Partitioning of beta diversity showed that nestedness contributed to the overall nematode diversity observed in Nyangati and Tebere rice fields. Results obtained from this study provide information that could aid in development and implementation of effective and environmentally sound nematode management practices in rice.

## CHAPTER ONE

### GENERAL INTRODUCTION TO THESIS

#### 1.1 Background information

Rice (*Oryza sativa*) is grown in several regions across the world and it acts as a source of calories to a huge population (Skamnioti & Gurr, 2009). Production of rice is mainly concentrated in Asia (India and China) due to the high human population (Skamnioti & Gurr, 2009). The main rice exporters include the United States and rice production in the region was at 10 billion kg in 2017 in an area of 1,004,430 hectares (Childs, 2017). Rice is regarded as a food security crop and in Kenya it was first introduced in 1907. After wheat and maize, rice is the most important grain and production is mainly by smallholder farmers who rely on it for subsistence and as a cash crop (Dawe & Slayton, 2010). It is cultivated in different types of cropping systems but the largest production systems are in irrigated areas (Ouma-Onyango, 2014). Irrigation schemes in Kenya account for 80% of the rice production while rainfed areas contribute 20%. The urban population in Kenya consumes large quantities of rice compared to those in the rural areas. Changes in eating habits have resulted in a 12% annual increase in the consumption rate of rice compared to maize (1%) and wheat (4%). The annual production rate of rice in Kenya was at 45,000-80,000 tonnes which is lower than the consumption at the national level which is 300,000. In order to meet the deficit, the government imports the rice which in 2008 cost Ksh. 7 billion and Ksh. 14 billion in 2018. Increasing rice production in Kenya is therefore necessary since it will reduce imports and improve smallholder farmers' livelihoods through creation of employment, improved food security and economic status (Dawe & Slayton, 2010). Decline in rice production may be as a result of various abiotic and biotic constraints such as changing climatic patterns, diseases and pests (Dawe & Slayton, 2010).

Some of the pests that contribute to this decline include nematodes, and other diseases such as rice blast. Plant-parasitic nematodes PPN are microscopic worms that attack most plants including rice which lead to about 20% yield losses(Kumar et al., 2020). They also have a wide distribution in Kenyan rice fields and feature as a major constraint to rice productivity (Namu *et al.*, 2018). Different PPN affect rice and they include *Meloidogyne* spp. *Aphelenchoides besseyi*, *Ditylenchus angustus*, *Hirschmanniella* spp., *Heterodera* spp. and *Pratylenchus* spp. The nematodes can infect the foliar or root system and *Aphelenchoides* spp. can survive in seeds for a long period of time (McGawley and Overstreet, 1998). Once the PPN infect rice, they cause mechanical damage that affect the physiological processes in the plant which affects productivity(Bridge *et al.*, 2005). The white tip disease is caused by *Aphelenchoides* spp. and its characterized by stunting, necrosis, deformed and small panicles and growth retardation (Yoshii & Yamamoto, 1950; Todd & Atkins, 1958). Cropping practices in rice agrosystems can influence the abundance of PPN including *Aphelenchoides* spp. which can negatively affect the yields (Gnamkoulamba *et al.*, 2018). In addition, since some PPN such as *Aphelenchoides* spp. are seed borne they can remain in the seeds for many years and farmers using the seeds as planting material continuously propagate the white tip disease(Sikora, *et al.*, 2005). Information on the abundance and diversity of PPN will provide a basis for their management.

## **1.2 Statement of the problem**

In tropical and sub-tropical countries such as Kenya, agriculture is the backbone of the economy and it provides food for subsistence and commercial use with a major focus on staple foods such as maize, wheat, and rice. Recently, the increase in rice consumption rate by about 12% annually, has surpassed other staple foods that have an annual increase in demand of about 3-4% (Dawe & Slayton, 2010). This implies that farming techniques and management of rice crop should be improved in order to minimize yield losses. Rice cultivation in Kenya is practiced in paddy irrigation schemes by both the National Rice Board and private farmers. Production is hindered by low technical knowhow including identification of pests and diseases that cause low yields (Dawe & Slayton, 2010). Rice production in Kenya has been declining in the past few years, including in Mwea, Kirinyaga County which produces over 50% of the local rice

(80,000 metric tonnes annually valued at around Ksh. 5 billion). Contributing to this decline are PPN that are widely distributed in Kenya (Namu *et al.*, 2018). The International Rice Research Institute (2004), cites PPN as one of the main contributors of yield losses. In Kenya, there is paucity of information on the effect of PPN on rice yield. This is because PPN damage remain invariably hidden by many other limiting factors that prevail in agriculture, especially the biotic and abiotic stress factors that simultaneously affect the crop (Sikora & Fernandez, 2005). In order to effectively control PPN information on their abundance and diversity is crucial.

### **1.3 Justification**

In developing countries like Kenya, with a projected population increase of about 2.7% per year, the issue of food security is of great concern. Focus has been on increasing food production such as rice whose rate of consumption of about 12% per year, has proven to surpass that of other staple foods; maize and wheat, 3-4% (Ministry of Agriculture and Cooperatives, 2012). Despite the importance rice in contributing to food security, there are several constraints to rice production in Kenya which include, pests such as the quela birds, rodents, diseases and PPN (Allarangaye *et al.*, 2006). Plant-parasitic nematodes, including some of the top ten PPN of economic importance globally, pose a major threat to rice yields. In Kirinyaga County, there are no studies on the nematode soil food web, gamma diversity and related partition components in rice fields. This information is critical for management of PPN in rice. This study therefore aimed at evaluating these aspects and the results that will be obtained will serve as a basis for designing sustainable management strategies for controlling PPN and improving rice production.

## **1.4 Objectives**

### **1.4.1 General objectives**

To examine gamma diversity components of nematodes associated with rice in Mwea, Kenya.

### **1.4.2 Specific objectives**

1. To determine the prevalence and abundance of rice nematodes in Mwea.
2. To evaluate the nematode-based soil food web in rice fields in Mwea.
3. To assess alpha and beta diversity of nematodes in Mwea rice fields based on additive diversity partitioning.

## **1.5 Null hypotheses**

1. In Mwea, prevalence and abundance of rice nematodes is similar.
2. There are no differences in nematode-based soil food web in Mwea rice fields.
3. Gamma diversity of nematodes in Mwea rice fields cannot be additively partitioned into alpha and beta diversity.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Production and importance of rice in Kenya

In Kenya, after wheat and maize, rice is an important staple food crop. Rice consumption rate has increased by 12% compared to that of maize and wheat which is estimated to be about 3-4%. This current consumption rate, which stands at about 300,000 metric tonnes per year is high, way above the estimated production of 45,000-80,000 tonnes per year, leaving a huge gap that is met through imports (Ministry of Agriculture and cooperatives, 2012). In Kenya rice is grown in Nyanza, Central, Coast and Nyanza regions. In Central province, Mwea produces the highest amount annually. Most of the rice grown in Kenya is in irrigation schemes (90%) while the rest is produced through rainfed conditions. Nearly all rice imports in Kenya are from far East, with Pakistan contributing about 74% of the total imports (Huang *et al.*, 1972). Other imports are from Vietnam (7%), Thailand (4%), India (4%), and also minimal imports from neighboring East African Community countries (Report, 2020).

Kenya being among the developing countries with a relatively high population increase (2.7% p.a.) (Oluoko-Odingo, 2011), food security is a concern which rice helps to curb, thus proving to be a very essential cereal crop in the country. About 300,000 farmers provide labor in rice fields and they rely on rice production as a source of livelihood. The increased consumption calls for an increase in rice production in order to curb the issue of food insecurity (Onyango, 2014). This will lead to an increase in smallholder farmers' income and reduce the rice import bill (thus saving the country's revenue expenditure). Rice production in Kenya also helps in proper land use since most areas that support rice growth are plain lands with soils that are poorly drained and therefore prone to frequent flooding, thus limited to most agricultural activities.

## 2.2 Rice cropping practices

In Kenya, rice is an important cereal crop with a high rate of consumption. It is mainly produced and consumed in Asian countries with high population densities such as Asia and India (Mendes & Godoy, 2017). Most rice growing countries plant rice in paddy ecosystems due to the water-demanding nature of rice but other regions grow rice in both upland and lowland conditions (Figure 2.1; Tantawi, 2004). Rice farming techniques can however vary and in some cases it can involve incorporation of other crops. For instance, in Asian countries such as India, rice farming follows the rice-wheat cropping system that involves practices that enable management of nutrients alongside abiotic factors that impact rice production (Subash *et al.*, 2014).

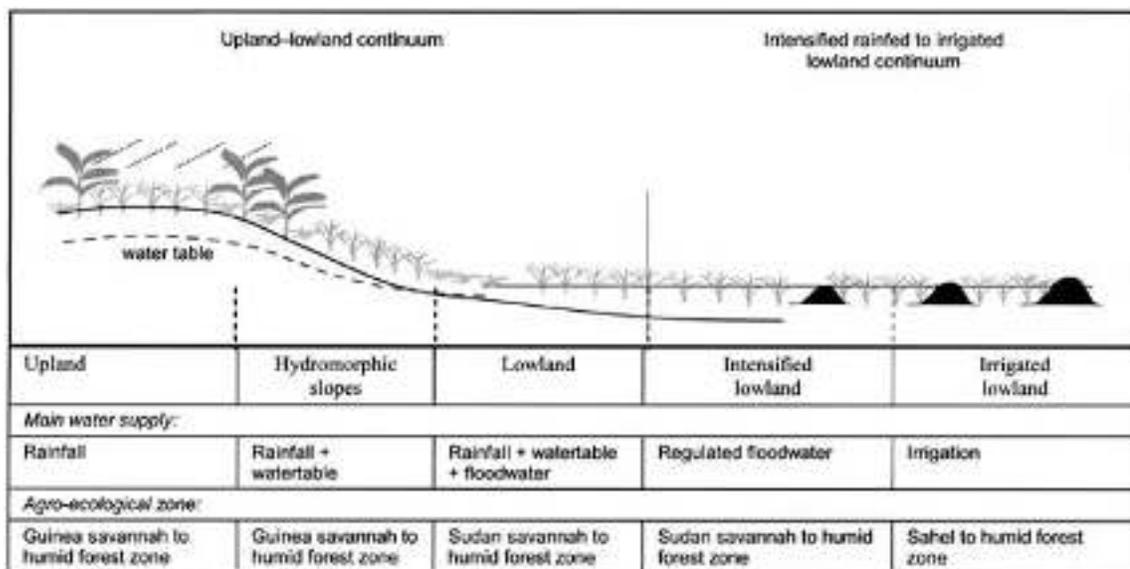


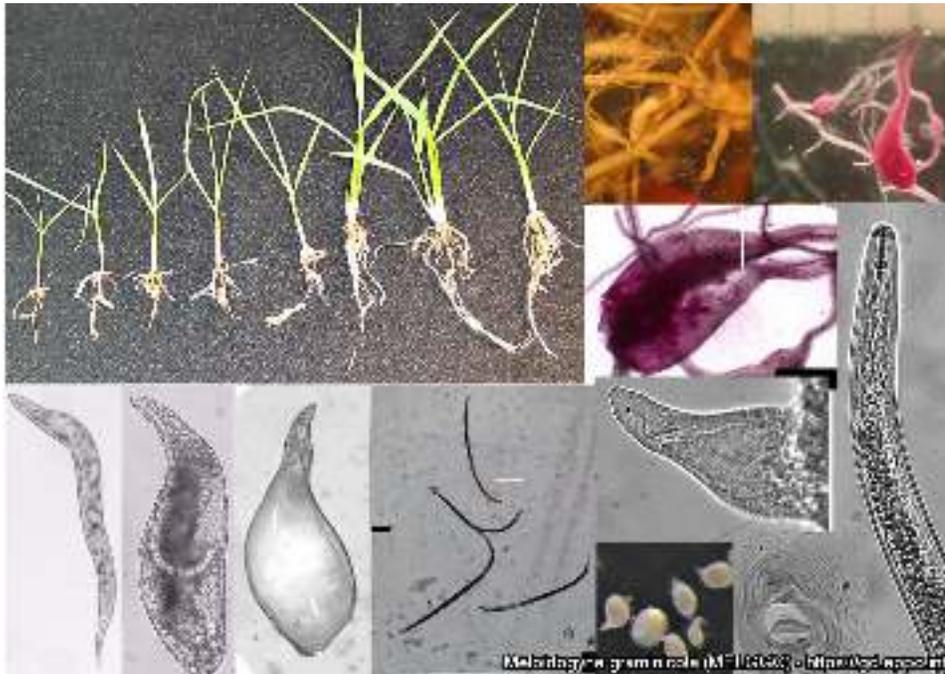
Figure 2.1: Rice cropping systems along an upland (rainfed) and lowland (irrigated) continuum (Tantawi, 2004).

In Kenya, rice farming is done in liberalized irrigation schemes like Mwea Tebere schemes, where irrigation is rotational and is facilitated majorly by the Nyamindi and Thiba rivers. In Mwea, farmers are being encouraged to adopt the System of Rice Intensification (SRI) management. This is because SRI was proven to increase yields by 33%, and has a seed reduction use by about 87%, and saves water by about 28% (Omwenga *et al.*, 2014). The main management practices of rice in Mwea include use of fertilizers, organic amendments and pesticides. Due to the contribution of rice towards achieving food security, it's important to evaluate the incidence, distribution, and diversity of the PPN in order to design sustainable control strategies.

### **2.3 Plant-parasitic nematodes in rice**

Like any other agricultural crop, rice production is faced by a number of challenges that are an obstacle to achieving the expected yields. Despite the importance of rice in Kenya, its production has remained lower than the rate of consumption. The main production constraints are pests including plant-parasitic nematodes. Infection of rice by PPN affects growth and yield due to the changes that occur in the physiological processes (Sikora & Fernandez, 2005). The main PPN in rice include, stem, cyst, lesion, root-knot, white tip and rice root nematodes. *Meloidogyne graminicola* is the main RKN species that infects rice (Figure 2.2 ;Sikora & Fernandez, 2005). The symptoms and extent of effect of the white tip nematode on rice are seen when the infested seed is planted and matures. Upon planting of infested seed the nematode becomes activated and moves to the meristematic areas of the rice during tillering, where it attacks areas within the shoot (Kyndt *et al.*, 2014). This is then followed by movement of the nematodes within the plant up to the tips of the leaves resulting in whitening (Sikora & Fernandez, 2005). After this, there is migration of the nematode to the panicle and spikelets where they start feeding on cells (Hoshino & Togashi, 2021). The nematode can also move between leaves under optimal moisture conditions, and can it can cause severe damage to the rice (Bekal *et al.*, 2003). *Aphelenchoides* spp. succeeds as a seed-borne nematode due to its ability to withstand low moisture conditions over a long period of time (Sikora & Fernandez, 2005). Other nematodes such as *Meloidogyne*

*graminicola* also reduce yields after formation of giant cells within the plant that impede the transport of water and nutrients (Mantelin et al., 2017).

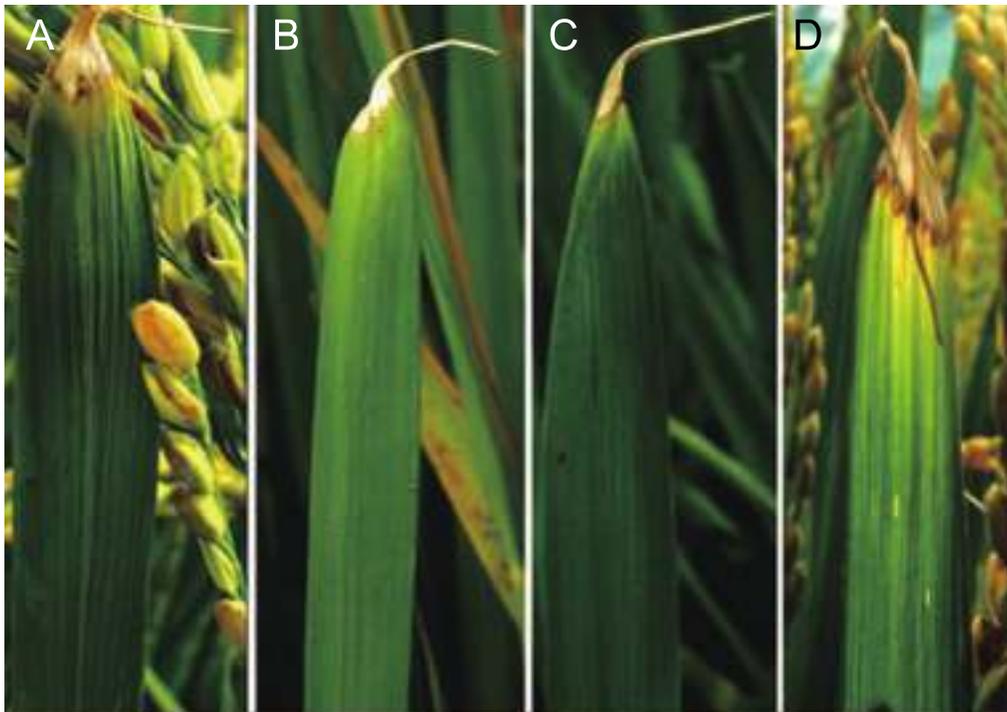


**Figure 2.2: *Meloidogyne graminicola* infection in rice (Source EPPO Global Database, 2019)**

### **2.3.1 Incidence of *Aphelenchoides* species in dry rice grains**

*Aphelenchoides* species is an important seed-borne pathogen which is considered a quarantine pest in many countries (Nico et al., 2002). Being seed-borne, *Aphelenchoides* spp. can be disseminated with the seed and therefore can be replanted and cause damage to rice cultivars which result in yield reduction which is estimated at 29-71% (Mendes & Godoy, 2017). Infested seeds have no visible symptoms and therefore not easily detected for elimination during planting (Çelik *et al.*, 2020). Through infested seeds, the nematodes rapidly spread in rice fields. Some species can withstand desiccation and they can survive in seed in a quiescent state (OEPP/EPPO, 2004). When in dry seeds, these nematodes are in anhydrobiosis and are activated by rehydration, thus upon planting the nematodes cause damage to the plants. They ectoparasitically feed on tender tissue and at the end of the season they get into a cryptobiosis state below the seed hulls (Croll,

1975). Slow rate of drying and aggregation enhances the survival of the nematode(Huang & Huang, 1974). *Aphelenchoides* tend to have an exponential growth pattern whereby, during early growth they occur in low numbers in the sheath as they feed around the apical meristem. The nematode population increases at tillering and is abundant during the reproductive phase of the plant. At this point, the nematodes feed on the reproductive structures of the plant since they are capable of entering the spikelets before anthesis. Later, reproduction of the nematodes ceases as maturation of the seed proceeds(Huang *et al.*, 1977). The nematodes cause the white-tip disease in rice (Figure 2.3(Hui et al., 2014).



**Figure 2.3: White tip disease on four rice varieties (a-d). Source, Hui et al., 2014)**

## 2.4 Additive diversity partitioning of nematodes

Additive partitioning of gamma diversity provides an insight into the alpha (within-community diversity) and beta (among-communities diversity) diversity of an ecosystem. Beta diversity can be further partitioned into nestedness and turnover which have specific linkages with biodiversity effects (Figure 2.4; Mori et al., 2018). It also aids in examining the determinants of diversity within a community at different spatial scales (Williams *et al.*, 2002). Basically, it involves partitioning gamma diversity into the alpha and beta components. Additively partitioning diversity implies that measurements of diversity within communities at a higher level are as a result of the combined differences at lower levels. Patterns of species diversity can be examined through additive partitioning and this method has been increasingly used in the past few years in assessing importance of diversity components (Wagner *et al.*, 2000).

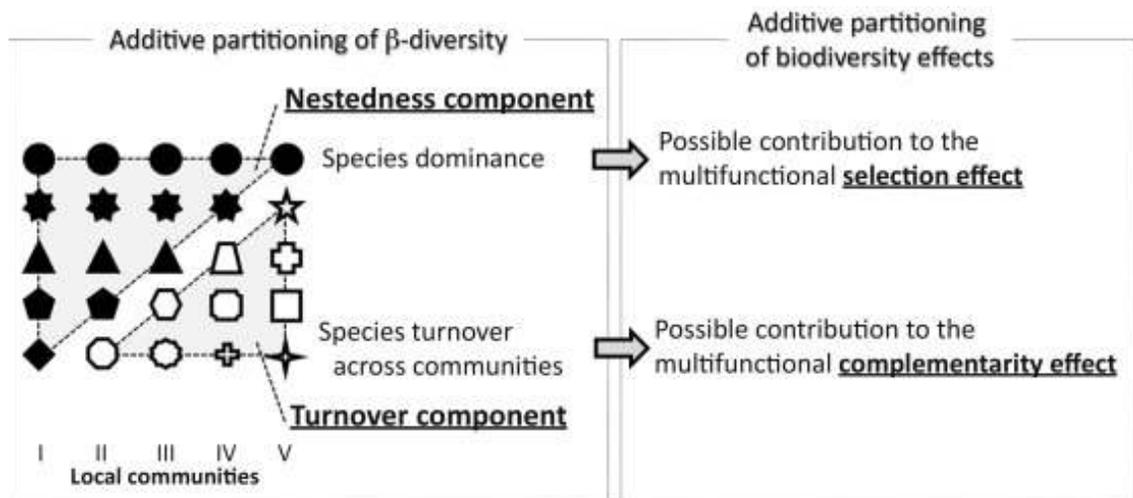


Figure 2.4: Linkage between biodiversity effects and additively partitioned beta diversity (Source, Mori et al., 2018).

Diversity and distribution of PPN vary between cropping systems (Heckman *et al.*, 1994). Agricultural intensification, land use change and new technologies may contribute to shifts in abundance of existing and emerging nematode species under different rice agro-ecosystems (Pascual *et al.*, 2014). Cultivation practices like excessive tillage, use of herbicides and pesticides result in a potentially negative impact on structure of the nematode community thus creating spatial changes in their assemblage. Resultant disturbed soils in agricultural fields due to cropping practices lead to disturbances that negatively affect nematode communities and the soil health (Daramola *et al.*, 2021). Based on this, it is important to examine the diversity patterns of nematode communities in rice in order to sustainably produce rice through effective control of economically damaging PPN.

## CHAPTER THREE

### Determination of the prevalence and abundance of rice nematodes in Mwea

#### 3.0 ABSTRACT

In Kenya, the rice consumption rate has increased surpassing that of other staple foods. Despite the importance of rice, its production has remained low over the years. This poor production can be attributed to various abiotic constraints that include PPN. Effective control requires that there is sufficient information on the nematode diversity and distribution. This study was conducted to determine the prevalence and abundance of nematodes in Nyangati and Tebere in Mwea, Kirinyaga county. Soil samples were collected from 30 rice fields in Nyangati and Tebere. Nematodes were extracted from soil and identified to the genus level. Soil physico-chemical properties were also determined. Differences in the abundance of nematode genera between the regions were examined using one-way analysis of variance. Spatial distribution of nematode communities in Nyangati and Tebere was examined using Jaccard index-based non-metric multidimensional scaling. The relationship between soil properties and abundance of nematode genera was also evaluated. A total of 17 nematode genera were identified in both regions. *Aphelenchoides* ( $F=4.13$ ;  $P=0.052$ ) and *Longidorus* ( $F=5.56$ ;  $P=0.026$ ) occurred in greater proportions in Tebere, and the abundance of *Helicotylenchus* ( $F=6.11$ ;  $P=0.020$ ) was higher in Nyangati. The omnivore, *Prodorylaimus* occurred in high numbers in the two regions. In both regions, omnivores made up the highest proportion of the free-living nematodes. The two regions could not be unequivocally ordered due to crossing of the Renyi diversity profiles. *Aphelenchoides* was positively correlated to electrical conductivity while *Helicotylenchus* was negatively correlated. The study gives baseline information on rice nematode communities, including economically damaging PPN, which can improve their management.

### 3.1 INTRODUCTION

In sub-Saharan Africa (SSA) there is a projected population increase by 2050 which translates to an increase in food demand. This will also lead to extreme food production pressure especially on smallholder farmers who are significant contributors to production of food in SSA (Kim et al., 2021). In view of this, most nations have opted to put more efforts in mass production of various staple foods including rice (Kyndt et al., 2014; Bandumula, 2018). There are several challenges to food production including climate change, biotic factors such as nematodes, among others. Plant-parasitic nematodes are among the pests that pose an enormous threat to global food security of more than US\$157 billion (Hassan et al., 2013). After wheat and maize, rice is an important grain in Kenya, and the consumption rate has steadily increased by about 12% surpassing that of maize and wheat (Ministry of Agriculture and cooperatives, 2012). It is grown in Kirinyaga, Kisumu, Mombasa and Tana River Counties (Ouma-Onyango, 2014), where Mwea, Kirinyaga County largely contributes to production by over 50%. Thus, rice production makes a significant contribution to food security and it's also a source of livelihood for the farmers. Despite the high demand of rice in Kenya and its significance as a whole, rice production has remained low leading to increased importation (Ouma-Onyango, 2014; Ministry of Agriculture, 2008).

Among the factors attributed to the low rice yields, are PPN that serve as a major constraint that account for 20% of losses in rice (Kumar et al., 2020). The PPN have a wide distribution in Kenyan rice fields and feature as a major constraint to productivity (Namu et al., 2018). The PPN can rapidly increase under optimal conditions and they remain active all-year round posing a great risk to crop production (Sikora et al., 2018; Luc et al., 2005). Rice nematodes cause disruptions in the normal physiological processes in the plant which affects the yield (Bridge et al., 2005). *Aphelenchoides* infests dry seeds and has the ability to reduce seed viability and retard germination (Tülek et al., 2014). In addition to the PPN, there are free-living nematodes that are beneficial since some groups in higher trophic levels such as predators and omnivores suppress PPN. Information on prevalence and distribution patterns of rice nematodes in Mwea can be used in implementation of PPN management schemes. This

study therefore aimed at examining the prevalence and abundance of nematodes in Mwea.

## **3.2 Materials and methods**

### **3.2.1 Site description**

The study sites were in Nyangati and Tebere regions in Mwea, Kirinyaga County. Nyangati has an altitudinal elevation of 1279m while that of Tebere is 1155m. The study sites lie in the lowland areas of Mwea, that have vertisol soils with high water holding capacity, thus having the ability to support efficient rice growth (Schaaf *et al.*, 2013). Mwea has an average temperature of between 14°C - 31°C with an average rainfall of 930mm which may not sufficiently support rice growth. Therefore, rice growth is supported by irrigation from Thiba and Nyamindi rivers (Akoko *et al.*, 2020).

### **3.2.2 Soil sampling, nematode extraction and identification**

Within the study sites of Nyangati and Tebere, fields were selected that had been exclusively cultivated with rice for at least 10 years. Soil samples were collected in thirty fields, 15 each in Tebere and Nyangati. The soil samples were collected in fields that had been planted with Basmati 370 rice variety, four months after planting. Soil sampling followed Wiesel *et al.* (2015) protocol whereby samples were collected from W-shaped "sample walks". A 3kg composite sample was collected at 25cm depth from each field. Extraction of nematodes was done using the modified Baermann technique which was followed by fixation. Identification of the nematodes was done to the genus level using a compound microscope and with the aid of morphological keys (Luc, Sikora, *et al.*, 2005; Mekete *et al.*, 2012). Soil physico-chemical properties were assessed at Kenya Agricultural and Livestock Research Organization, National Agricultural Research Laboratories.

### **3.2.8 Data analysis**

The average number of nematode counts from triplicate samples was used for subsequent analyses. Transformation of the nematode count data was done prior to data analysis to meet the required criteria for normality. Nematode functional guilds and colonizer-persister values were used to categorize the nematodes. Differences in the abundance of nematode genera were examined using one-way analysis of variance. Spatial distribution of nematode communities in Nyangati and Tebere was examined using Jaccard index-based non-metric multidimensional scaling (NMDS). Differences between the two regions were assessed using permutational multivariate analysis of variance (PERMANOVA) and permutational multivariate analysis of dispersion (PERMDISP) (Clarke, 1993, Anderson, 2001, 2006). Relationships between nematode genera abundance and soil properties were determined using canonical correspondence analysis (CCA). Vegan package in R was used for NMDS and CCA analyses. All analyses were done in R version 4.0.2 (R Core Team, 2020).

### 3.3 RESULTS

Cumulatively, there were seventeen nematode genera in Tebere and Nyangati rice fields. Bacterivores were predominantly in the Ba<sub>2</sub> functional guild and herbivores mainly in Pp<sub>3</sub>. *Panagrellus* and *Rhabditis* were the only enrichment opportunist bacterivores. *Aphelenchoides* (F=4.13; P=0.052) and *Longidorus* (F=5.56; P=0.026) occurred in greater proportions in Tebere, and the abundance of *Helicotylenchus* (F=6.11; P=0.020) was higher in Nyangati. The omnivore, *Prodorylaimus* occurred in high numbers in both regions (Table 3.1).

**Table 3.1. Mean number ( $\pm$  standard error) of nematode genera in 250g of soil from Nyangati and Tebere, Mwea.**

**Cp-Colonizer-persister values from 1-5. Pr – predators, Ba – bacterivores, Fu-fungivores, Pp – herbivores and Om – omnivores.**

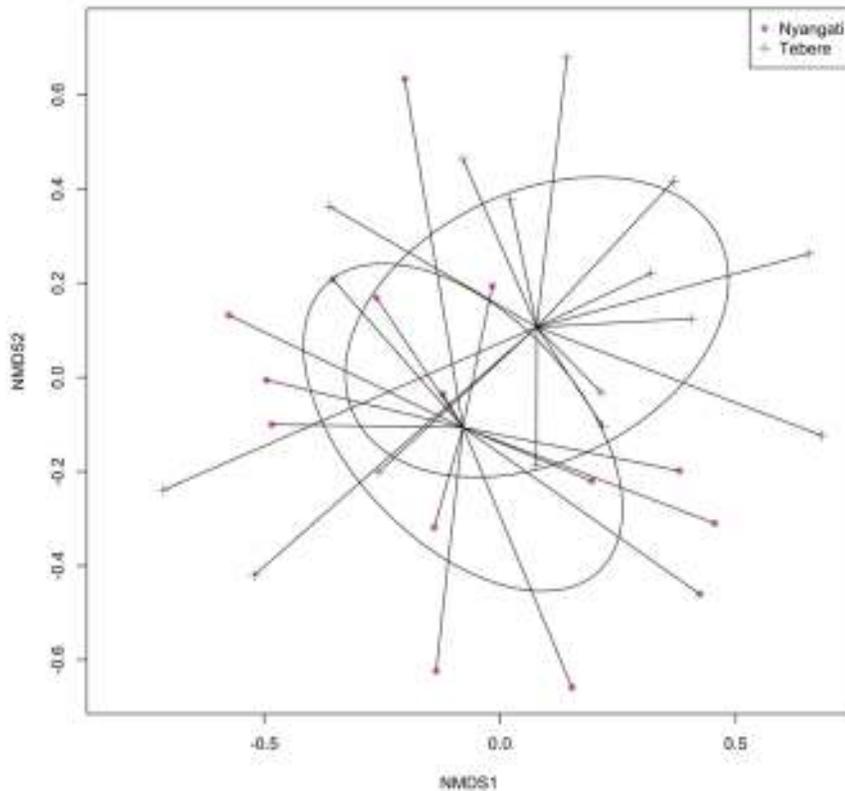
Genus	Cp value	Functional guild	Nyangati		Tebere		F value	P value
			Mean	SE	Mean	SE		
<i>Acrobeloides</i>	2	Ba <sub>2</sub>	2.60	2.60	5.27	3.01	0.90	0.351
<i>Anaplectus</i>	2	Ba <sub>2</sub>	13.13	5.65	4.00	2.14	1.10	0.304
<i>Aphelenchoides</i>	2	Fu <sub>2</sub>	1.33	1.33	15.73	6.42	4.13	0.052*
<i>Aporcelaimus</i>	5	Om <sub>5</sub>	30.00	12.36	7.87	4.62	1.22	0.278
<i>Cephalobus</i>	2	Ba <sub>2</sub>	4.00	2.14	2.67	1.82	0.23	0.638
<i>Eucephalobus</i>	2	Ba <sub>2</sub>	3.93	2.84	1.33	1.33	0.45	0.510
<i>Helicotylenchus</i>	3	Pp <sub>3</sub>	70.40	18.23	15.87	5.16	6.11	0.020*
<i>Heterocephalobus</i>	2	Ba <sub>2</sub>	9.13	5.67	17.07	5.71	2.11	0.157
<i>Labronema</i>	4	Om <sub>4</sub>	20.87	8.18	9.20	5.35	0.92	0.346

<i>Longidorus</i>	5	Pp <sub>5</sub>	14.33	9.02	23.67	6.39	5.56	0.026*
<i>Meloidogyne</i>	3	Pp <sub>3</sub>	10.53	4.65	10.47	6.30	0.36	0.552
<i>Mesodorylaimus</i>	4	Om <sub>4</sub>	33.93	10.71	34.00	12.73	0.30	0.591
<i>Mylonchulus</i>	4	Pr <sub>4</sub>	2.67	1.82	2.60	2.60	0.19	0.669
<i>Panagrellus</i>	1	Ba <sub>1</sub>	7.87	6.57	3.93	3.93	0.28	0.602
<i>Pratylenchus</i>	3	Pp <sub>3</sub>	9.20	6.60	0	0	3.32	0.079
<i>Prodorylaimus</i>	4	Om <sub>4</sub>	57.40	13.25	58.73	13.74	0.01	0.965
<i>Rhabditis</i>	1	Ba <sub>1</sub>	19.80	5.07	22.20	8.90	0.58	0.453

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\* = P < 0.05.

Nematode communities in Nyangati and Tebere were different with an average dissimilarity of 71.8% (Figure 3.1; PERMANOVA,  $R^2 = 0.06$ ,  $F = 1.8$ ,  $P = 0.02$ ; PERMDISP,  $F = 0.17$ ,  $P = 0.69$ ). Nematode genera that influenced the observed dissimilarity were *Helicotylenchus*, *Prodorylaimus*, *Mesodorylaimus*, *Aporcelaimus*, *Longidorus*, *Rhabditis* and *Labronema*.



**Figure 3.1: Non-metric multidimensional scaling ordination of nematode communities in rice fields in Nyangati and Tebere, Mwea (PERMANOVA:  $P = 0.02$ ; NMDS Stress = 0.2)**

Phosphorus, pH and electrical conductivity (EC) were significantly different in the two regions. Tebere had the highest pH ( $P=0.005$ ) and EC ( $P=0.0001$ ) while P ( $P=0.001$ ) was high in Nyangati (Table 3.2). The first two axes in the CCA accounted for 18.2% of the variation. *Aphelenchoides* was positively correlated to EC while *Helicotylenchus* was negatively correlated (Figure 3.2).

**Table 3.2: Soil physico-chemical properties of Nyangati and Tebere rice fields, Mwea.**

Soil property	Nyangati		Tebere		F value	P value
	Mean	SE	Mean	SE		
pH	6.20	0.16	6.92	0.18	9.32	0.005**
Total Nitrogen %	0.27	0.01	0.25	0.01	2.32	0.139
Total Organic Carbon %	3.07	0.10	2.81	0.16	1.99	0.170
Phosphorus ppm	38.40	2.39	23.80	3.28	12.93	0.001**
Potassium meq%	0.23	0.03	0.40	0.09	3.55	0.070
Calcium meq%	19.56	3.07	24.72	4.29	0.96	0.336
Magnesium meq%	5.58	0.08	5.44	0.17	0.56	0.459
Manganese meq%	0.88	0.07	0.66	0.04	7.31	0.01*
Copper ppm	3.06	0.25	2.90	0.43	0.11	0.743
Iron ppm	206.26	32.09	146.50	46.08	1.13	0.296
Zinc ppm	2.44	0.17	2.80	0.33	0.95	0.338
Sodium meq%	1.02	0.31	1.24	0.29	0.27	0.611
Electrical Conductivity mS/cm	-	-	0.74	0.06	138.72	<0.0001***
Sand (%)	16.80	0.26	26.00	4.86	3.57	0.069
Clay (%)	71.20	1.68	62.00	4.86	3.20	0.085
Silt (%)	12.00	1.62	12.00	1.22	0.01	1

\*\* = P < 0.001, \*\*\* = P < 0.0001.

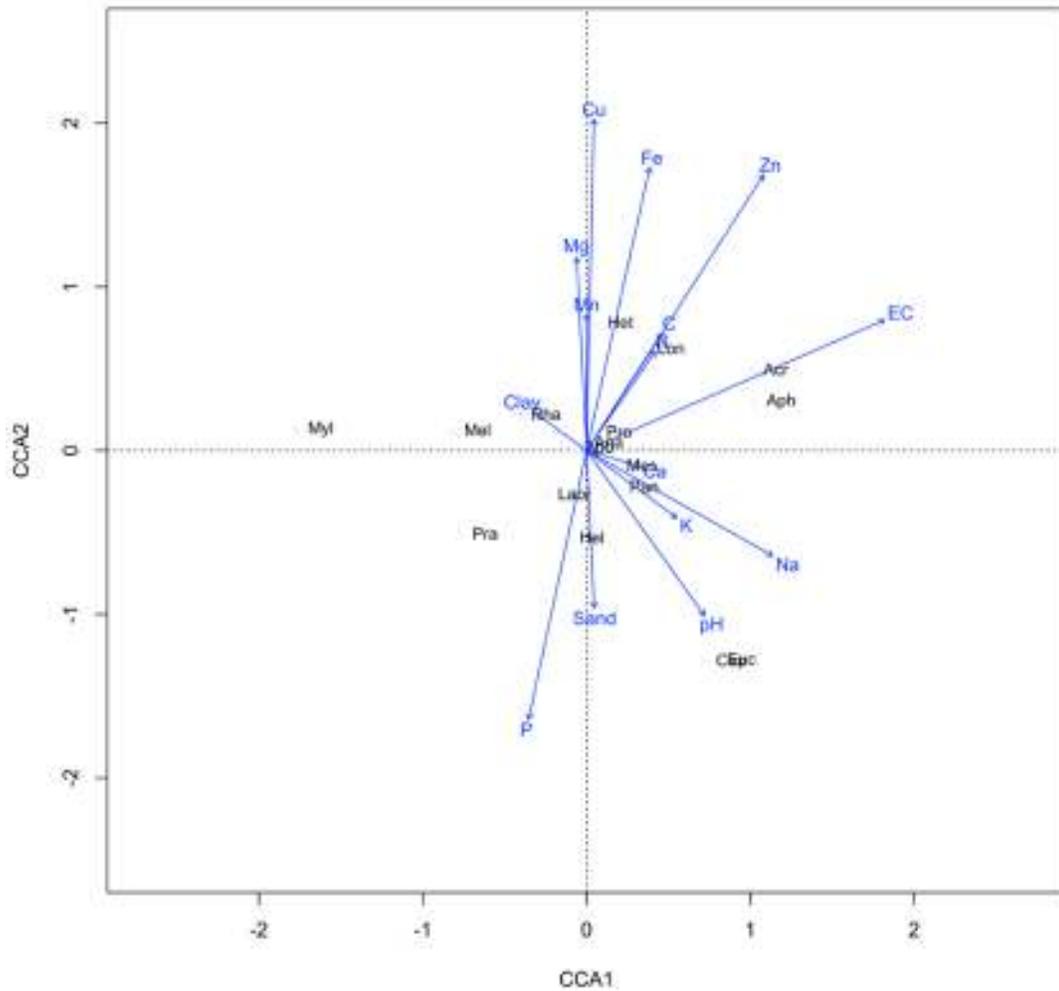
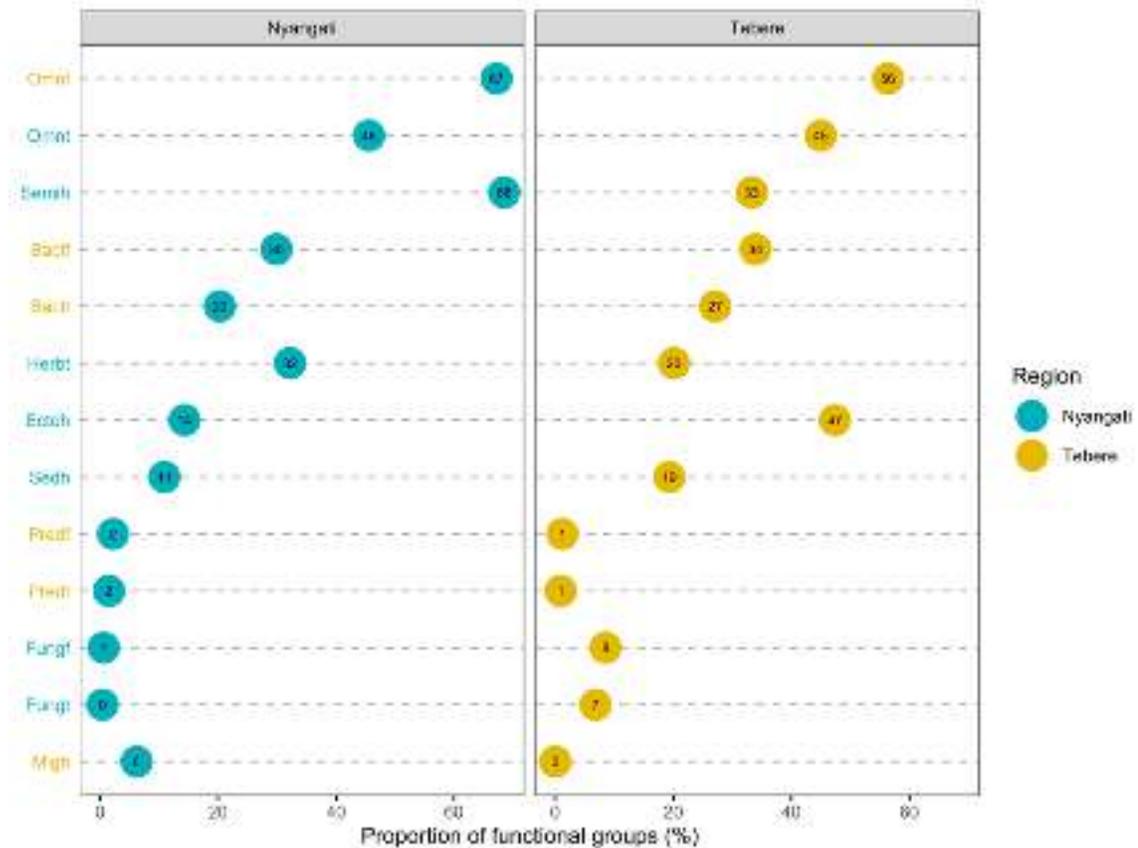


Figure 3.2: Canonical correspondence analysis of nematodes in Nyangati and Tebere rice fields, Mwea. First and second axis eigenvalues are 0.21 and 0.20, respectively.

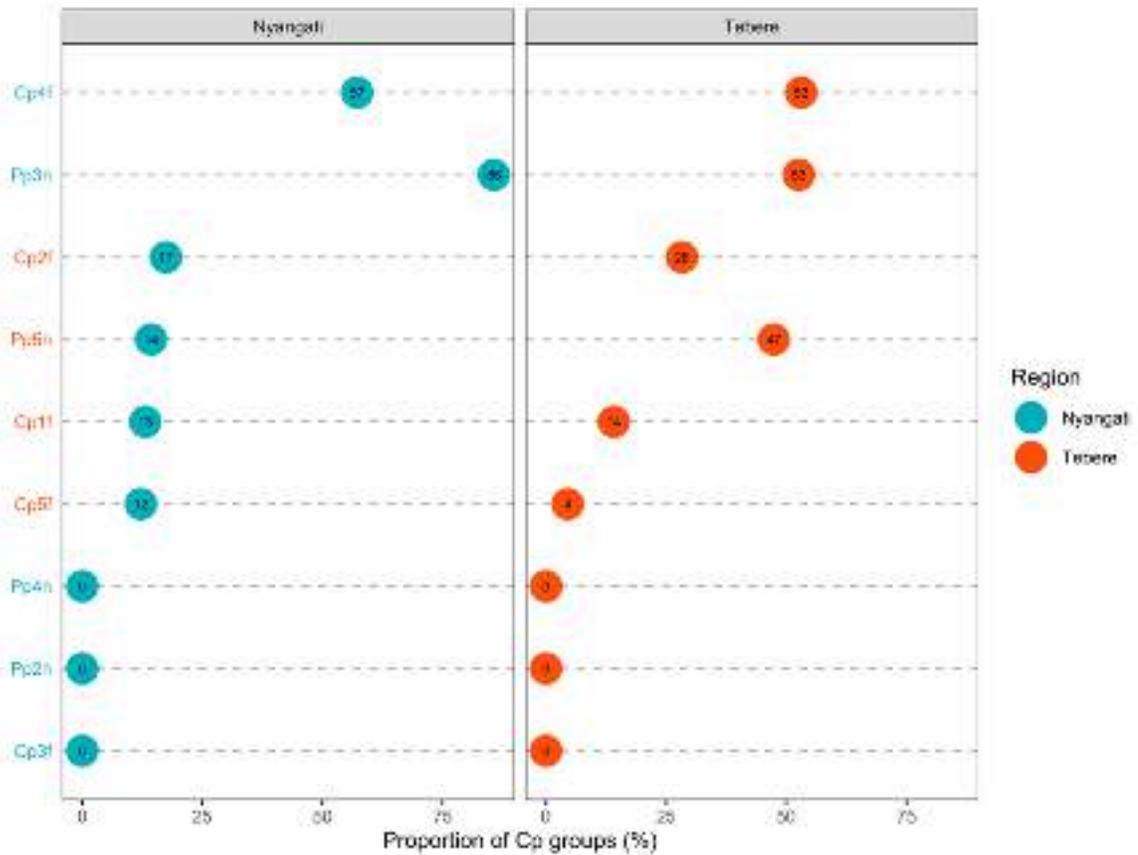
*Acr-Acrobelloides, Ana-Anaplectus, Aph-Aphelenchoides, Apo-Aporcelaimus, Cep-Cephalobus, Euc-Eucephalobus, Hel-Helicotylenchus, Het-Heterocephalobus, Labr-Labronema, Lon-Longidorus, Mel-Meloidogyne, Mes-Mesodorylaimus, Myl-Mylonchulus, Pan-Panagrellus, Pra-Pratylenchus, Pro-Prodorylaimus, Rha-Rhabditis*

From the entire nematode community, herbivores occurred in greater numbers in Nyangati (32%) compared with Tebere (20%). In both regions, omnivores made up the highest proportion of the free-living nematodes (Figure 3.3). Out of the total population of herbivores, Nyangati and Tebere had a greater proportion (86%) of Pp3 and Pp5 (47%) nematodes, respectively (Figure 3.4).



**Figure 3.3: Proportion of nematode trophic groups in Nyangati and Tebere rice growing regions. Herbt-Herbivores, % of total number of nematodes, Fungf-Fungivores, % of total number of nematodes, Fungh-Fungivores, % of free-living, Bactf-Bacterivores, % of total number of nematodes, Bacth-Bacterivores, % of free-living, Predt-Predators, % of total number of nematodes, Predh-Predators, % of free-living, Omnt-Omnivores, % of total number of nematodes, Omnf-Omnivores, % of free-living, Sedh-Sedentary parasites, % of herbivores, Migh-Migratory**

endoparasites, % of herbivores, Semih-Semi-endoparasites, % of herbivores, Ectoh-Ectoparasites, % of herbivores



**Figure 3.4: Proportion of nematode Cp groups in Nyangati and Tebere rice growing regions, Mwea. Cp1f-Cp1 % of free-living, Cp2f-Cp2 % of free-living, Cp3f-Cp3% of free-living, Cp4f-Cp4 % of free-living, Cp5f-Cp5 % of free-living, Pp2h-Pp2 % of herbivores, Pp3h-Pp3 % of herbivores, Pp4h-Pp4 % of herbivores, Pp5h-Pp5 % of herbivores.**

### 3.4 DISCUSSION

Irrigated rice cropping systems are an ecosystem with environmental variables that differentially shape soil communities (Okada et al., 2011, Nguyen et al., 2021). In this study, *Panagrellus* and *Rhabditis* were the only bacterivorous enrichment opportunists. *Rhabditis* has been previously reported in paddy areas (Korenko and Schmidt, 2007) but Korobushkin et al. (2019) did not observe the genus in irrigated rice fields. The abundance of enrichment opportunists in the family Rhabditidae may be attributed to a heightened breakdown of organic matter by bacteria and the availability of labile food sources (Okada et al., 2011). In this study, the number of *Aphelenchoides* and *Longidorus* was significantly higher in Tebere while *Helicotylenchus* population was greater in Nyangati. However, *Aphelenchoides* made up a small proportion of the entire nematode community; 0.4% in Nyangati and 6.7% in Tebere. This nematode is generally prevalent in upland rice fields (Okada et al., 2011, Nguyen et al., 2020) while *Longidorus* is more common in irrigated areas (Schmidt et al., 2015). The PPN *Helicotylenchus* made up the highest proportion (22.6%) of nematode genera in Nyangati. High prevalence of *Helicotylenchus* was also reported in the Philippines, where it occurred in 80% of the sampled regions (Pascual et al. 2014). According to Coyne et al. (2001) *Helicotylenchus* is positively correlated to irrigation compared to *Meloidogyne incognita* and *Pratylenchus zae* as similarly observed by Bambaradeniya et al. (2004). Control of *Helicotylenchus* in Mwea is imperative since it significantly affects rice grain yield (Audebert et al., 2000) and concomitant infection of paddy rice by *Criconebella onoensis* and *H. dihystra* causes 63-72% yield losses (Chinapen et al., 1988). Only one predatory genus, *Mylonchulus* was present in both regions probably due to the negative effects of flooding on predaceous nematodes (Korobushkin et al., 2019).

The omnivores, *Prodorylaimus*, *Labronema*, *Mesodorylaimus* and *Aporcelaimus* were present in both regions. They belong to cp4 and cp5 groups which are sensitive to disturbances—the observed high number is therefore unexpected although it has been observed in other studies (Okada et al. 2011, Nguyen et al. 2021). Sinh et al. (2021) hypothesize that an increased population of omnivores in paddy rice fields receiving

chemical fertilizers may be due to an increase in food supply. Nematodes in higher trophic groups have a regulatory role against PPN (Sánchez-Moreno and Ferris, 2018), and it is therefore important that low levels of disturbance are maintained in Nyangati and Tebere in order to sustain a structured food web that suppresses PPN. In this study, regional variability may have been a stronger driver of differences in specific nematode genera. This was shown in the NMDS where certain nematode genera had a greater contribution to the dissimilarity between regions. Some of the genera showed correlation with soil properties. Soil pH and EC, which are linked to salinity, are properties that greatly influence nematode communities in paddy rice fields. Nguyen et al. (2020) observed that bacterial and fungal feeders were positively correlated to EC while PPN showed a negative correlation which was not significant. Enrichment opportunists in the cp2 class can tolerate soils with high EC (Nguyen et al., 2021). This may explain the positive correlation that was observed between *Aphelenchoides* and EC. The tolerance of this genera to high salinity was also reported in rice fields with alluvial soils containing high salt content (Nguyen et al., 2021).

### **3.5 CONCLUSION**

Nematodes communities in Nyangati and Tebere are different at the genus, cp and trophic levels. Distribution of nematode genera in the two regions is influenced by soil properties. Economically important PPN were identified in the study and integrated nematode management schemes should therefore be put in place for their control. The relatively high number of nematodes in higher trophic groups should be enhanced further through the use of appropriate farming practices.

## CHAPTER FOUR

### Evaluation of the nematode-based soil food web in rice fields in Mwea

#### 4.0 ABSTRACT

The nematode soil food web plays an important role in the provision of ecosystem services. Rice cultivation in Mwea is shifting to intensive use of fertilizers and pesticides that may affect the complexity of the nematode food web. This study examined the nematode diversity and food web structure in 30 irrigated rice fields in Nyangati and Tebere in Mwea, Kirinyaga county. Soil samples were collected from each field for extraction of nematodes which were identified to the genus level. One-way analysis of variance was used to evaluate the differences in the indices and metabolic footprints in Nyangati and Tebere. The Maturity Index (MI) ranged from 3.03 to 3.34 while the MI2-5 was 3.39-3.72. Channel and basal indices were below 30% while the EI and SI were above 50%. There was no variation in metabolic footprints except for fungivore footprint which was significantly higher ( $F=4.58$ ;  $P=0.041$ ) in Tebere. The soil food web in both regions was structured, moderately enriched, and with low to moderate disturbance. Based on these results, agricultural practices that enhance specific components of the food web and increase the suppressive ability of soil against PPN should be implemented in rice fields in Nyangati and Tebere.

#### 4.1 INTRODUCTION

Agricultural intensification affects the soil food web (Sánchez-Moreno et al., 2011) which influences crop productivity (Wang et al., 2012; Su et al., 2021). A global meta-analysis showed that the application of pesticides and chemical-based fertilizers lowers the structural complexity of the nematode soil food web (Puissant et al., 2021). On the other hand, judicious use of inorganic fertilizers enhances the food web structure and increases soil suppressiveness against PPN (Liu et al., 2016). In some cases, nematode functional guilds have a greater contribution to the structure of the soil food web compared to other microorganisms as demonstrated by Li et al. (2016) in an agricultural field trial. There are several PPN associated with rice such as *Helicotylenchus*, *Trichodorus*, *Xiphinema* (Coyne et al., 1999), *Meloidogyne*, *Hirschmanniella*, *Heterodera*, *Pratylenchus*, *Aphelenchoides* and *Ditylenchus* (Kyndt et al., 2014). Nematode genera that cause significant yield losses in rice include *Aphelenchoides*, *Ditylenchus*, *Criconemoides*, *Heterodera*, *Hirschmanniella*, *Hoplolaimus*, *Meloidogyne*, *Paralongidorus*, *Pratylenchus* and *Xiphinema* (Peng et al., 2018).

In Kenya, rice, the third most important staple crop, is grown in Kirinyaga, Kisumu, Mombasa and Tana River Counties (Ouma-Onyango, 2014; Fukuta et al., 2019). Despite the 12% annual increase in consumption (300,000 tonnes), yield is comparatively low (45,000 to 80,000 tonnes) and the gap is covered through import (Ministry of Agriculture, 2008; Ouma-Onyango, 2014). Yield decreases have been reported despite increased application of nitrogen fertilizers (Njinju et al., 2018). There are several biotic constraints (Bakker, 1970; Abo & Sy, 1997; Kihoro et al., 2013) in Kenyan rice production systems that include PPN (Pili et al., 2016). In addition to these constraints, irrigated areas that account for 80% of rice production in Kenya, pose additional stress such as high salinity and low fertility. Certain economically damaging nematodes prefer irrigated areas (Makihara et al., 2018). For instance, the number of *Hirschmanniella oryzae* (Gilces et al., 2016) and *Meloidogyne graminicola* second-stage juveniles were higher in irrigated areas compared to upland fields in Myanmar (Win et al., 2011). Similarly, in Côte d'Ivoire, the number of *Basiria*, *Filenchus* and *Coslenchus* was greater in high moisture paddy fields (Coyne et al., 2001). Irrigation in rice cropping

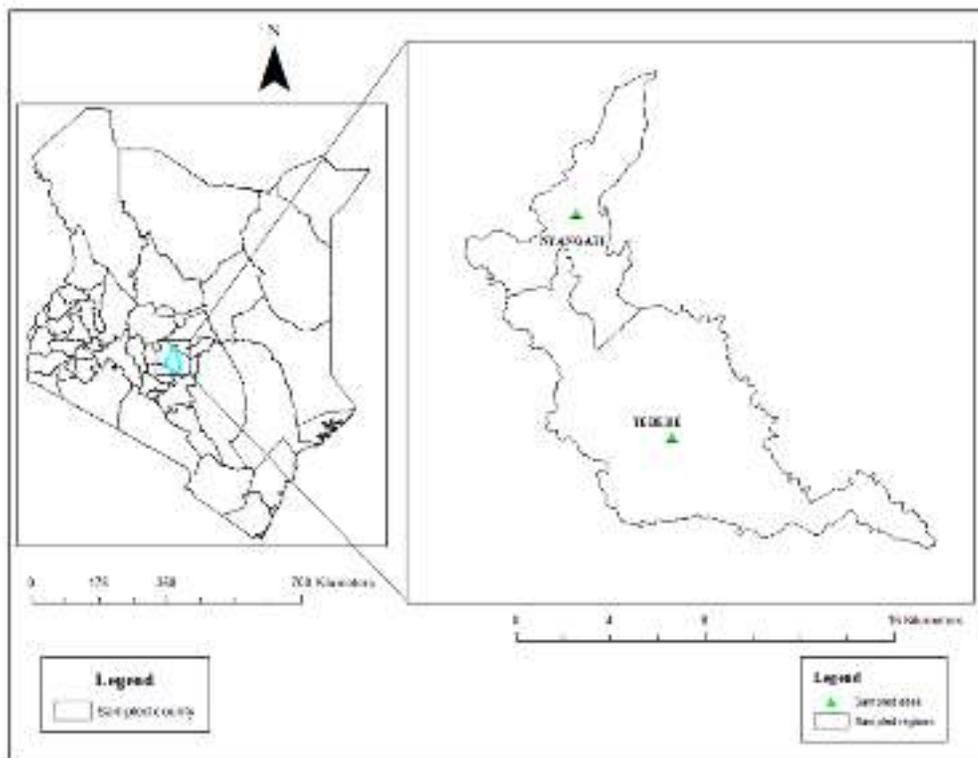
systems also affects the nematode food web (Lu et al., 2017) which has a regulatory role against PPN (Sánchez-Moreno & Ferris, 2007).

Disturbance in rice fields alters the food web structure by reducing nematode trophic levels, number of predatory nematodes, enrichment and structure indices (Korobushkin et al., 2019). Wang et al., (2019) reported alterations in nematode trophic groups due to elevated carbon dioxide in rice paddy fields with increased plant parasitic index. Okada et al., (2011) revealed that flooding increases the abundance of *k* strategists, lowers the enrichment index and increases the structure index. Salinity in irrigated rice fields affects nematode assemblages and trophic groups and reduces the complexity of the food web structure (Chau et al., 2021). In order to sustainably control PPN, maintain soil health and related ecosystem functions in Kenyan rice ecosystems, it is important to have information on nematode diversity and its contribution to the soil food web. This study therefore examined the soil food web indices and metabolic footprints in irrigated rice fields in Mwea.

## **4.2 Materials and methods**

### **4.2.1 Site description and soil sampling**

Irrigated rice fields in Nyangati (0°36'47.4"S 37°21'11.4"E) and Tebere (0°38'10.9"S 37°21'44.0"E) regions of Mwea, Kirinyaga County (Figure 4.1) were sampled. Annually, the region has two rainy seasons with approximately 930mm rainfall and average temperatures between 14°C - 31°C (Akoko et al., 2020). For this study, fields that were cultivated with rice for at least 10 years were selected. Soil samples were collected from fields cultivated with the rice variety Basmati 370 at four months after planting. Thirty fields in Nyangati (15) and Tebere (15) were sampled. The sampled fields had received basal fertilizer input (NPK; 17:17:17) and ammonium sulfate top dressing after 45 days of transplanting. In addition, insects were controlled with applications of Oshothion 50EC and Diazol 60EC. Based on the sampling protocol described by Wiesel et al. (2015), soil samples from each field were collected from three sample walks at 25cm depth. Composite 3kg soil sample from each field was used for subsequent analyses.



**Figure 4.1: Study areas at Nyangati and Tebere, in Mwea, Kirinyaga County, Kenya**

#### **4.2.2 Nematode analyses**

From the composite sample, three 250g sub-samples were used for nematode extraction using the modified Baermann technique (Hooper, 1986). Nematodes were fixed according to Hooper (1970) and identified to the genus level (Bongers, 1988, Mai and Mullin, 1996) using a compound microscope. Nematodes were assigned to specific cp groups as described by Ferris et al. (2001).

### **4.2.3 Data analysis**

From the triplicate samples, the average number of nematodes was used in subsequent analyses. Nematode based indices (plant-parasitic index; PPI, maturity index of nematodes belonging to colonizer-persister group 1 – 5; MI, Maturity index excluding cp1 nematodes; MI 2-5, structure index; SI, enrichment index; EI, basal index; BI and channel index; CI) and metabolic footprints were determined using the Nematode Indicators Joint Analysis online program (Sieriebriennikov et al. 2014). One-way ANOVA was used to evaluate the differences in the indices and metabolic footprints in Nyangati and Tebere using  $\log(x+1)$  transformed count data. Significantly different means were separated using Tukey honestly significant difference post hoc test. All analyses were done in R version 4.0.2 (R Core Team, 2020).

### 4.3 RESULTS

Rice fields in Nyangati had a higher MI and MI2-5 although they were not significantly different from Tebere (Figure 4.2 and 4.3). The MI ranged from 3.03 to 3.34 while the MI2-5 was 3.39-3.72. The fields in Nyangati and Tebere showed different levels of soil food web complexity (Figure 4.4). Most of the fields had nematode genera belonging to cp 3-5 and an average number had cp 1 nematodes (Figure 4.5). Tebere had a higher PPI although it was not significantly different from Nyangati.

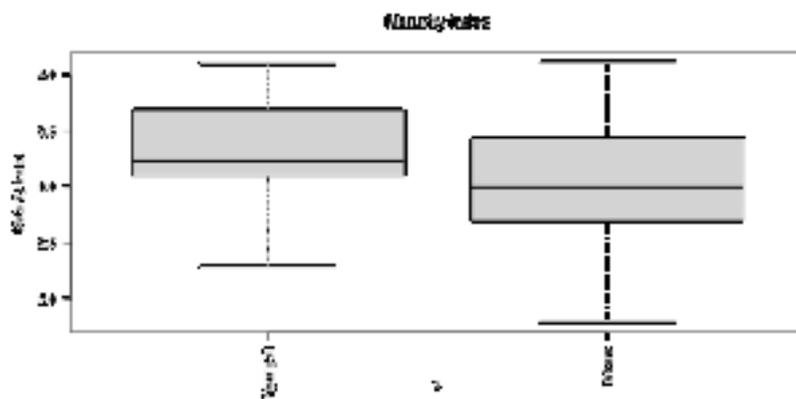
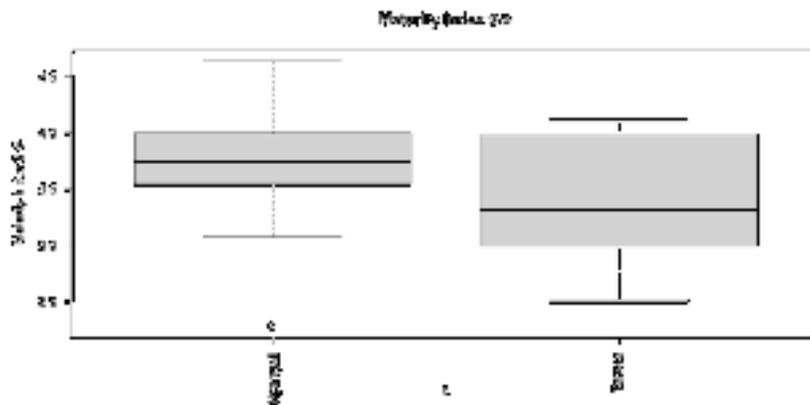
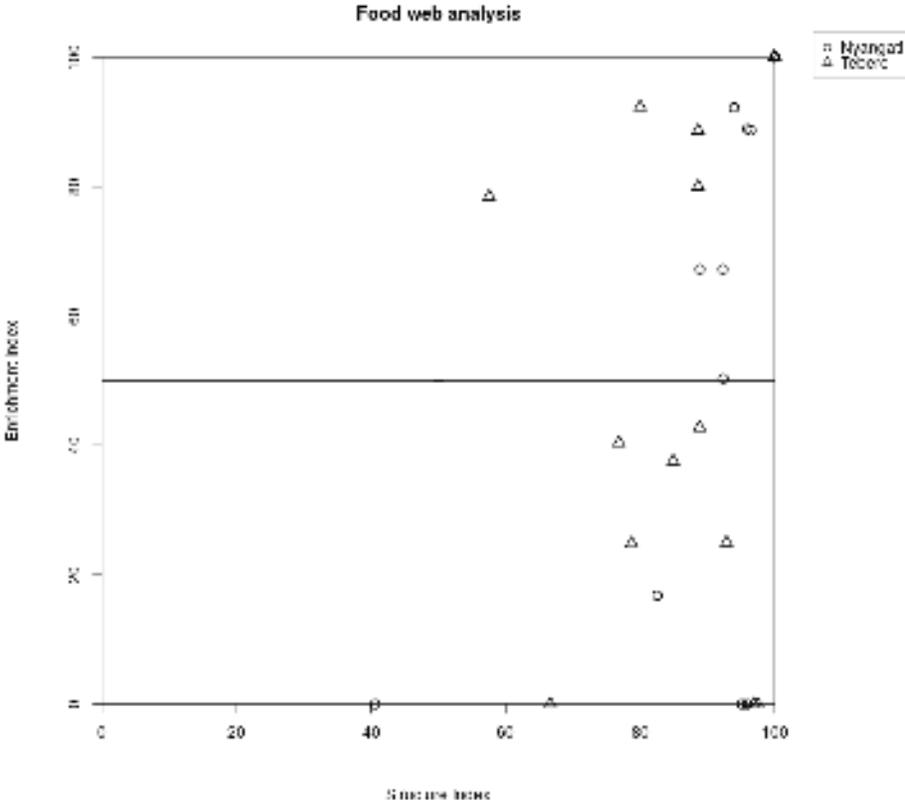


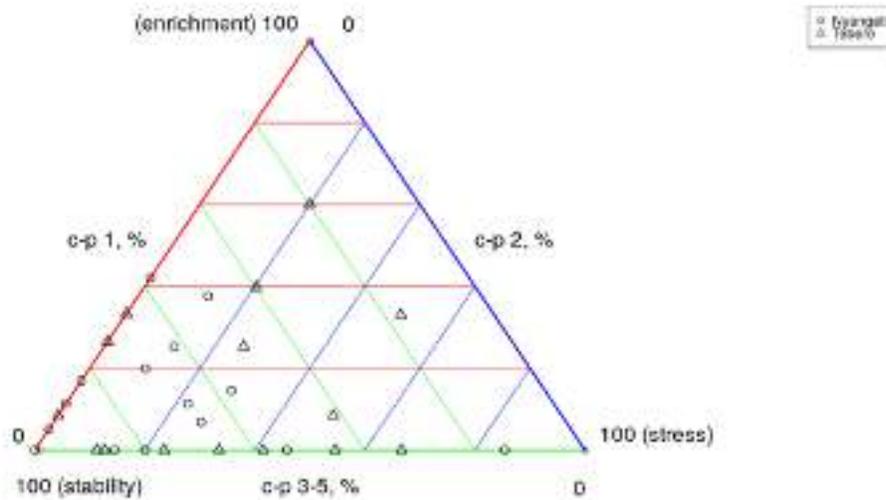
Figure 4.2: Maturity index (MI) in rice fields in Nyangati and Tebere regions in Mwea.



**Figure 4.3: Maturity index 2-5 (MI 2-5) in rice fields in Nyangati and Tebere regions in Mwea.**



**Figure 4.4: Soil food web condition in rice fields in Nyangati and Tebere in Mwea.**



**Figure 4.5: Nematode groups (colonizer-persister 1-5) in rice fields in Nyangati and Tebere in Mwea**

Channel and basal indices were below 30% while the EI and SI were above 50%. There was no variation in metabolic footprints except for fungivore footprint which was significantly higher ( $F_{1,28}=4.58$ ;  $P=0.041$ ) in Tebere (Table 4.1). The fungivore and predator footprints had low values in both regions. Based on the EI and SI, the soil food web in both regions was structured, moderately enriched, and with low to moderate disturbance (Figure 4.6).

**Table 4.1: Soil food web indices and metabolic footprints (log-transformed) in Nyangati and Tebere rice fields**

Index/footprint	Nyangati		Tebere		F value	P value
	Mean	SE	Mean	SE		
Maturity Index (MI)	3.34a	0.13	3.03a	0.17	2.28	0.142
Maturity Index 2-5 (MI2-5)	3.72a	0.14	3.39a	0.14	2.55	0.122
Plant Parasitic Index (PPI)	2.99a	0.24	3.8a	0.34	1.20	0.283
Channel Index (CI)	6.67a	6.67	28.5a	11.66	3.26	0.082
Basal Index (BI)	7.82a	3.87	9.75a	2.44	1.27	0.269
Enrichment Index (EI)	58.09a	11.02	54a	10.07	0.04	0.844
Structure Index (SI)	91.62a	3.86	86.54a	3.28	0.48	0.495
Composite footprint	6.02a	0.20	5.68a	0.21	1.43	0.241
Enrichment footprint	2.81a	0.51	2.69a	0.51	0.03	0.869
Structure footprint	5.28a	0.30	4.59a	0.28	2.78	0.106
Herbivore footprint	3.38a	0.48	3.88a	0.46	0.57	0.455
Fungivore footprint	0.06a	0.06	0.48b	0.19	4.58	0.041*
Bacterivore footprint	3.83a	0.33	3.8a	0.32	0.00	0.952
Predator footprint	0.31a	0.21	0.2a	0.20	0.16	0.696
Omnivore footprint	5.26a	0.31	4.4a	0.40	2.97	0.096

Means with the same letter across the row are not significantly different.\* =  $P < 0.05$ .

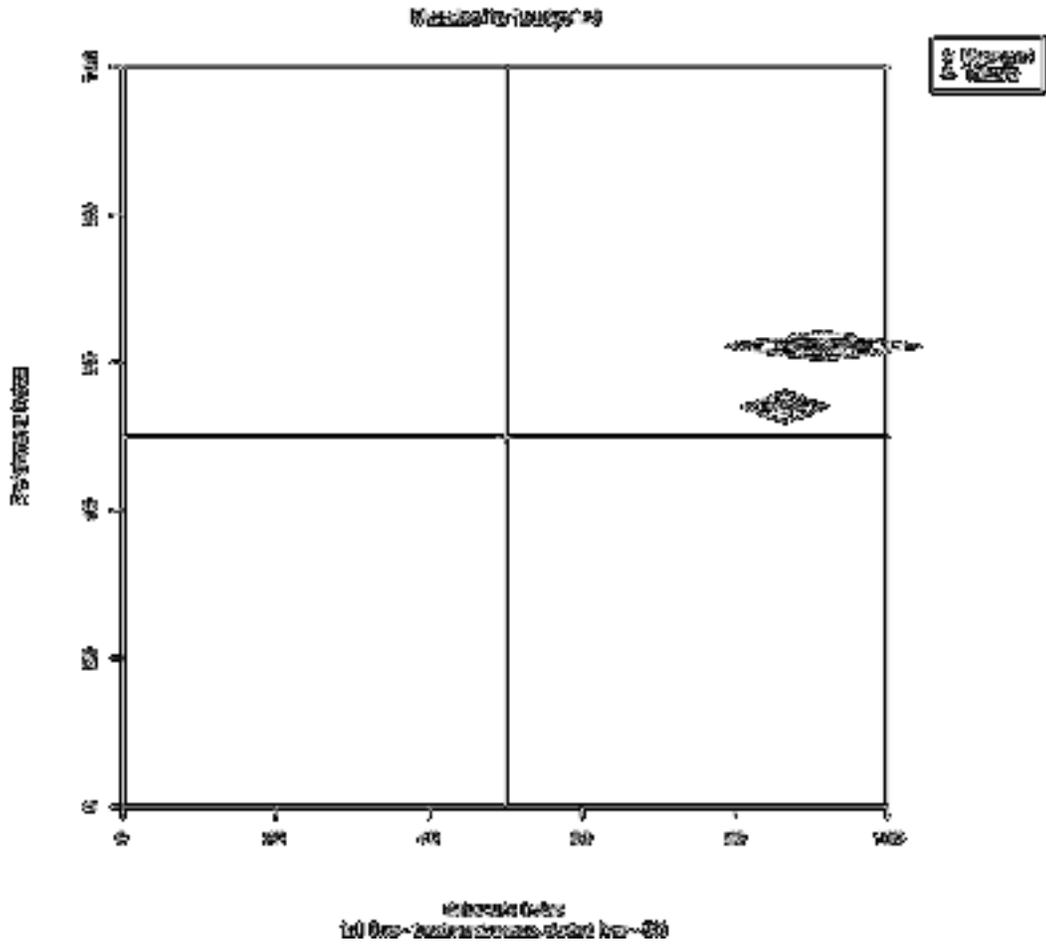


Figure 4.6: Soil food web condition in Nyangati and Tebere rice fields based on the enrichment and structure indices.

#### 4.4 DISCUSSION

The condition of an ecosystem can be inferred from the maturity index that is based on nematode communities (Bongers, 1990). In this study the MI ranged from 3.03 to 3.34 while the MI2-5 was 3.39-3.72 which is an indication of an intermediate level of maturity in the soil food web. A similar range was reported by Chau et al. (2021) and they observed that the values were greater in alluvial soil that was not affected by salt. Contrary to the observations in this study, Okada et al. (2011) observed maturity index in the range of 2-2.4 in the upper layer of paddy rice. The type of rice cropping system can affect the MI as reported by Landi et al. (2018). They found that the MI in organic rice systems was  $<2$  while that in conventional systems was  $>3$ . In agroecosystems, the MI and MI2-5 may be low due to disturbances but this was contrary to what was observed in this study but has been reported elsewhere. For instance, a comparison of MI in undisturbed systems and rice fields with high disturbance characterized by water logging, use of fertilizers and machinery, showed a negative correlation between the MI and level of disturbance (Korenko & Schmidt, 2006).

In this study there was a moderately high percentage of nematode genera belonging to cp3-5. These groups of nematodes have been reported in irrigated rice fields (Okada et al., 2011). In a different study, higher numbers of cp3 nematodes were present in paddy fields with and without salt while the population of cp5 nematodes was greater in alluvial soil that did not have salt intrusion (Chau et al., 2021). The higher cp groups are sensitive to anthropogenic disturbances and their reproduction rate is low (Ferris et al., 2001). The observed population of cp3-5 nematodes in this study may be due to several factors including the type of resources in the rice cropping systems and the presence of other soil organisms that affect the predator-prey interactions within the soil food web (Masson et al., 2022). The proportions of nematode cp groups in an ecosystem influences specific nematode indices (Sánchez-Moreno & Ferris, 2018).

Soil food web indices were similar across the two regions with the CI and BI having values below 30% while the EI and SI were above 50%. Contrary to these observations, Korobushkin et al. (2019) reported significant regional effects on EI and SI in rice fields. Similar to observations made in this study, Okada et al. (2011) reported

high SI values with great abundance of cp 3-5. Moderate values of EI that were observed in both Nyangati and Tebere are expected due to fertilizer application that increases the level of fertility and consequently the number of enrichment opportunists (Ferris et al., 2001). In addition, under irrigated conditions in rice fields, bacterial decomposition is dominant and organic matter from rice litter acts as a source of nutrients to cp1 bacterivores which is reflected in high EI (Okada et al., 2011). Rice cultivation as with other forms of agriculture comprise a source of disturbance to soil nematode communities and food web structure (Sánchez-Moreno & Ferris, 2007). Based on the EI, there was moderate disturbance in Nyangati and Tebere, probably due to fertilizers and pesticides application (Puissant et al., 2021); however, the soil food web showed some level of complexity due to the presence of omnivorous nematodes which also contributed to the observed high SI (Ferris et al., 2001). In both regions, the channel index was low which is indicative of low levels of complex material and the reduced role of fungivores in decomposition (Ferris et al., 2001). Fungivore footprint was also reduced which may be due to the anaerobic conditions in paddy fields that inhibit fungal growth (Lu et al., 2017).

## **CONCLUSION**

The soil food web structure in both regions was enriched due to the presence of enrichment opportunists and it was also structured with moderate disturbance. The intensive use of fertilizers and pesticides in Mwea rice fields is increasing (Njinju et al., 2018; Ma et al., 2021) which forms a potential source of disturbance that can reduce the complexity of the nematode soil food web (Sánchez-Moreno et al., 2011). Several other factors that were not assessed in the current study such as climatic variables and their interactions with nematodes, soil properties and other organisms may also affect nematode community dynamics in the rice fields. Changes in cropping practices and the type of rice cultivar may also increase PPN damage in rice (Peng et al., 2018). The baseline information on the status of nematode soil food web provided in this study can be used to address the emerging issues and in the development of sustainable rice cropping systems through integrated nematode management.



## CHAPTER FIVE

### **Assessment of alpha and beta diversity of nematodes in Mwea rice fields based on additive diversity partitioning**

#### **5.0 ABSTRACT**

In Kenya, rice is an important cereal crop with high economic value, serving as the 3<sup>rd</sup> most consumed staple food. Nematode diversity in cropping systems can have implications on crop productivity. This study therefore aimed at determining the alpha and beta diversity of nematode communities in rice fields in Mwea, Kirinyaga County. Soil samples were collected from 15 fields each in Nyangati and Tebere. Nematodes were extracted from the soil before fixation and morphological identification to the genus level. Gamma diversity was additively partitioned into  $\alpha$  and  $\beta$  components in the R package *vegan*. Contribution of nestedness and turnover to beta diversity was computed using the *betapart* package in R software. The two regions could not be unequivocally ordered due to crossing of the Renyi diversity profiles along the scale parameter. Genus richness partitioning, showed that  $\alpha$  and  $\beta$  components contributed 33.7% and 66.3% of the  $\gamma$  diversity, respectively. For Shannon and Simpson diversity, the largest contribution to overall diversity was from  $\alpha$  component. Partitioning of beta diversity showed that nestedness contributed to the overall nematode diversity observed in Nyangati and Tebere rice fields. Within Nyangati and Tebere, turnover had the highest contribution to beta diversity. The variations in nematode diversity should be considered in management of plant-parasitic nematodes.

#### **5.1 INTRODUCTION**

Rice (*Oryza sativa*) is grown in a wide range of climatic conditions across the world and is an important source of calories (Seck et al., 2012; Muthayya et al., 2014). Production has increased due to the high demand from the expanding human population (Skamnioti

& Gurr, 2009). Development of rice production systems has been prioritized by most governments in SSA due to its role in alleviating poverty. Expansion of rice production in SSA is envisaged due to the availability of suitable land for cultivation (Balasubramanian et al., 2007). In Kenya, rice, the third most important staple crop, is grown in different regions (Fukuta *et al.*, 2019; Ouma-Onyango, 2014) as a subsistence and cash crop (Evans *et al.*, 2018). Despite its importance, there are several abiotic and biotic production constraints. Among the biotic factors are PPN which cause significant yield losses. (Arif *et al.*, 2018; Gnamkoulamba *et al.*, 2018). The infection of rice by PPN in Kenya may be exacerbated by other stresses such as flooding, drought, salinity, weeds and diseases (Balasubramanian et al., 2007). Some of the diseases that affect rice in Kenya include rice blast caused by *Magnaporthe Oryzae* (Kihoro et al., 2013) and brown spot disease (Nganga et al., 2022).

Agricultural practices can influence nematode diversity which may affect the trophic groups and functional guilds with implications on the soil food web (Sánchez-Moreno et al., 2008). Rice is associated with different nematode communities. Nematode genera ranged from 6-18 in the European regions of Primorsky Krai, Kalmykia and Krasnodarsky Krai (Korobushkin et al., 2019). In Ivory Coast, there were 80 nematode species in upland rice fields under different farm management practices (Coyne et al., 1998). The nematode genera *Tobrilus*, *Rhabdolaimus*, *Mesodorylaimus* and *Hirschmanniella* were associated with paddy rice. Namu et al. (2018) reported 22 genera in upland and irrigated rice fields in Ombeyi, Kirogo, Nyangati, Thiba, Kakola, Kombura and Mwambe regions of Kenya. Additive diversity partitioning can be used to provide insights into the determinants of the species distribution and diversity at spatial and temporal scales (Veech et al., 2002). The total  $\gamma$  diversity of a region can be partitioned into  $\alpha$  and  $\beta$  components which represent diversity within and among communities, respectively (Lande, 1996). Such diversity analyses can provide information on the ecological drivers of nematode communities in rice cropping systems which can be used in designing control measures of economically damaging PPN. The aim of this study was to examine the alpha and beta diversity of nematode communities in Mwea rice fields.

## **5.2 Materials and methods**

### **5.2.1 Site description**

Soil sampling was done in 30 fields in two regions (Nyangati and Tebere) located at Mwea, Kirinyaga County. The area has an average rainfall and temperature range of 930mm and 14-31<sup>0</sup>C, respectively. Using the sampling technique by Wiesel et al. (2015), 3kg composite sample was collected from each field four months after planting of Basmati 370 rice variety. By the time of sampling, the rice fields had received basal fertilizer input (NPK; 17:17:17) and ammonium sulfate top dressing after 45 days of transplanting. In addition, insecticide applications of Oshothion 50EC and Diazol 60EC had been done.

### **5.2.2 Nematodes extraction and identification**

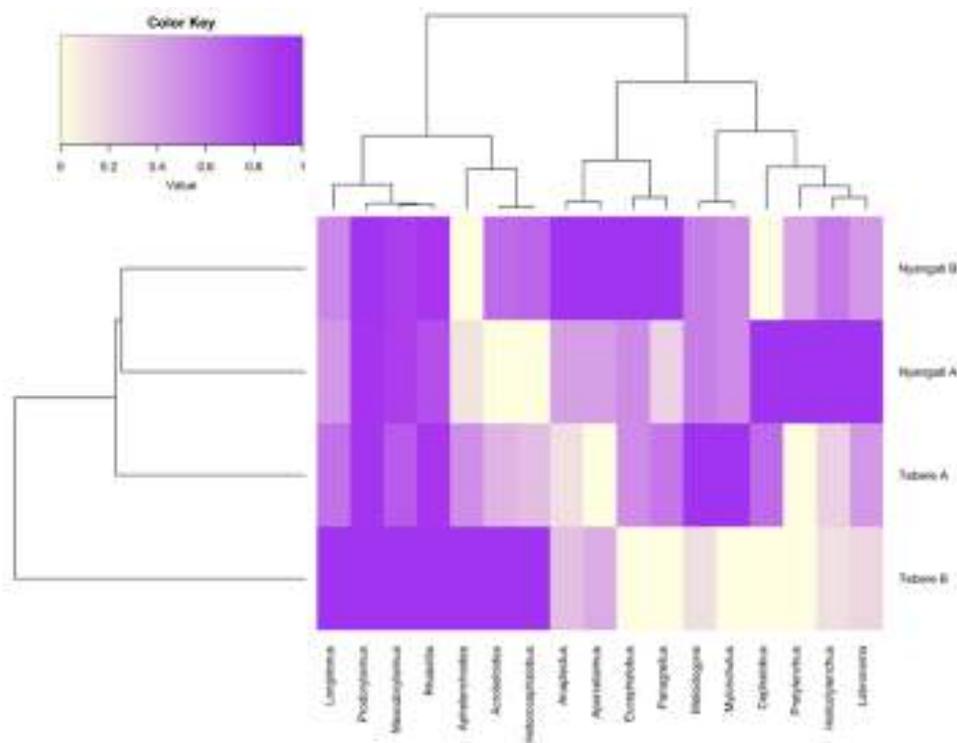
Extraction of nematodes from soil samples was done using the modified Baermann technique which was followed by fixation (Hooper et al., 2005; Karuri et al., 2017). Identification of the nematodes was done to genus level using a compound microscope and with the aid of morphological keys (Luc, Sikora, *et al.*, 2005; Mekete *et al.*, 2012).

### **5.2.3 Data analysis**

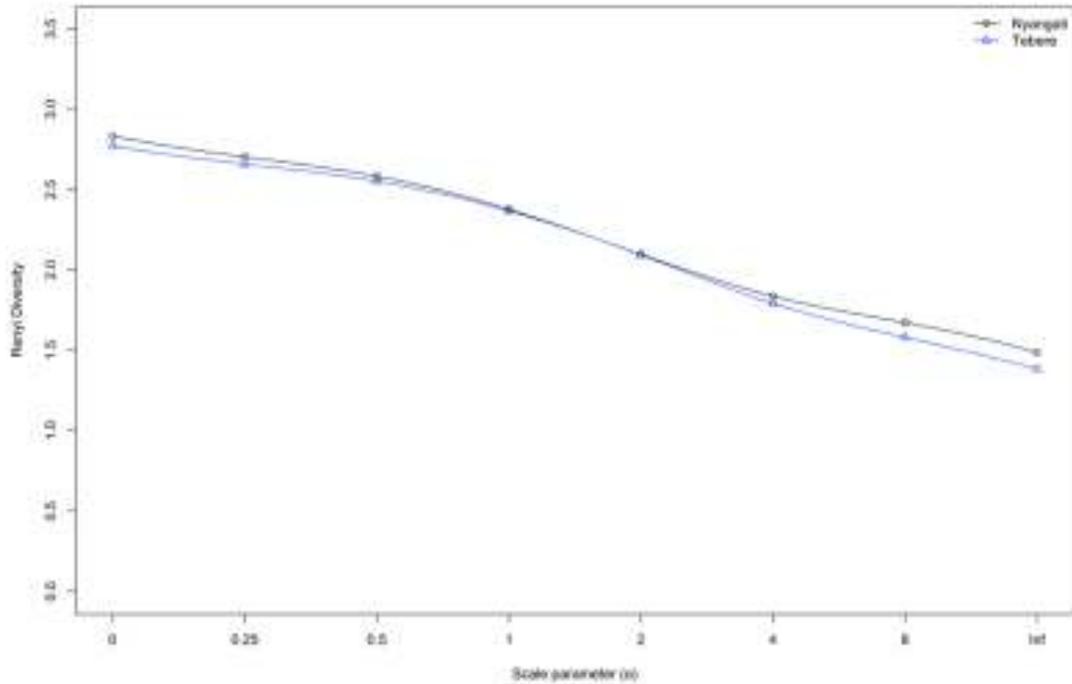
Additive diversity partitioning of gamma diversity into its  $\alpha$  and  $\beta$  components was done using the *adipart* function in R package *vegan*. Contribution of turnover and nestedness to beta diversity was computed using the *betapart* package in R software. The distribution of nematode communities in Nyangati and Tebere was examined using heatmap analysis. Renyi diversity ordering of nematode genera (Tóthmérész, 1995) in the two regions was conducted using the *renyi* function in R *vegan* package.

### 5.3 RESULTS

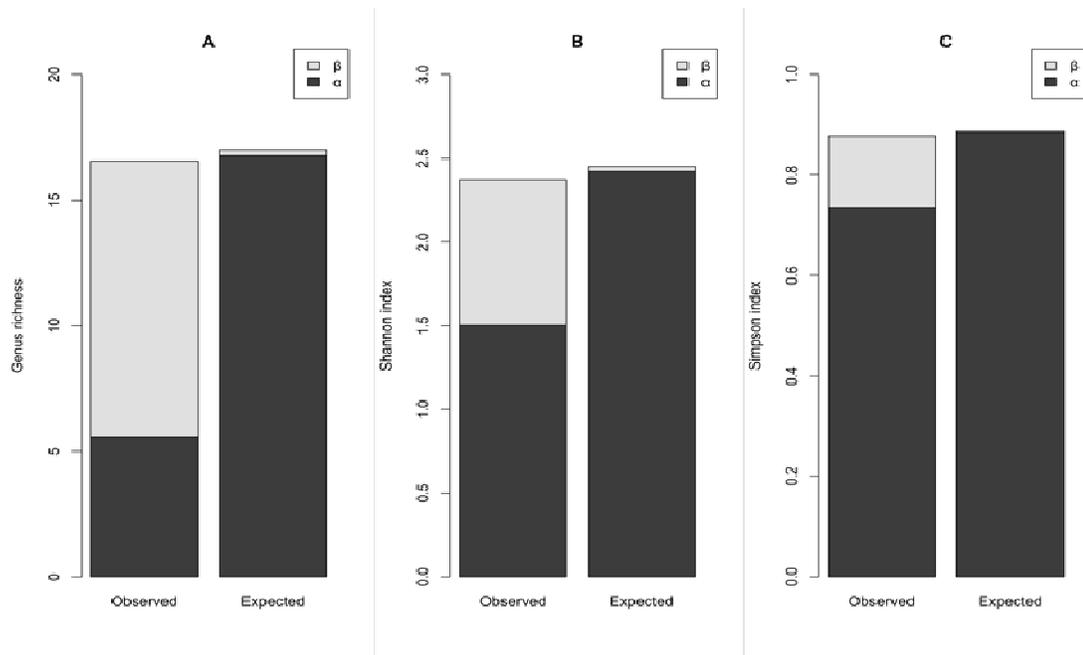
The abundance of some nematode genera was different in Nyangati (Nyangati A and B sampling blocks) and Tebere (Tebere A and B sampling blocks) rice fields. From the Renyi diversity profiles, the two regions could not be unequivocally ordered due to crossing of the profiles along the scale parameter (Figure 5.2). The PPN *Helicotylenchus* and *Pratylenchus* were more prevalent in Nyangati A while *Meloidogyne* occurred in high numbers in Tebere A (Figure 5.1). Genus richness partitioning, showed that  $\alpha$  and  $\beta$  components contributed 33.7% and 66.3% of the  $\gamma$  diversity, respectively. For Shannon and Simpson diversity indices, the largest contribution to overall diversity was from  $\alpha$  component. Observed  $\beta$  diversity for Shannon (37.5% of  $\gamma$  diversity) and Simpson (15.9% of  $\gamma$  diversity) diversity indices was higher than expected ( $P < 0.0001$ ) (Figure 5.3).



**Figure 5.1: Heatmap of nematode communities in Nyangati and Tebere rice fields, Mwea.**



**Figure 5.2: Renyi diversity profiles of nematode communities in Nyangati and Tebere rice fields, Mwea. Scale parameter values are 0- species richness, 1- Shannon index, 2- logarithm of the reciprocal Simpson index, and infinity- Berger-Parker index.**



**Figure 5.3: Additive  $\gamma$  diversity partitioning (alpha,  $\alpha$  and beta,  $\beta$ ) components) of A) nematode genus richness B) Shannon diversity and C) Simpson diversity indices in Nyangati and Tebere rice fields, Mwea.**

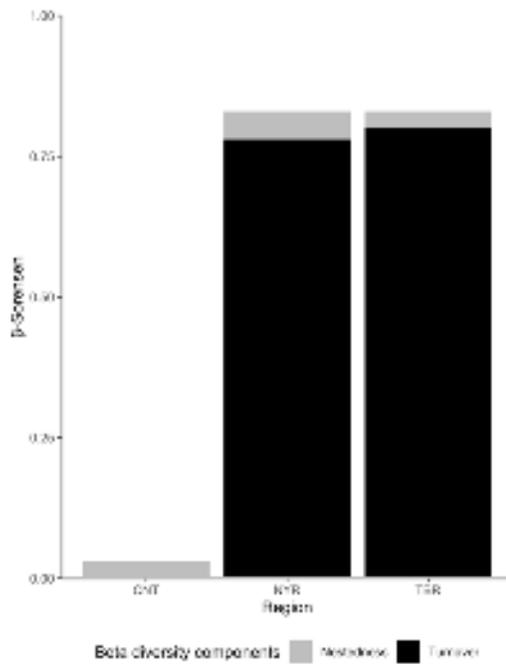
Partitioning of beta diversity showed that nestedness ( $\beta_{sne}$ ) contributed to the overall nematode diversity observed in Nyangati and Tebere rice fields. Within Nyangati and Tebere, turnover ( $\beta_{sim}$ ) had the highest contribution to beta diversity ( $\beta_{sor}$ ) (Table 5.1 and 5.2; Figure 5.4).

**Table 5.1: Nestedness and turnover components of beta diversity in Nyangati and Tebere rice fields, Mwea**

$\beta_{\text{sor}}$ component	Value
$\beta_{\text{sne}}$	0.03
$\beta_{\text{sim}}$	0
$\beta_{\text{sor}}$	0.03
% contribution of $\beta_{\text{sne}}$ to $\beta_{\text{sor}}$	100
% contribution of $\beta_{\text{sim}}$ to $\beta_{\text{sor}}$	0

**Table 5.2: Nestedness and turnover components of beta diversity within Nyangati and Tebere rice fields Mwea**

$\beta_{\text{sor}}$ component	Nyangati	Tebere
$\beta_{\text{sne}}$	0.05	0.03
$\beta_{\text{sim}}$	0.78	0.80
$\beta_{\text{sor}}$	0.83	0.83
% contribution of $\beta_{\text{sne}}$ to $\beta_{\text{sor}}$	6	4
% contribution of $\beta_{\text{sim}}$ to $\beta_{\text{sor}}$	94	96



**Figure 5.4: Nestedness and turnover components of beta diversity in Nyangati and Tebere rice fields, Mwea. CNT-Nyangati and Tebere rice fields, NYR- Nyangati rice fields, TBR- Tebere rice fields.**

## 5.4 DISCUSSION

Variations in nematode communities can be at different levels such as fields and regions (Neher et al., 1995). In this study, nematode genus diversity differed in Nyangati and Tebere rice fields with variations in abundance of the PPN *Helicotylenchus*, *Pratylenchus* and *Meloidogyne*. Crossing of Renyi diversity profiles of nematode communities in the two regions may be as a result of similarities in some nematode genera in the two regions. However, there were variations in the distribution of some nematode genera which could be due to several reasons that are either field- or region-specific. A study by Nguyen et al. (2020) showed that soil physico-chemical properties affected the abundance of nematode communities in rice fields. Similarly, there were strong correlations between N, P, pH and nematode communities in rain-fed rice in Nigeria (Oladele et al., 2021). The same study demonstrated that application of biochar and inorganic NPK fertilizer modified the nematode assemblages.

The root-knot nematode, *Meloidogyne* which occurred in high abundance in Tebere A causes high yield losses in rice and the major species *M. graminicola* can withstand flooded conditions and is adapted to most rice cropping systems. Depending on the temperature conditions, *M. graminicola* life cycle is 19-27 days with female nematodes developing after 14 days after infection by juveniles. Transport of water and nutrients is disrupted by formation of giant cells, which leads to low yields (Mantelin et al., 2017). *Pratylenchus* which was also observed in this study has been reported to affect rice productivity but mainly under upland conditions (Protand Savary 1993). Genus richness partitioning, showed that  $\alpha$  and  $\beta$  components contributed 33.7% and 66.3% of the  $\gamma$  diversity, respectively. The high contribution of  $\beta$  diversity has been reported in other studies. In rice growing regions in Russia, region strongly influenced the genus richness (Korobushkin et al., 2019) while prevalence of *Meloidogyne graminicola* ranged between 64-94% in lowland rice fields in Myanmar (Win et al., 2011). Similarly, regional variability was observed in the abundance of *Heterodera sachari*, *Meloidogyne incognita*, *Mesocriconema curvatum*, *M. onoens* and *Xiphinema ifacolum* in Liberia.

Results that were observed from this study further showed that the largest contribution to overall Shannon and Simpson diversity indices was from  $\alpha$  component. Okada et al. (2011) also observed that the Shannon and inverse of Simpson's indices did not vary between different rice fields. Compared with other ecosystems, Shannon diversity index was reported to be lower in rice cropping systems (Korenko and Schmidt, 2007; Nguyen et al., 2020). In this study, there was variable contribution of turnover and nestedness to the  $\beta$  diversity. Agricultural practices and soil physico-chemical properties act as habitat filters that determine nematode  $\beta$  diversity (Archidona-Yuste et al., 2020). The observed patterns in the contribution of  $\beta$  diversity components could be due to the environmental factors and the rate of change of nematode communities at different spatial scales (Li et al., 2020). In addition, agriculture results in homogenization of nematode communities due to reduced species turnover (Archidona-Yuste et al., 2021).

## **CONCLUSION**

Nematode communities in rice agro-ecosystems in Nyangati and Tebere have different patterns of  $\alpha$  and  $\beta$  diversity. The contribution of turnover and nestedness also differed within and between the two regions. In order to develop rice cropping systems with reduced levels of PPN that cause yield losses, it is important to put in place site-specific nematode management schemes that considers the type of PPN genera.

## CHAPTER SIX

### GENERAL OVERVIEW (SYNTHESIS)

#### 6.1 INTRODUCTION

This chapter gives a summary of the main results, proposed recommendations and the way forward concerning nematode diversity in rice cropping systems in Mwea, Kirinyaga County. The main objective of this study was to examine diversity components of nematodes associated with rice in Nyangati and Tebere regions in Mwea. The nematode abundance, diversity and soil food web condition was assessed. Below are the specific objectives:

1. To determine the prevalence and abundance of rice nematodes in Mwea.
2. To evaluate the nematode-based soil food web in rice fields in Mwea.
3. To assess alpha and beta diversity of nematodes in Mwea rice fields based on additive diversity partitioning.

#### 6.2 SUMMARY OF THE MAJOR FINDINGS

##### 6.2.1 Determination of the prevalence and abundance of rice nematodes in Mwea

Soil samples were collected from 30 fields within two major sampling sites of Nyangati (15) and Tebere (15) in Mwea, Kirinyaga County. Nematodes were extracted from the soil followed by identification to the genus level. The results revealed that:

- There were 17 nematode genera in both regions with *Aphelenchoides* and *Longidorus* occurring in greater proportions in Tebere.
- The population of *Helicotylenchus* was high in Nyangati and *Prodorylaimus* occurred in high numbers in the two regions.
- In both regions, omnivores made up the highest proportion of the free-living nematodes.
- The two regions could not be unequivocally ordered due to crossing of the Renyi diversity profiles.
- *Aphelenchoides* was positively correlated to electrical conductivity while *Helicotylenchus* was negatively correlated.

### **6.2.2 Evaluation of the nematode-based soil food web in rice fields in Mwea**

Thirty rice fields in Mwea were selected for soil sampling. The fields had been exclusively cultivated with rice for 10 years. Nematodes were extracted from the soil samples and morphologically identified. Nematode data was used to compute different soil food web indices and metabolic footprints. The results were as follows:

- The MI ranged from 3.03 to 3.34 while the MI2-5 was 3.39-3.72.
- Channel and basal indices were below 30% while the EI and SI were above 50%.
- There was no variation in metabolic footprints except for fungivore footprint which was significantly higher in Tebere.
- The soil food web in both regions was structured, moderately enriched, and with low to moderate disturbance.

### **6.2.3 Assessment of alpha and beta diversity of nematodes in Mwea rice fields based on additive diversity partitioning**

Diversity analysis of nematode communities was assessed based on data from 30 rice fields in Nyangati and Tebere. The gamma diversity was additively partitioned into  $\alpha$  and  $\beta$  diversities. The  $\beta$  diversity was further assessed by determining the turnover and nestedness components. The following were the results;

- **The two regions could not be unequivocally ordered due to crossing of the Renyi diversity profiles along the scale parameter.**
- **Genus richness partitioning, showed that  $\alpha$  and  $\beta$  components contributed 33.7% and 66.3% of the  $\gamma$  diversity, respectively. For Shannon and Simpson diversity indices, the largest contribution to overall diversity was from the  $\alpha$  component.**
- **Partitioning of beta diversity showed that nestedness contributed to the overall nematode diversity observed in Nyangati and Tebere rice fields.**
- Turnover had the highest contribution to beta diversity within Nyangati and Tebere.

### **6.3 RECOMMENDATIONS, IMPLICATIONS AND WAY FORWARD**

- Abundance and prevalence of nematodes in Nyangati and Tebere was variable with some economically important PPN occurring in high proportions. These nematodes may pose a risk to rice production in Mwea and their management should be prioritized.
- Nematode abundance in rice is affected by soil properties which can interact with other environmental variables thereby affecting the population dynamics. Further investigations into the factors that influence the spatial and temporal distribution of nematodes in rice in Kenya is required.
- The nematode-based soil food web in rice fields in Nyangati and Tebere was structured, moderately enriched, and with low to moderate disturbance. Due to the increasing intensive use of fertilizers and pesticides in Mwea rice fields, the level of disturbance may increase which could reduce the complexity of the nematode soil food web. It will be crucial to continuously monitor the soil food web condition in the rice growing regions.
- The diversity of nematodes within and between fields in Mwea was different and there are several potential drivers of the variability. Additional assessment of these factors is needed. In order to develop rice cropping systems with reduced levels of PPN that cause yield losses, it is important to put in place site-specific nematode management schemes that considers the type of PPN genera.

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