University of Yaoundé I
****
Université de Yaoundé I

Faculty of Science
****
Faculté des Sciences

DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY
DÉPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES

LABORATORY OF PARASITOLOGY AND ECOLOGY
LABORATOIRE DE PARASITOLOGIE ET ÉCOLOGIE

Distribution and damaging activities of termites and inventory of their natural enemies in cocoa agroforestry systems of Cameroon

Thesis presented as part of fulfilment for obtaining the degree of

Doctorate/Ph.D. in Animal Biology

Option**: Parasitology and Ecology**

Speciality**: Pest management and biological control**

By

DJUIDEU TCHOUAMOU Christian Landry

Registration n°: **11Q0141**

Master of Science

Publicly defended on July 10, 2023 before the jury composed of:

President: FOMENA Abraham, Professor, University of Yaoundé 1;

Rapporteurs: KEKEUNOU Sévilor, Professor, University of Yaoundé 1;

BISSELEUA DAGHELA Hervé, Director of Research, World Cocoa Foundation

Members: NJIOKOU Flobert, Professor, University of Yaoundé 1;

MONY NTONE Ruth, Associate Professor, University of Yaoundé 1;

TCHUINKAM Timoléon, Associate Professor, University of Dschang;

Year 2023

UNIVERSITE DE YAOUNDE I
UNIVERSITY OF YAOUNDE I

DEPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES
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CAMEROUN

FACULTE DES SCIENCES
FACULTY OF SCIENCE

ATTESTATION DE CORRECTION

Conformément à l'autorisation de soutenance de la thèse de Doctorat/Ph.D N°129-2023/UYI/CRFD_SVSE /URFD-SV/Ad/ du 08 juin 2023 de Monsieur le Recteur de l'Université de Yaoundé I, la thèse intitulée « **Distribution and damaging activities of termites and inventory of their natural enemies in cocoa agroforestry systems of Cameroon** » a été présentée et soutenue publiquement le lundi **10 juillet 2023** par l'étudiant **DJUIDEU TCHOUAMOU Christian Landry**, Matricule **11Q0141**. Le document final a été corrigé suivant les recommandations du jury.

En foi de quoi la présente attestation est délivrée pour servir et valoir ce que de droit.

Fait à Yaoundé, le

Le Président du Jury

Les Examinateurs

Ruth Mony-Ntone
Maître de Conférences

Le Chef de Département

Charles Félix Bilong Bilong
Professeur

University of Yaoundé I
****
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MONY NTONE Ruth, Associate Professor, University of Yaoundé 1;

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Year 2023

UNIVERSITÉ DE YAOUNDÉ I **Faculté des Sciences** Division de la Programmation et du Suivi des Activités Académiques		THE UNIVERSITY OF YAOUNDE I **Faculty of Science** Division of Programming and Follow-up of Academic Affaires
LISTE DES ENSEIGNANTS PERMANENTS		**LIST OF PERMANENT TEACHING STAFF**

ACADEMIC YEAR 2022/2023
(Per Department and per Grade)
UPDATE from 31 MAY 2023

ADMINISTRATION

DEAN: TCHOUANKEU Jean- Claude, *Assistant Professor*
VICE-DEAN / DPSAA: ATCHADE Alex de Théodore, *Professor*
VICE-DEAN / DSSE: NYEGUE Maximilienne Ascension, *Professor*
VICE-DEAN / DRC: ABOSSOLO ANGUE Monique, *Assistant Professor*
Head of Administrative and Financial Division: NDOYE FOE Florentine Marie Chantal, *Assistant Professor*
Head of Division of Academic Affairs, Research and tuition of DAARS: AJEAGAH Gideon AGHAINDUM, *Professor*

1- DEPARTMENT OF DE BIOCHEMISTRY (BC) (43)

N°	FULL NAMES	GRADE	OBSERVATIONS
1.	BIGOGA DAIGA Jude	Professor	In service
2.	FEKAM BOYOM Fabrice	Professor	In service
3.	KANSCI Germain	Professor	In service
4.	MBACHAM FON Wilfred	Professor	In service
5.	MOUNDIPA FEWOU Paul	Professor	*Head of Department*
6.	NGUEFACK Julienne	Professor	In service
7.	NJAYOU Frédéric Nico	Professor	In service
8.	OBEN Julius ENYONG	Professor	In service
9.	ACHU Merci BIH	Assistant Professor	In service
10.	ATOGHO Barbara MMA	Assistant Professor	In service
11.	AZANTSA KINGUE GABIN BORIS	Assistant Professor	In service
12.	BELINGA née NDOYE FOE F. M. C.	Assistant Professor	*Head of DAF / FS*
13.	DJUIDJE NGOUNOUE Marceline	Assistant Professor	In service
14.	DJUIKWO NKONGA Ruth Viviane	Assistant Professor	In service
15.	EFFA ONOMO Pierre	Assistant Professor	*VD/FS/Univ Ebwa*
16.	EWANE Cécile Annie	Assistant Professor	In service
17.	KOTUE TAPTUE Charles	Assistant Professor	In service
18.	LUNGA Paul KEILAH	Assistant Professor	In service
19.	MBONG ANGIE M. Mary Anne	Assistant Professor	In service
20.	MOFOR née TEUGWA Clotilde	Assistant Professor	*Dean of FS / UDs*
21.	NANA Louise épouse WAKAM	Assistant Professor	In service
22.	NGONDI Judith Laure	Assistant Professor	In service
23.	TCHANA KOUATCHOUA Angèle	Assistant Professor	In service
24.	AKINDEH MBUH NJI	Senior Lecturer	In service
25.	BEBEE Fadimatou	Senior Lecturer	In service
26.	BEBOY EDJENGUELE Sara Nathalie	Senior Lecturer	In service
27.	DAKOLE DABOY Charles	Senior Lecturer	In service
28.	DONGMO LEKAGNE Joseph Blaise	Senior Lecturer	In service
29.	FONKOUA Martin	Senior Lecturer	In service
30.	FOUPOUAPOUOGNIGNI Yacouba	Senior Lecturer	In service

31.	KOUOH ELOMBO Ferdinand	Senior Lecturer	In service
32.	MANANGA Marlyse Joséphine	Senior Lecturer	In service
33.	OWONA AYISSI Vincent Brice	Senior Lecturer	In service
34.	Palmer MASUMBE NETONGO	Senior Lecturer	In service
35.	PECHANGOU NSANGOU Sylvain	Senior Lecturer	In service
36.	WILFRED ANGIE ABIA	Senior Lecturer	In service

37.	BAKWO BASSOGOG Christian Bernard	Assistant Lecturer	In service
38.	ELLA Fils Armand	Assistant Lecturer	In service
39.	EYENGA Eliane Flore	Assistant Lecturer	In service
40.	MADIESSE KEMGNE Eugenie Aimée	Assistant Lecturer	In service
41.	MANJIA NJIKAM Jacqueline	Assistant Lecturer	In service
42.	MBOUCHE FANMOE Marceline Joëlle	Assistant Lecturer	In service
43.	WOGUIA Alice Louise	Assistant Lecturer	In service

2- DEPARTMENT OF ANIMAL BIOLOGY AND PHYSIOLOGY (BPA) (52)

1.	AJEAGAH Gideon AGHAINDUM	Professor	*DAARS/FS*
2.	BILONG BILONG Charles-Félix	Professor	*Head of Department*
3.	DIMO Théophile	Professor	In service
4.	DJIETO LORDON Champlain	Professor	In service
5.	DZEUFIET DJOMENI Paul Désiré	Professor	In service
6.	ESSOMBA née NTSAMA MBALA	Professor	*HD and Vice Dean/FMSB/UYI*
7.	FOMENA Abraham	Professor	In service
8.	KEKEUNOU Sévilor	Professor	In service
9.	NJAMEN Dieudonné	Professor	In service
10.	NJIOKOU Flobert	Professor	In service
11.	NOLA Moïse	Professor	In service
12.	TAN Paul VERNYUY	Professor	In service
13.	TCHUEM TCHUENTE Louis Albert	Professor	*Inspector in Charge / Progr. Coord./MINSANTE*
14.	ZEBAZE TOGOUET Serge Hubert	Professor	In service

15.	ALENE Désirée Chantal	Assistant Professor	*Vice Dean/ Uty Ebwa*
16.	BILANDA Danielle Claude	Assistant Professor	In service
17.	DJIOGUE Séfirin	Assistant Professor	In service
18.	GOUNOUE KAMKUMO Raceline épse FOTSING	Assistant Professor	In service
19.	JATSA BOUKENG Hermine épse MEGAPTCHE	Assistant Professor	In service
20.	LEKEUFACK FOLEFACK Guy B.	Assistant Professor	In service
21.	MAHOB Raymond Joseph	Assistant Professor	In service
22.	MBENOUN MASSE Paul Serge	Assistant Professor	In service
23.	MEGNEKOU Rosette	Assistant Professor	In service
24.	MOUNGANG Luciane Marlyse	Assistant Professor	In service
25.	NOAH EWOTI Olive Vivien	Assistant Professor	In service
26.	MONY Ruth épse NTONE	Assistant Professor	In service
27.	NGUEGUIM TSOFACK Florence	Assistant Professor	In service
28.	NGUEMBOCK	Assistant Professor	In service
29.	TAMSA ARFAO Antoine	Assistant Professor	In service

30.	TOMBI Jeannette	Assistant Professor	In service

31.	ATSAMO Albert Donatien	Senior Lecturer	In service
32.	BASSOCK BAYIHA Etienne Didier	Senior Lecturer	In service
33.	ETEME ENAMA Serge	Senior Lecturer	In service
34.	FEUGANG YOUMSSI François	Senior Lecturer	In service
35.	FOKAM Alvine Christelle Epse KENGNE	Senior Lecturer	In service
36.	GONWOUO NONO Legrand	Senior Lecturer	In service
37.	KANDEDA KAVAYE Antoine	Senior Lecturer	In service
38.	KOGA MANG DOBARA	Senior Lecturer	In service
39.	LEME BANOCK Lucie	Senior Lecturer	In service
40.	MAPON NSANGOU Indou	Senior Lecturer	In service
41.	METCHI DONFACK MIREILLE FLAURE EPSE GHOUMO	Senior Lecturer	In service
42.	MVEYO NDANKEU Yves Patrick	Senior Lecturer	In service
43.	NGOUATEU KENFACK Omer Bébé	Senior Lecturer	In service
44.	NJUA Clarisse YAFI	Senior Lecturer	*HD /Uty Bamenda*
45.	NWANE Philippe Bienvenu	Senior Lecturer	In service
46.	TADU Zephyrin	Senior Lecturer	In service
47.	YEDE	Senior Lecturer	In service
48.	YOUNOUSSA LAME	Senior Lecturer	In service

49.	AMBADA NDZENGUE GEORGIA ELNA	Assistant Lecturer	In service
50.	KODJOM WANCHE Jacguy Joyce	Assistant Lecturer	In service
51.	NDENGUE Jean De Matha	Assistant Lecturer	In service
52.	ZEMO GAMO Franklin	Assistant Lecturer	In service

3- DEPARTMENT OF PLANT BIOLOGY AND PHYSIOLOGY (BPV) (34)

1.	AMBANG Zachée	Professor	*Head of Department*
2.	DJOCGOUE Pierre François	Professor	In service
3.	MBOLO Marie	Professor	In service
4.	MOSSEBO Dominique Claude	Professor	In service
5.	YOUMBI Emmanuel	Professor	In service
6.	ZAPFACK Louis	Professor	In service

7.	ANGONI Hyacinthe	Assistant Professor	In service
8.	BIYE Elvire Hortense	Assistant Professor	In service
9.	MAHBOU SOMO TOUKAM. Gabriel	Assistant Professor	In service
10	MALA Armand William	Assistant Professor	In service
11	MBARGA BINDZI Marie Alain	Assistant Professor	*DAAC /UDla*
12	NDONGO BEKOLO	Assistant Professor	In service
13	NGALLE Hermine BILLE	Assistant Professor	In service
14	NGODO MELINGUI Jean Baptiste	Assistant Professor	In service
15	NGONKEU MAGAPTCHE Eddy L.	Assistant Professor	*CT / MINRESI*
16	TONFACK Libert Brice	Assistant Professor	In service
17	TSOATA Esaïe	Assistant Professor	In service
18	ONANA JEAN MICHEL	Assistant Professor	In service

19.	DJEUANI Astride Carole	Senior Lecturer	In service
20.	GONMADGE CHRISTELLE	Senior Lecturer	In service
21.	MAFFO MAFFO Nicole Liliane	Senior Lecturer	In service

22.	NNANGA MEBENGA Ruth Laure	Senior Lecturer	In service
23.	NOUKEU KOUAKAM Armelle	Senior Lecturer	In service
24.	NSOM ZAMBO EPSE PIAL ANNIE CLAUDE	Senior Lecturer	*In Mission/UNESCO MALI*
25.	GODSWILL NTSOMBOH NTSEFONG	Senior Lecturer	In service
26.	KABELONG BANAHO Louis-Paul-Roger	Senior Lecturer	In service
27.	KONO Léon Dieudonné	Senior Lecturer	In service
28.	LIBALAH Moses BAKONCK	Senior Lecturer	In service
29.	LIKENG-LI-NGUE Benoit C	Senior Lecturer	In service
30.	TAEDOUNG Evariste Hermann	Senior Lecturer	In service
31.	TEMEGNE NONO Carine	Senior Lecturer	In service
32.	MANGA NDJAGA JUDE	Assistant Lecturer	In service
33.	DIDA LONTSI Sylvere Landry	Assistant Lecturer	In service
34.	METSEBING Blondo-Pascal	Assistant Lecturer	In service

4- DEPARTMENT OF INORGANIC CHEMISTRY (CI) (28)

1.	GHOGOMU Paul MINGO	Professor	*Minister Delegate PR*
2.	NANSEU NJIKI Charles Péguy	Professor	In service
3.	NDIFON Peter TEKE	Professor	*CT MINRESI*
4.	NENWA Justin	Professor	In service
5.	NGAMENI Emmanuel	Professor	*Dean FS/Univ. Ngaoundere*
6.	NGOMO Horace MANGA	Professor	*Vice Chancelor/UB*
7.	NJOYA Dayirou	Professor	In service

8.	ACAYANKA Elie	Assistant Professor	In service
9.	EMADAK Alphonse	Assistant Professor	In service
10	KAMGANG YOUBI Georges	Assistant Professor	In service
11	KEMMEGNE MBOUGUEM Jean C.	Assistant Professor	In service
12	KENNE DEDZO GUSTAVE	Assistant Professor	In service
13	MBEY Jean Aime	Assistant Professor	In service
14	NDI NSAMI Julius	Assistant Professor	*Head of Department*
15	NEBAH Née NDOSIRI Bridget NDOYE	Assistant Professor	*Senator/SENAT*
16	NJIOMOU C. épse DJANGANG	Assistant Professor	In service
17	NYAMEN Linda Dyorisse	Assistant Professor	In service
18	PABOUDAM GBAMBIE AWAWOU	Assistant Professor	In service
19	TCHAKOUTE KOUAMO Hervé	Assistant Professor	In service
20	BELIBI BELIBI Placide Désiré	Assistant Professor	*Head of Service/ ENS Bertoua*
21	CHEUMANI YONA Arnaud M.	Assistant Professor	In service
22	KOUOTOU DAOUDA	Assistant Professor	In service

23.	MAKON Thomas Beauregard	Senior Lecturer	In service
24.	NCHIMI NONO KATIA	Senior Lecturer	In service
25.	NJANKWA NJABONG N. Eric	Senior Lecturer	In service
26.	PATOUOSSA ISSOFA	Senior Lecturer	In service
27.	SIEWE Jean Mermoz	Senior Lecturer	In service

28.	BOYOM TATCHEMO Franck W.	Assistant Lecturer	In service

5- DEPARTMENT OF ORGANIC CHEMISTRY (CO) (37)			
1.	Alex de Théodore ATCHADE	Professor	*Vice-Dean / DPSAA*
2.	DONGO Etienne	Professor	*Vice-Dean/FSE/UYI*
3.	NGOUELA Silvère Augustin	Professor	*Head of Department UDS*
4.	PEGNYEMB Dieudonné Emmanuel	Professor	*Director/ MINESUP/ Head of Department*
5.	WANDJI Jean	Professor	In service
6.	MBAZOA née DJAMA Céline	Professor	In service
7.	AMBASSA Pantaléon	Assistant Professor	In service
8.	EYONG Kenneth OBEN	Assistant Professor	In service
9.	FOTSO WABO Ghislain	Assistant Professor	In service
10	KAMTO Eutrophe Le Doux	Assistant Professor	In service
11	KENMOGNE Marguerite	Assistant Professor	In service
12	KEUMEDJIO Félix	Assistant Professor	In service
13	KOUAM Jacques	Assistant Professor	In service
14	MKOUNGA Pierre	Assistant Professor	In service
15	MVOT AKAK CARINE	Assistant Professor	In service
16	NGO MBING Joséphine	Assistant Professor	*Head of Unit MINRESI*
17	NGONO BIKOBO Dominique Serge	Assistant Professor	*C.E.A/ MINESUP*
18	NOTE LOUGBOT Olivier Placide	Assistant Professor	*DAAC/Uty Bertoua*
19	NOUNGOUE TCHAMO Diderot	Assistant Professor	In service
20	TABOPDA KUATE Turibio	Assistant Professor	In service
21	TAGATSING FOTSING Maurice	Assistant Professor	In service
22	TCHOUANKEU Jean-Claude	Assistant Professor	*Dean /FS/ UYI*
23	YANKEP Emmanuel	Assistant Professor	In service
24	ZONDEGOUMBA Ernestine	Assistant Professor	In service
25	MESSI Angélique Nicolas	Senior Lecturer	In service
26	NGNINTEDO Dominique	Senior Lecturer	In service
27	NGOMO Orléans	Senior Lecturer	In service
28	NONO NONO Éric Carly	Senior Lecturer	In service
29	OUAHOUO WACHE Blandine M.	Senior Lecturer	In service
30	OUETE NANTCHOUANG Judith Laure	Senior Lecturer	In service
31	SIELINOU TEDJON Valérie	Senior Lecturer	In service
32	TCHAMGOUE Joseph	Senior Lecturer	In service
33	TSAFFACK Maurice	Senior Lecturer	In service
34	TSAMO TONTSA Armelle	Senior Lecturer	In service
35	TSEMEUGNE Joseph	Senior Lecturer	In service
36	MUNVERA MFIFEN Aristide	Assistant Lecturer	In service
37	NDOGO ETEME Olivier	Assistant Lecturer	In service

6- DEPARTMENT OF INFORMATICS (IN) (22)			
1.	ATSA ETOUNDI Roger	Professor	*Head of Division MINESUP*
2.	FOUDA NDJODO Marcel Laurent	Professor	*General Inspector/ MINESUP*
3.	NDOUNDAM Réné	Assistant Professor	In service
4.	TSOPZE Norbert	Assistant Professor	In service

5.	ABESSOLO ALO'O Gislain	Senior Lecturer	*Head of Unit MINFOPRA*
6.	AMINOU HALIDOU	Senior Lecturer	*Head of Department*
7.	DJAM Xaviera YOUH - KIMBI	Senior Lecturer	In service
8.	DOMGA KOMGUEM Rodrigue	Senior Lecturer	In service
9.	EBELE Serge Alain	Senior Lecturer	In service
10	HAMZA Adamou	Senior Lecturer	In service
11	JIOMEKONG AZANZI Fidel	Senior Lecturer	In service
12	KOUOKAM KOUOKAM E. A.	Senior Lecturer	In service
13	MELATAGIA YONTA Paulin	Senior Lecturer	In service
14	MESSI NGUELE Thomas	Senior Lecturer	In service
15	MONTHE DJIADEU Valery M.	Senior Lecturer	In service
16	NZEKON NZEKO'O ARMEL JACQUES	Senior Lecturer	In service
17	OLLE OLLE Daniel Claude Georges Delort	Senior Lecturer	*Deputy Director ENSET Ebolowa*
18	TAPAMO Hyppolite	Senior Lecturer	In service

19	BAYEM Jacques Narcisse	Assistant Lecturer	In service
20	EKODECK Stéphane Gaël Raymond	Assistant Lecturer	In service
21	MAKEMBE. S . Oswald	Assistant Lecturer	*Director CUTI*
22	NKONDOCK. MI. BAHANACK.N.	Assistant Lecturer	In service

7- DEPARTMENT OF MATHEMATICS (MA) (33)

1.	AYISSI Raoult Domingo	Professor	*Head of Department*

2.	KIANPI Maurice	Assistant Professor	In service
3.	MBANG Joseph	Assistant Professor	In service
4.	MBEHOU Mohamed	Assistant Professor	In service
5.	MBELE BIDIMA Martin Ledoux	Assistant Professor	In service
6.	NOUNDJEU Pierre	Assistant Professor	*Head of Service Curriculums & Diplomas /FS/UYI*
7.	TAKAM SOH Patrice	Assistant Professor	In service
8.	TCHAPNDA NJABO Sophonie B.	Assistant Professor	*Director/AIMS Rwanda*
9.	TCHOUNDJA Edgar Landry	Assistant Professor	In service

10	AGHOUKENG JIOFACK Jean Gérard	Senior Lecturer	*Head of Unit MINEPAT*
11	BOGSO ANTOINE Marie	Senior Lecturer	In service
12	CHENDJOU Gilbert	Senior Lecturer	In service
13	DJIADEU NGAHA Michel	Senior Lecturer	In service
14	DOUANLA YONTA Herman	Senior Lecturer	In service
15	KIKI Maxime Armand	Senior Lecturer	In service
16	LOUMNGAM KAMGA Victor	Senior Lecturer	In service
17	MBAKOP Guy Merlin	Senior Lecturer	In service
18	MBATAKOU Salomon Joseph	Senior Lecturer	In service
19	MENGUE MENGUE David Joël	Senior Lecturer	*Head Dpt /ENS Université d'Ebolowa*
20	MBIAKOP Hilaire George	Senior Lecturer	In service
21	NGUEFACK Bernard	Senior Lecturer	In service
22	NIMPA PEFOUKEU Romain	Senior Lecturer	In service
23	OGADOA AMASSAYOGA	Senior Lecturer	In service

24	POLA DOUNDOU Emmanuel	Senior Lecturer	*En stage*
25	TCHEUTIA Daniel Duviol	Senior Lecturer	In service
26	TETSADJIO TCHILEPECK M. Eric.	Senior Lecturer	In service

27	BITYE MVONDO Esther Claudine	Assistant Lecturer	In service
28	FOKAM Jean Marcel	Assistant Lecturer	In service
29	GUIDZAVAI KOUCHERE Albert	Assistant Lecturer	In service
30	MANN MANYOMBE Martin Luther	Assistant Lecturer	In service
31	MEFENZA NOUNTU Thiery	Assistant Lecturer	In service
32	NYOUMBI DLEUNA Christelle	Assistant Lecturer	In service
33	TENKEU JEUFACK Yannick Léa	Assistant Lecturer	In service

8- DEPARTMENT OF MICROBIOLOGY (MIB) (24)

1.	ESSIA NGANG Jean Justin	Professor	*Head of Department*
2.	NYEGUE Maximilienne Ascension	Professor	*VICE-DEAN / DSSE*

3.	ASSAM ASSAM Jean Paul	Assistant Professor	In service
4.	BOUGNOM Blaise Pascal	Assistant Professor	In service
5.	BOYOMO ONANA	Assistant Professor	In service
6.	KOUITCHEU MABEKU Epse KOUAM Laure Brigitte	Assistant Professor	In service
7.	RIWOM Sara Honorine	Assistant Professor	In service
8.	NJIKI BIKOÏ Jacky	Assistant Professor	In service
9.	SADO KAMDEM Sylvain Leroy	Assistant Professor	In service

10	ESSONO Damien Marie	Senior Lecturer	In service
11	LAMYE Glory MOH	Senior Lecturer	In service
12	MEYIN A EBONG Solange	Senior Lecturer	In service
13	MONI NDEDI Esther Del Florence	Senior Lecturer	In service
14	NKOUDOU ZE Nardis	Senior Lecturer	In service
15	TAMATCHO KWEYANG Blandine Pulchérie	Senior Lecturer	In service
16	TCHIKOUA Roger	Senior Lecturer	*Head of Service of Tuition*
17	TOBOLBAÏ Richard	Senior Lecturer	In service

18	NKOUE TONG Abraham	Assistant Lecturer	In service
19	SAKE NGANE Carole Stéphanie	Assistant Lecturer	In service
20	EZO'O MENGO Fabrice Télésfor	Assistant Lecturer	In service
21	EHETH Jean Samuel	Assistant Lecturer	In service
22	MAYI Marie Paule Audrey	Assistant Lecturer	In service
23	NGOUENAM Romial Joël	Assistant Lecturer	In service
24	NJAPNDOUNKE Bilkissou	Assistant Lecturer	In service

9. DEPARTMENT OF PHYSICS (PHY) (43)

1.	BEN- BOLIE Germain Hubert	Professor	In service
2.	DJUIDJE KENMOE épouse ALOYEM	Professor	In service
3.	EKOBENA FOUDA Henri Paul	Professor	*Vice-Chancelor. Uty Ndéré*

4.	ESSIMBI ZOBO Bernard	Professor	In service
5.	HONA Jacques	Professor	In service
6.	NANA ENGO Serge Guy	Professor	In service
7.	NANA NBENDJO Blaise	Professor	In service
8.	NDJAKA Jean Marie Bienvenu	Professor	*Head of Department*
9.	NJANDJOCK NOUCK Philippe	Professor	In service
10.	NOUAYOU Robert	Professor	In service
11.	SAIDOU	Professor	*Head of Station/IRGM/MINRESI*
12.	TABOD Charles TABOD	Professor	*Dean FS/Univ/Bda*
13.	TCHAWOUA Clément	Professor	In service
14.	WOAFO Paul	Professor	In service
15.	ZEKENG Serge Sylvain	Professor	In service
16.	BIYA MOTTO Frédéric	Assistant Professor	*DG/HYDRO Mekin*
17.	BODO Bertrand	Assistant Professor	In service
18.	ENYEGUE A NYAM épse BELINGA	Assistant Professor	In service
19.	EYEBE FOUDA Jean sire	Assistant Professor	In service
20.	FEWO Serge Ibraïd	Assistant Professor	In service
21.	MBINACK Clément	Assistant Professor	In service
22.	MBONO SAMBA Yves Christian U.	Assistant Professor	In service
23.	MELI'I Joelle Larissa	Assistant Professor	In service
24.	MVOGO ALAIN	Assistant Professor	In service
25.	NDOP Joseph	Assistant Professor	In service
26.	SIEWE SIEWE Martin	Assistant Professor	In service
27.	SIMO Elie	Assistant Professor	In service
28.	VONDOU Derbetini Appolinaire	Assistant Professor	In service
29.	WAKATA née BEYA Annie Sylvie	Assistant Professor	*Director/ENS/UYI*
30.	WOULACHE Rosalie Laure	Assistant Professor	*Internship since February 2023*

31.	ABDOURAHIMI	Senior Lecturer	In service
32.	AYISSI EYEBE Guy François Valérie	Senior Lecturer	In service
33.	CHAMANI Roméo	Senior Lecturer	In service
34.	DJIOTANG TCHOTCHOU Lucie Angennes	Senior Lecturer	In service
35.	EDONGUE HERVAIS	Senior Lecturer	In service
36.	FOUEJIO David	Senior Lecturer	*Head of Unit MINADER*
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40.	TEYOU NGOUPO Ariel	Senior Lecturer	In service
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42.	NGA ONGODO Dieudonné	Assistant Lecturer	In service
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6.	NZENTI Jean-Paul	Professor	In service
7.	ONANA Vincent Laurent	Professor	*Head of Department/Uty. Eb.*
8.	YENE ATANGANA Joseph Q.	Professor	*Head of Div. /MINTP*
9.	ABOSSOLO née ANGUE Monique	Assistant Professor	*Vice-Dean / DRC*
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11.	EKOMANE Emile	Assistant Professor	*Head Div./Uty Ebolowa*
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13.	FUH Calistus Gentry	Assistant Professor	*Sec. Of State/MINMIDT*
14.	GANNO Sylvestre	Assistant Professor	In service
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16.	MBIDA YEM	Assistant Professor	In service
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18.	NGO BIDJECK Louise Marie	Assistant Professor	In service
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20.	NJILAH Isaac KONFOR	Assistant Professor	In service
21.	NYECK Bruno	Assistant Professor	In service
22.	TCHAKOUNTE Jacqueline épse NUMBEM	Assistant Professor	*Head of Unit /MINRESI*
23.	TCHOUANKOUE Jean-Pierre	Assistant Professor	In service
24.	TEMGA Jean Pierre	Assistant Professor	In service
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26.	ANABA ONANA Achille Basile	Senior Lecturer	In service
27.	BEKOA Etienne	Senior Lecturer	In service
28.	ESSONO Jean	Senior Lecturer	In service
29.	EYONG John TAKEM	Senior Lecturer	In service
30.	MAMDEM TAMTO Lionelle Estelle, épouse BITOM	Senior Lecturer	In service
31.	MBESSE Cécile Olive	Senior Lecturer	In service
32.	METANG Victor	Senior Lecturer	In service
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38.	TEHNA Nathanaël	Senior Lecturer	In service
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40.	MBANGA NYOBE Jules	Senior Lecturer	In service
41.	KOAH NA LEBOGO Serge Parfait	Assistant Lecturer	In service
42.	NGO'O ZE ARNAUD	Assistant Lecturer	In service
43.	TENE DJOUKAM Joëlle Flore, épouse KOUANKAP NONO	Assistant Lecturer	In service

Statistical distribution of Lecturers at the Faculty of Science of the University of Yaoundé I

NUMBER OF LECTURERS					
DEPARTMENT	**Professors**	**Assistant Professors**	**Senior Lecturers**	**Assistant Lecturers**	**Total**
BCH	8 (01)	15 (11)	13 (03)	7 (05)	**43 (20)**
BPA	14 (01)	16 (09)	18 (04)	4 (02)	**52 (16)**
BPV	6 (01)	12 (02)	13 (07)	3 (00)	**34 (10)**
CI	7 (01)	15 (04)	5 (01)	1 (00)	**28 (06)**
CO	6 (01)	18 (04)	11 (04)	2 (00)	**37 (09)**
IN	2 (00)	2 (00)	14 (01)	4 (00)	**22 (01)**
MAT	1 (00)	8 (00)	17 (01)	7 (02)	**33 (03)**
MIB	2 (01)	7 (03)	8 (04)	7 (02)	**24 (10)**
PHY	15 (01)	15 (04)	11 (01)	2 (00)	**43 (06)**
ST	8 (00)	17 (03)	15 (04)	3 (01)	**43 (08)**
Total	**69 (07)**	**125 (40)**	**125 (30)**	**40 (12)**	**359 (89)**

In a nutshell, a total of **359 (89)** distributed as follows:

- Professors **69 (07)**
- Assistant Professors **125 (40)**
- Senior Lecturers **125 (30)**
- Assistant Lecturers **40 (12)**

( ) = Number of women **89**

DEDICATION

This piece of work is dedicated to my ***entire family****. Hope they find in this masterpiece the fulfilment of years of hard work and self-sacrifice. May the good times come now!*

To my beloved Dad, ***MBARGA Bruno Dieudonné****, who passed away during the finalization of this thesis.*

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LIST OF ABBREVIATIONS

A&K: Attract and Kill

CAFS: Cocoa agroforestry systems

CIA: Chloroform – Isoamyl acid

CTAB: Hexadecyltrimethylammonium bromide

DNA: Deoxyribonucleic acid

dNTPs: Deoxyribonucleotide triphosphates

EDTA: Ethylenediaminetetraacetic acid

EPF: Entomopathogenic fungi

HCl: Hydrochloric acid

HPVA: Heinze polyvinylic alcohol

HS: Humification score

IRAD: Institute of Agricultural Research for Development

IPM: Integrated pest management

ITS: Internal transcribed spacer

MgCl2: Magnesium chloride

NaCl: Sodium chloride

PCR: Polymerase Chain Reaction

PDA: Potato dextrose agar

PDB: Potato dextrose broth

PVP-40: Polyvinylpyrrolidone 40

RLFP: Restriction Fragment Length Polymorphism

SE: Standard error of the mean

TAE: Tris acetate EDTA

WAVE: Central and West African Virus Epidemiology

ABSTRACT

Termites are increasingly recognized as a major constraint to cocoa production in West and Central Africa. Their attacks and damages in cocoa agroforestry systems and cocoa trees may significantly affect tree survival and yield. Termite control relied almost exclusively on persistent organochlorine insecticides which are currently under restrictive use due to increasing concern over damage to human health and the environment. Previous studies reported the limited effect of some entomopathogenic fungi as biological control agents in the field. In addition very little is known about the type of termite responsible for damages to cocoa trees or how environmental conditions and land-use management shape their bio-ecology including that of biological control agents such as mites and parasitic flies. This study was therefore undertaken to assess the damaging activities of termites and to screen biological control agents against key termite pests of cocoa in order to develop new control formulation. The first objective of the study focused on identifying and characterizing the different functional groups of termites in cocoa plantations through a sampling of termites on infestation sites of randomly selected cocoa trees. The study then evaluated the type of ecosystem services and disservices that termites provide to cocoa by quantifying termites' role on soil fertility and estimating the yield gap induced by their damages. The study further isolated, characterized and identified fungi and parasitic mites and flies as potential biological control agents using molecular tools and techniques. This study identified fifty four termite species associated with cocoa agroforestry systems with the rustic shaded systems recording the highest termite taxonomic and functional diversity (37 species). The reduction of shade trees coupled with increasing temperatures resulted to a significant increase in the number of cocoa trees attacked by termites. The study further found that cocoa trees were infested both aboveground and belowground but shade trees tend to reduce belowground infestation. The Taylor's power law was the best-fitted model to describe the aggregative dispersion pattern of termites on cocoa trees and help to estimate sampling plans using Green's sequential sampling. The study provided detailed information on the damage patterns of key genera *Microtermes, Microcerotermes, Ancistrotermes, Nasutitermes* and *Odontotermes* in cocoa agroforestry systems. Termite infestations affected tree growth, pod formation and flowering. Termites reduced cocoa yield by about 29% and could reach 55% in case of severe infestation. The study also isolated fifty nine species of potential biological control agents of termites using morphological and molecular tools. They included 26 fungi species, 19 mite species and 14 parasitoid flies. Fungi were mainly Ascomycota species among which *Aspergillus* spp. were the most frequently isolated fungi. Mites belonged all to Super-Order Acariforme and were

mainly composed of acarid species. Parsitoid flies isolated from termites were mainly the family Phoridae and included *Megaselia* spp. and *Melaloncha* sp.. A more diversified community of biological control agents was recorded in heavily shaded cocoa agroforestry systems. The study did not isolate any mites on termites collected in full sun cocoa agroforestry systems. This study is the first to document potential damage patterns, sampling plans and biological control agents of termites on cocoa. Results provided could be used by researchers and farmers to assess the severity of termite damages in plantations and to develop IPM programs to manage termite pest species in cocoa agroforestry systems. This study also highlighted the contribution of shade trees in limiting termite outbreaks in cocoa agroforestry systems. The study provided new insight on good candidate biological control agents (*Cordyceps tenuipes, Penicillium paxilli, P. citrinum* and *Fusarium oxysporum, Acotyledon* sp. and *Melaloncha* sp.) that can further be explored in an Attract-and-Kill strategy to manage termites in agricultural lands or in environments where termites are potential threats.

Keywords: Termite outbreaks, cocoa intensification, pest severity, belowground damage, yield loss, parasitoids, entomopathogenic fungi.

RÉSUMÉ

Les termites sont de plus en plus reconnus comme une contrainte majeure à la production de cacao en Afrique de l'Ouest et du Centre. Leurs attaques et dégâts dans les systèmes agroforestiers de cacao et sur les cacaoyers peuvent affecter de manière significative la survie et le rendement des plants. La lutte contre les termites reposait presque exclusivement sur des insecticides organochlorés persistants qui font actuellement l'objet d'une utilisation restrictive en raison des préoccupations croissantes concernant les dommages à la santé humaine et à l'environnement. Des études antérieures ont rapporté l'effet limité de certains champignons entomopathogènes en tant qu'agents de lutte biologique sur le terrain. En outre, on sait très peu de choses sur le type de termite responsable des dégâts sur les cacaoyers ou sur la manière dont les conditions environnementales et la gestion des terres façonnent leur bioécologie, y compris celle des agents de lutte biologique tels que les acariens et les mouches parasites. Cette étude a donc été entreprise pour évaluer les dégâts des termites et pour dépister les agents de lutte biologique contre les principaux termites ravageurs du cacao afin de développer de nouvelles formulations de traitement. Le premier objectif de l'étude portait sur l'identification et la caractérisation des différents groupes fonctionnels de termites dans les cacaoyères à travers un échantillonnage des termites au niveau des sites d'infestation des cacaoyers aléatoirement choisis. L'étude a ensuite évalué le type de services écosystémiques et de contre-services que les termites fournissent au cacaoyer en quantifiant le role des termites sur la fertilité des sols et en estimant la perte de rendement induite par leurs dégâts. L'étude a en outre isolé, caractérisé et identifié les champignons, les acariens et mouches parasites comme agents potentiels de lutte biologique à l'aide d'outils et de techniques moléculaires. Cette étude a identifié cinquante-quatre espèces de termites associées aux systèmes agroforestiers cacaoyers, les systèmes rustiques ombragés enregistrant la plus grande diversité taxonomique et fonctionnelle des termites (37 espèces). La réduction des arbres d'ombrage couplée à l'augmentation des températures a entraîné une augmentation significative du nombre de cacaoyers attaqués par les termites. L'étude a en outre révélé que les cacaoyers étaient infestés à la fois au-dessus et au-dessous du sol, mais que les arbres d'ombrage ont tendance à réduire l'infestation souterraine. La loi de puissance de Taylor a été le modèle le mieux adapté pour décrire le modèle de dispersion agrégative des termites sur les cacaoyers et aider à estimer les plans d'échantillonnage à l'aide de l'échantillonnage séquentiel de Green. L'étude a fourni des informations détaillées sur les modes de dégâts des genres clés *Microtermes*, *Microcerotermes*, *Ancistrotermes*, *Nasutitermes* et *Odontotermes* dans les systèmes agroforestiers de cacao. Les infestations par les termites ont affecté la croissance des arbres, la formation des cabosses et la

floraison. Les termites ont réduit le rendement du cacao d'environ 29 % et pouvant atteindre 55 % en cas d'infestation sévère. L'étude a également isolé cinquante-neuf espèces d'agents potentiels de lutte biologique des termites à l'aide d'outils morphologiques et moléculaires. Ils comprenaient 26 espèces de champignons, 19 espèces d'acariens et 14 mouches parasitoïdes. Les champignons sont constitués principalement des espèces du Phylum Ascomycota parmi lesquelles *Aspergillus* spp. ont été les champignons les plus fréquemment isolés. Les acariens ont appartenu tous au Super-Ordre des Acariformes et ont été principalement composés d'espèces d'Acaridae. Les mouches parasitoïdes isolées des termites ont appartenu toutes à la famille des Phoridae et comprenaient les espèces *Megaselia* spp. et *Melaloncha* sp.. Une communauté plus diversifiée d'agents de lutte biologique a été enregistrée dans les systèmes agroforestiers cacaoyers fortement ombragés. L'étude n'a pas isolé d'acariens sur les termites récoltés dans les systèmes agroforestiers cacaoyers en plein soleil. Cette étude est la première à documenter les modes de dégâts potentiels, les plans d'échantillonnage et les agents de lutte biologique des termites sur le cacaoyer. Les résultats fournis pourraient être utilisés par les chercheurs et les agriculteurs pour évaluer la gravité des dégâts causés par les termites dans les plantations et pour développer des programmes de lutte intégrée pour gérer les espèces de termites nuisibles dans les systèmes agroforestiers de cacao. Cette étude a également mis en évidence la contribution des arbres d'ombrage dans la limitation des explosions de termites dans les systèmes agroforestiers cacaoyers. L'étude a fourni de nouvelles informations sur les bons agents de lutte biologique candidats (*Cordyceps tenuipes*, *Penicillium paxilli*, *P. citrinum* et *Fusarium oxysporum*, *Acotyledon* sp. et *Melaloncha* sp.) qui peuvent être explorés plus tard dans la stratégie Attract-and-Kill pour lutter contre les termites dans les terres agricoles ou dans des environnements où les termites sont des menaces potentielles.

Mots-clés : Explosion de termites, intensification du cacao, la sévérité des ravageurs, dégâts souterrains, perte de rendement, parasitoïdes, champignons entomopathogènes.

INTRODUCTION

Introduction

Insect pests are among the most important threats to crop production in Africa. Up to 100% yield loss are reportedly due to pest attacks on several annual and perennial crops (Makundi, 2006; Ambele *et al.*, 2018a). They are responsible of important yield losses in cocoa farming through belowground and aboveground attacks (Bisseleua *et al.*, 2011; Ambele *et al.*, 2018a; Mahot *et al.*, 2019). Despite a substantial improvement in pest control means throughout the last century, mainly based on chemicals, pest outbreaks remain recurrent in agricultural fields with the concur of poor land-use and climate change (Brodie *et al.*, 2012; Dhanush *et al.*, 2015). The suitability of chemicals in pest control is now highly questioned for two main reasons: (1) pests have developed resistance mechanisms against almost all chemical families frequently used against them with notable decrease in their effectiveness (Georghiou, 2012; Roush & Tabashnik, 2012) and (2) chemicals are dangerous for non-target beneficial organisms in farm landscape with dramatic consequences on ecosystems services such as pollination (Pimentel & Edwards, 1982; Losey & Vaughan, 2006). In addition, chemicals also affect indirectly human health through their accumulation in edible plant organs, especially in leaves, fruits and roots (Gilden *et al.*, 2010; Yadav, 2010). This latter situation led chocolate companies around the world to encourage and support organic cocoa produced without chemical inputs. Organic cocoa receives higher prices than non-organic cocoa, and farmers producing it are often incentivized (Armengot *et al.*, 2016; Riedel *et al.*, 2019).

In the face of recurrent pest outbreaks and pesticide-related consequences, there is an increasing interest towards the development of more eco-friendly, long-lasting and profitable control means. The solution put forward is to copy natural control means to instill a continuous auto-control at long term (Wyckhuys *et al.*, 2019). Biological control is an essential part of integrated pest management strategies (Stenberg, 2017) and is central to ecosystem functioning and sustainable crop production (Wyckhuys *et al.*, 2019). Yet, despite their proven economic value and societal benefits this ecosystem service (Losey & Vaughan, 2006), it is often disregarded and substituted by chemically-synthesized insecticides because of farmers' ecological illiteracy and poor farming techniques (Bernhardt *et al.*, 2017; Jørgensen *et al.*, 2018; Wyckhuys *et al.*, 2019). Pest biocontrol is closely related to farm biodiversity; natural enemies often require synergistic or mutualistic actions with other organisms/enemies to trigger or optimize their control (Tscharntke *et al.*, 2011). Thus, the conservation of in-farm biodiversity is key for biological pest control (Clough *et al.*, 2010; Tscharntke *et al.*, 2011; Jouquet *et al.*, 2018) and a farming system ensuring a local conservation of natural enemies is of high interest (Sonwa *et al.*, 2005; Bisseleua *et al.*, 2013; Jagoret *et al.*, 2017).

Cocoa agroforestry systems are considered as the best system to ensure the conservation of biodiversity in farm and ensure a natural biocontrol of herbivores through numerous enemies such as birds, amphibians, wasps, ants, spiders, parasites and parasitoids (Tscharntke *et al.*, 2007, 2011; Bisseleua *et al.*, 2009, 2011, 2013; Clough *et al.*, 2010). Agroforestry systems also have key roles in important ecosystem processes such as soil fertilization, pollination and climate change mitigation (Tscharntke *et al.*, 2011). However, due to the assumption that agroforestry systems yield lower than unshaded systems, farmers are more and more abandoning this farming system as they remove shade trees within the farm. This trend resulted in a heterogeneity of cocoa cropping systems ranging from traditional shaded to full sun systems in Southern Cameroun (Bisseleua *et al.*, 2013). These changes in land-use towards unshaded systems lead to recurrent pest outbreaks such as mirids, borers and especially termites, main belowground pests of cocoa in Africa (Schroth *et al.*, 2004; Bisseleua *et al.*, 2011, 2013; Tscharntke *et al.*, 2011; Ambele *et al.*, 2018a, 2018b; Djuideu *et al.*, 2020).

Termites are major pests of cocoa in Africa (Wood, 1996; Rouland-Lefèvre, 2010; Tra-Bi, 2013). They cause significant damages to cocoa seedlings, young and mature trees to the extent of requiring replacement (Du & Truc, 2009; Tra-Bi *et al.*, 2015). However, infestation patterns of termites on cocoa are still not well known as well as their impact on marketable yield. Recent studies suggested that termites can be responsible of up to 50% of yield loss in a cocoa farm (Djuideu, 2017). Assessing their spatial dynamic and dispersion patterns is prior for an effective pest management (Bisseleua *et al.*, 2011). Termites are usually controlled using broad spectrum and persistent organochlorine insecticides (Logan *et al.*, 1990), nowadays subjected to serious limitations and increasing legal restrictions due to their harmful effects (Nyeko & Olubayo, 2005). In addition, termites have recently increased their ranges of damage on crops, probably due to climate change and poor land-use, and the control means available to farmers are ineffective to protect crops against their attacks (Djuideu *et al.*, 2020). With their cryptic behavior, an optimal control of termites need to consider their lifestyle while avoiding non-target beneficial insects. In this view, natural enemies are the best-suited candidates to build up effective biopesticides. Among enemies of termites, the main groups used in attempts of termite control for their proven pathogenicity are fungi (Blackwell & Rossi, 1986; Culliney & Grace, 2000; Rath, 2000), mites (Phillipsen & Coppel, 1977; Eickwort, 1990; Zhang *et al.*, 1995), nematodes (Wang *et al.*, 2002) and parasitoid flies (Sze *et al.*, 2008).

In Cameroon, cocoa represents the most exported and one of the most important cash crop which trade represents the main livelihood of several hundred thousand households

especially in rural areas, but farm yield is poor (Tchokote *et al.*, 2015). Numerous challenges are related to cocoa cultivation and one of the most important is pests and diseases which affect significantly the cocoa revenue. The livelihoods of cocoa-depending farmers and the rural development of Cameroon are seriously affected. Few studies have attempted to quantify the services and disservices of soil-dwelling pests such as termites in this system. The efficiency of entomopathogenic fungi (EPF) and other natural enemies against termites is very high against termites with up to 100% mortality induced in laboratory conditions. But the application of EPF during field experiments are often difficult with a diminished mortality compared to laboratory trials (Culliney & Grace, 2000). Many environmental and biological factors are incriminated such as a low shelf life, a poor establishment in soil, a high sensitivity to sun light and the avoidance behavior of insects in natural conditions (Przyklenk *et al.*, 2015). The avoidance of termites towards EPF applied may be linked to a poor co-evolutive process between the EPF strains and the target insect. Indeed, most EPF strains used in control of a specific insect pest are rarely isolated among natural enemies of the target insect. Moreover, Hussain *et al.* (2010) reported that *Coptotermes formosanus* can detect volatiles of most virulent EPF species and easily avoid contamination in field trials. Therefore, using naturally infesting EPF strains and natural enemies may enhance the probability of encounter between both species and increase the mortality in field trials.

Research questions

Our main research question is: What are the damage patterns of termites on cocoa and what are best biological weapons to control termites in cocoa agroforestry systems, capable to overcome their behavioral barrier and induce high mortality?

This study is guided by the following research questions:

- What are the termite species found in cocoa agroforestry systems and cocoa trees and how is land-use change and environmental factors affecting their bio-ecology?
- What type of ecosystem services and disservices these termites can provide to the cocoa agroforestry systems?
- Are these termites naturally controlled by other organisms? If yes, who are these organisms and where are they found?
- Is there any relationship between the land-use management in cocoa agroforestry systems, the environmental factors and the survival of these beneficial organisms?

Research hypothesis

Our general hypothesis is that biological control agents, naturally-infesting subterranean termites in their environment, are capable to bypass their avoidance behavior and are the best

weapons to control termite pest populations. The study was further guided by the following specific hypotheses:

- The current outbreak of termite species as pests is due the removal of shade cover in the cocoa agroforestry systems and climate variability.
- The shade tree removal in cocoa agroforestry systems encourages termite disservices on cocoa trees meanwhile reducing their ecosystem services provided in soil fertilization
- Termites display an aggregative dispersion pattern on cocoa trees that could help to set up sampling plans to monitor their level of attack
- The reduction of shade cover is also leading to the reduction of biological control agents of termites such as entomopathogenic fungi (EPF), parasitic mites and flies.

Aim and objectives

This study aimed to assess the damaging activities of termites and to screen biological control agents that could be used against key termite pests in cocoa agroforestry systems in order to develop new formulation.

More specifically, the study was about to:

(1) assess the taxonomic and functional diversity of termites in cocoa farms under different shade management regime and climate variability;

(2) document the ecosystem services and disservices provided by termites in cocoa farms and their dispersion pattern in relation to shade management;

(3) screen biological control agents (entomopathogenic fungi, parasitic mites and flies) of termite pest species using morphological and molecular tools and techniques;

(4) document the interactions between, environmental factors, shade management regime and the biological control agents (e.g. EPF, parasitic mites and flies).

This work prior presents an introduction that gives the context of the pest importance in cocoa farming, the disservices of termites, the limits of control means developed against them and need for innovative solutions. In the first chapter, a literature review gives all information on termite ecology, their services and disservices to cultivated crops, the importance of agroforestry and the range of control means available and ways forward. The second chapter details the methods and the material used to achieve this study's objectives, followed by a third chapter presenting results and discussion. This thesis's last section is the conclusion of relevant findings and future research perspectives and recommendations for future management initiatives of termites as threat.

CHAPTER I: LITERATURE REVIEW

I.1. Termites in cocoa: a long-lasting and evolving relationship

I.1.1. Services and disservices provided by termites

I.1.1.1. What are termites?

Termites (Blattodea: Termitoidae) are eusocial cockroaches (insects) that live in complex societies that can be modelled as "superorganisms" – where the individuals form part of a larger self-regulating entity (Figure 1) (Bignell *et al.*, 2010; Beccaloni & Eggleton, 2013). They are present in all ecosystems worldwide except in polar areas, but they are widely spread in Tropics especially in Africa (Emerson, 1928; Harris, 1954; Dibog *et al.*, 1998; Bignell *et al.*, 2010). Termites are morphologically and anatomically differentiated into three distinct castes which ensure different functions within the colony, similar to those found in multicellular organisms. The reproductive (kings, queens and nymphs) are responsible of the dispersal, the pair bonding and the fecundity; the workers ensure the foraging and feeding of the colony, the tending of immatures and the nest construction; and soldiers ensure only the defence of the colony against aggressors and predators (Eggleton, 2010). The termite group, formerly named Isoptera, is composed of more than 2929 living species regrouped in nine families and 16 subfamilies (Beccaloni & Eggleton, 2013; Krishna *et al.*, 2013).

- Termite classification from Beccaloni & Eggleton (2013)

Order **Blattodea** Brunner von Wattenwyl, 1882

Epifamily **Termitoidae** Latreille, 1802

Family **Mastotermitidae** Desneux, 1904 (1 genus, 1 species)

Family **Archotermopsidae** Engel, Grimaldi & Krishna, 2009 (3 genera, 6 species)

Family **Hodotermitidae** Desneux, 1904 (3 genera, 21 species)

Family **Stolotermitidae** Holmgren, 1910 (2 genera, 10 species)

Subfamily **Stolotermitinae** Holmgren, 2010 (1 genera, 7 species)

Subfamily **Porotermitinae** Emerson, 1942 (2 genera, 3 species)

Family **Kalotermitidae** Froggatt, 1897 (21 genera, 456 species)

Family **Stylotermitidae** Holmgren & Holmgren, 1917 (1 genus, 45 species)

Family **Rhinotermitidae** Froggatt, 1897 (12 genera, 315 species)

Subfamily **Coptotermitinae** Holmgren, 1910 (1 genus, 67 species)

Subfamily **Heterotermitinae** Froggatt, 1897 (2 genera, 168 species)

Subfamily **Prorhinotermitinae** Quennedey and Deligne, 1975 (1 gen., 11 sp.)

Subfamily **Psammotermitinae** Holmgren, 1911 (1 genus, 6 species)

Subfamily **Rhinotermitinae** Froggatt, 1897 (6 genera, 62 species)

Subfamily **Termitogetoninae** Holmgren, 1910 (1 genus, 2 species)

Family **Serritermitidae** Holmgren, 1910 (2 genera, 3 species)

Family **Termitidae** Latreille, 1802 (238 genera, 2072 species)

Subfamily **Apicotermitinae** Grassé and Noirot (42 genera, 2020species)

Subfamily **Cubitermitinae** Weidner (26 genera, 156 species)

Subfamily **Foraminitermitinae** Holmgren (3 genera, 10 species)

Subfamily **Macrotermitinae** Kemner (12 genera, 373 species)

Subfamily **Nasutitermitinae** Hare (77 genera, 596 species)

Subfamily **Sphaerotermitinae** Holmgren (1 genus, 1 species)

Subfamily **Syntermitinae** Engel and Krishna (15 genera, 99 species)

Subfamily **Termitinae** Latreille (61 genera, 637 species)

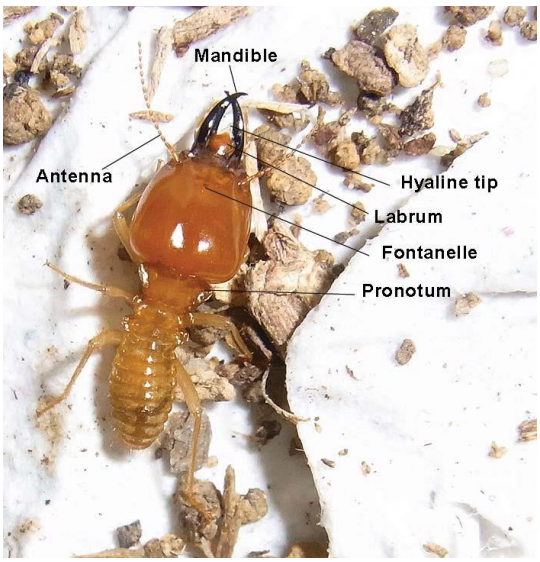


Figure 1. Morphology of a major soldier of *Macrotermes bellicosus* with the main diagnosis elements (Photo ©Djuideu, 2017)

I.1.1.2. Foraging and ecosystem services provided by termites

Termites are soil-dwelling insects and most important component of soil macrofauna (Lavelle *et al.*, 1993; Davies *et al.*, 2003). Among termite castes, workers are numerously and functionally the most important group. They do almost all tasks, live and work within the colony. During foraging activities, most workers are protected under sheeting or runways (also called galleries). Only a relatively few grass, microepiphyte and litter feeders forage unprotected on ground (e.g. *Pseudacanthotermes militaris* sometimes forage in litter without sheeting protection) (Eggleton, 2010). In those cases the foraging morphs are conspicuously

more sclerotized than the non-foraging morphs. Termites feed on living and dead plant material at all stages of decomposition (wood, grasses and humus) (Donovan *et al.*, 2001; Hyodo *et al.*, 2008; Eggleton, 2010), animal dung (Krishna *et al.*, 2013) and even plastic cables in rare occasions (Charpentier, 2005). Feeding preferences vary between higher taxa (genera, subfamilies and families) and even from a species to another within the same genus. Regarding their position on the humification gradient, four feeding groups were differentiated in termites (Donovan *et al.*, 2001; Bignell *et al.*, 2010). Group I feeds on dead wood and grass and have relatively simple guts. Group II feeds on wood, grass, leaf litter and microepiphytes and have more complex guts. Group III feeds on humus (i.e. soil-like material with recognizable plant material in it). Group IV feeds on soil (i.e. soil-like material with a high proportion of silica and no recognizable plant material). Regarding the feeding mode, i.e. how they digest the food foraged and ingested, termites can be classified in three groups (Grassé, 1986; Brune & Ohkuma, 2010):

- **Lower termites**: termites of this group are true wood feeders which depend on endosymbionts such as flagellated protozoa *Trichonympha grandis* or *Jaenia annectens* to feed on foraged wood. These termites lack specific enzymes to digest wood and then preserve these flagellate in their hind gut where they convert cellulose in wood into starches and sugars that termites can use as nutrients. This group include all families except Termitidae. The genera *Kalotermes*, *Neotermes, Coptotermes* and *Reticulitermes* are the most known as pests. They belong to feeding groups I and II.
- **Fungus growers**: termites of this group "cultivate" a fungus genus *Termitomyces* which decomposes externally dead plant material within the colony. These termites then feed on cultivated fungi in the same way that humans use the cattle. It is a termite-specific exosymbiosis also found in some fungus-growing ants. All members of this group belong to the subfamily Macrotermitinae (Termitidae), widely recognized as main crop pests in termite group. They all belong to feeding group II.
- **Higher termites**: termite of this group have developed specific enzymes to digest their food although they still contain microbial symbionts to help in digestion process. They are able to directly transform cellulose of wood into nutrients in their gut (*Microcerotermes, Nasutitermes*…) or to even feed on extremely decayed plant material or soil (*Cubitermes*, *Procubitermes*, *Ananteotermes*…). The members of this group belong to all subfamilies of Termitidae except Macrotermitinae and all the feeding groups are included.

Through their foraging behavior, termites play an important role in humification and fertilization of soil by transforming progressively organic matter into minerals to be absorbed by plant roots (Attignon *et al.*, 2005; Yamada *et al.*, 2006). The more termites of group III and IV are abundant in the environment, the more humifiation process is important (Davies *et al.*, 2003; Attignon *et al.*, 2005). Termites are of the greatest importance in litter decomposition and in recycling carbon and nutrients (Yamada *et al.*, 2006; Jouquet *et al.*, 2018). Konaté *et al.* (2003) showed that they have a positive impact on litter decomposition in fire-prone ecosystems where carbon and nutrients can be lost by fire. Termites also have an important impact on water dynamics in soil (Kaiser *et al.*, 2017). Indeed, termite foraging activity is often associated with the production of belowground galleries which increase soil macroporosity, create "preferential flow paths" and increase water infiltration in soil (Evans *et al.*, 2011; Kaiser *et al.*, 2017). A high diversity of termites in agricultural landscapes contributes to increase crop yield (Dibog *et al.*, 1999; Evans *et al.*, 2011).

At the landscape scale, termites also act as heterogeneity drivers when they produce aboveground mounds that appear like nutrient "hot-spots" or "fertility islands" in which primary productivity is locally increased and water flow improved. Depending on the feeding group (e.g., soil feeding termites vs fungus growers) and the pedoclimatic properties of the environment, these mounds can also have higher carbon and azote contents compared to the surrounding soil (Konaté *et al.*, 1999; Jouquet *et al.*, 2018). In addition, termites are an essential link in the ecosystem food chain with some endangered mammals (e.g. aardvarks, pangolins, anteaters and chimpanzees) which specialized their anatomy and behavior to feed almost exclusively on termites (Redford, 1987; Willis *et al.*, 1992; Hua *et al.*, 2015). Termites (winged termites) are also among the most consumed edible insects throughout the world, being essential component of human diet in Africa (Anankware *et al.*, 2015; Tamesse *et al.*, 2018).

I.1.1.3. Termite disservices to human assets

Despite their numerous ecosystem services, termites are more regarded as pests than beneficial insects in human collective consciousness. They become serious issues when they attack crops and buildings, and consequently their positive roles are often overshadowed by their pest status (Su & Scheffrahn, 2000; Verma *et al.*, 2009; Shanbhag & Sundararaj, 2013). Termites threaten agriculture in the tropics where billions of US$ are annually spent on their prevention and extermination (Mugerwa, 2015; Jouquet *et al.*, 2018).

From more than 2900 species identified to date, between 180 and 300 termite species are pests for buildings and crops (Verma *et al.*, 2009). All these species belong to wood-feeding

and litter harvester groups (groups I and II). The fungus-growing Macrotermitinae species (Termitidae, especially from the genera *Macrotermes*, *Odontotermes*, *Ancistrotermes*, and *Microtermes*) and the Rhinotermitidae species (especially the genera *Reticulitermes* and *Coptotermes*) contain most of the pest species (Pearce, 1997; Jouquet *et al*., 2018). The most notorious pest species of termites are generally those that have been introduced into new geographical areas, usually as a result of human activity, and became invasive. Particularly menacing examples are *Cryptotermes brevis*, *Cryptotermes domesticus*, *Cryptotermes dudleyi*, *Coptotermes formosanus*, *Coptotermes gestroi*, *Reticulitermes flavipes*, and *Reticulitermes lucifugus* (Krishna *et al.*, 2013). Due to the global warming, temperate regions are particularly exposed to the invasion of tropical and subtropical termite pest species. Termites can cause damage to crops, human constructions, pastures and forestry as well as to non-cellulosic materials such as electrical cables (Charpentier, 2005; Mugerwa, 2015; Jouquet *et al.*, 2018). A wide variety of crops are affected by termite attacks, including trees in plantations and orchards, coconuts, palms, sugar cane, rice, maize, wheat, sorghum, groundnuts, coffee, tea, cocoa, yam, cassava and cotton (Rouland-Lefèvre, 2010). Fungus-growing termites are responsible for the majority of crop damage and 90% of tree mortality in South African forests (Mitchell, 2002). Records on crop yield losses induced by termites vary from 3 to 100% in Africa and Asia (Umeh & Ivbijaro, 1997; Mitchell, 2002; Sekamatte *et al.*, 2003; Joshi, 2005; Verma *et al.*, 2009). Termite attacks can also affect crop quality as reported by Black & Okwakol (1997) who noticed that the scarification of crop tubers by termites can reduce their market value and increase the toxin contents in groundnuts.

The presence of termites in cocoa farms has been documented since the 1950s (Harris, 1954) but their damage to cocoa trees were not documented. The first signs of termite damage on cocoa was reported by Sands (1972) who described them as minor, illustrated by galleries running off the tree. Entwhistle (1972) the same year published the first catalog of major pests in cocoa where termites were not mentioned. In early 2000s, published studies suggested that termites are becoming major pests in cocoa because of the increase of damage observed and the neglected belowground attacks that go often unnoticed (Sands, 1998a; Vos *et al.*, 2003). There is more than 19 termite species identified as pest of cocoa worldwide and the most damaging ones belong to the genus *Coptotermes* (e.g. *C. sjostedti* in Cote d'Ivoire) (Krishna *et al.*, 2013; Tra-Bi, 2013). Termites are nowadays considered as major cocoa pests in main cocoa producing countries in Africa such as Côte d'Ivoire (Tra-Bi, 2013; Tra-Bi *et al.*, 2015), Ghana (Asare & David, 2010) and Cameroon (Ambele *et al.*, 2018b; Djuideu *et al.*, 2020). In Cameroon, termites are reportedly responsible of up to 50% on yield loss in cocoa farms (Djuideu *et al.*,

2020). Moreover, termites are supposed to take part in the contamination of cocoa trees by pathogens such as *Phytophthora megakarya* during their foraging activities as they transport earth containing fungi spore with legs and mouthparts in galleries (Vos *et al.*, 2003).

I.1.2. Termite infestation and damage patterns on cocoa

Termite infestations are not as devastative as infestations of others major cocoa pests such as mirids (*Sahlbergella singularis* and *Distantiella theobromae*) in Africa or pod borers (*Conopomorpha cramerella*) in South-East Asia (Vos *et al.*, 2003; Bisseleua *et al.*, 2011; Mahot *et al.*, 2019). Indeed, compared to these pests, termites rarely attack fruits (pods) and they mainly feed on the wooden part of the tree such as bark, sapwood, heartwood and roots (Cowie *et al.*, 1989). Termite attacks on cocoa trees go unnoticed by farmers most of the time, until the damage increase considerably and trees wither and die (Vos *et al.*, 2003). Also, termites could weaken the posture of the tree by their damage and it could easily collapses in case of very bad weather (Asare & David, 2010). This evolution of termite-cocoa relationship suggests that there is at least one driving factor that increases the susceptibility of cocoa trees to termite attacks or/and increase pest population range. The change in land-use, the ageing of cocoa orchards or climate change are possible factors put forward but no or few studies have been carried to assess these hypotheses. In addition, termite attacks are more severe on introduced or exotic plant species or varieties as it is the case of cocoa which was introduced in West Africa in late 19th century (Braudeau, 1969; Logan *et al.*, 1990). Their attacks are not widespread to all the plants within the farm but they are usually more important on weak/stressed plants suffering from disease, lack or too much water, or inappropriate soil properties (Logan *et al.*, 1990; Jouquet *et al.*, 2018). Depending on the damaging termite species and the attacked plant structures we can distinguish two main types of termite infestation (Figure 2) (Vos *et al.*, 2003; Ambele *et al.*, 2018a):

- **Aboveground infestation**: termites of this group build of conspicuous galleries (earthruns) running off the cocoa tree and sometimes epigeal nests. In these galleries, workers are eating up and perforate the bark to also feed on trunk core beneath it. Attacks can result in the wilting and drying of attacked branched that need to be cut down to avoid the spread of the pest all over the tree. Also, the galleries can form large earth plaster on the trunk that can mechanically prevent the flowering and pod formation by covering large areas of trunk and branches where flowers and pods can emerge. The main species of this group are belonging to genera *Coptotermes*, *Microcerotermes* and *Nasutitermes*.
  In this group, we also note wood-borne termites which live inside wood cavity where the colony has settled down. Their presence is hardly noticeable until the tree completely

dries off. The species incriminated are belonging to family Kalotermitidae such as *Cryptotermes havilandi* (Congo), *Neotermes gestri* (Equatorial Guinea) and *Neotermes arburiensis* (Nigeria).

- **Belowground infestation**: termites of this group damage mainly cocoa roots and, rarely, build thin galleries on trunks going up to 60 cm in which they feed on bark and perforate. Damaging species build subterranean nests near or inside the root system of the tree and move back and forth between the nest and the roots to feed on. Damaged roots are tunneled and dry. The consequences of belowground attacks are noticed aboveground through the generalized drying of the plant and the abundant fall of leaves. The tree could die in a few years if the infestation persists. The species incriminated are belonging to genus *Microtermes*, *Ancistrotermes*, *Macrotermes* and *Odontotermes*. Subterranean termites are the main pests of seedlings and young trees leading to up to 90% replacement after transplanting in farm (Du & Truc, 2009).

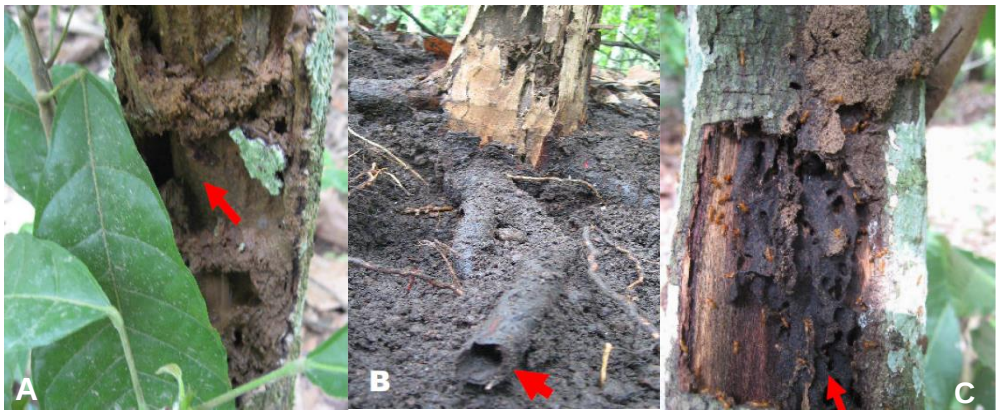


Figure 2. Examples of damage caused by termites on cocoa trees (red arrows). **A**) Major damage on cocoa trunk; **B**) cocoa root attacked by termites; **C**) minor damage on cocoa trunk under a scraped gallery (Photo © Tra Bi, 2013).

I.1.3. Roles of cocoa agroforestry systems: enhancing termite-mediated services and mitigating disservices

I.1.3.1. Cocoa agroforestry systems as a multifunctional landscape

The first farms established in Cameroon in early 1900s by Germans were designed in the traditional complex agroforestry system (Braudeau, 1969) (Figure 3). An agroforestry system is a form of agro-ecosystems characterized by a diversity and combination of shade trees below which a wide range of crop and food crops are grown for goods and services (Schroth *et al.*, 2004). Agroforestry systems in West Africa are mainly characterized by the combination of a diversity of trees in association with cocoa, coffee, oil palm, fruit trees and food crops

(Norgrove *et al.*, 2009). Such multistrata agroforestry systems with a diverse canopy of shade tree cover and herbaceous cover provide a diversity of ecosystem function and services similar to forest ecosystems (Bos *et al.*, 2007; Clough *et al.*, 2009; Tscharntke *et al.*, 2011).

Figure 3. A cocoa agroforestry system of southern Cameroon (Photo ©Djuideu, 2017)

Ecosystem functions provided by agroforestry systems include light and nutrient regulation (Isaac *et al.*, 2007); mitigation of greenhouse gas emission and climate changes (Tscharntke *et al.*, 2011); regulation of temperature and precipitation (Schwendenmann *et al.*, 2010); and provision of a diversity of microhabitats for small organisms (Clough *et al.*, 2010). Additional functional properties include enhancing the soil fertility of the farm (Dibog *et al.*, 1999; Asara, 2015); ensuring food security to the community by providing a diversity of food and nutrients such as fruits (Schroth *et al.*, 2004; Bisseleua *et al.*, 2013); ensuring conservation biological control of key pests and diseases (Clough *et al.*, 2010; Tscharntke *et al.*, 2011) and providing a wildlife friendly habitats for forest-dwelling species (Sonwa *et al.*, 2007; Tscharntke *et al.*, 2011).

In Cameroon nowadays, cocoa agroforestry systems are progressively converted into less shaded or unshaded systems due to the assumption that cocoa agroforestry systems yield lesser than monocrop systems (Jagoret *et al.*, 2009; Bisseleua *et al.*, 2013). This assumption does not take into account two main factors: (1) the short-term yield of monoculture systems (less than 25 years old) compared to agroforestry systems (still producing at 100 years) (Jagoret *et al.*, 2016), and (2) associated shade trees can provide additional revenue to farmers from the sale of fruits and timber (Bisseleua *et al.*, 2013; Djuideu *et al.*, 2020).

I.1.3.2. Agroforestry systems enhance termite-mediated services

Termites are desiccation-tolerant insects that require a high level of humidity in their environment (Grassé, 1982; Oberst *et al.*, 2018). They like warm and humid environments like primary forests, especially beneficial species that are highly sensitive to temperature and humidity shifts (Dibog *et al.*, 1998; Eggleton *et al.*, 1999, 2002a; Deblauwe *et al.*, 2008; Norgrove *et al.*, 2009). Forests with their thick shade of trees stabilize microclimatic and pedologic conditions for beneficial termites which thrive better than in open vegetation. In addition, forest cover regulates the intensity of sunlight reaching the ground (avoiding soil insolation) that allows highly sensitive termites to move upper in soil layers (less than 10 cm depth). Thus, the increased abundance in upper soil layers of light-avoiding beneficial species could increase the decomposition speed and induce a rapid soil turnover (Cabrera & Rust, 1996). Agroforestry systems by mimicking forest environment contribute to enhance beneficial termite activities in the same way and stimulate litter decomposition (Schroth *et al.*, 2004; Tscharntke *et al.*, 2011). Numerous studies showed that termite diversity is very high in complex shaded cocoa agroforestry systems and the community is mostly constituted of beneficial species (Norgrove *et al.*, 2009; Tra-Bi, 2013; Ambele *et al.*, 2018b). Furthermore, shade trees provide huge amount of leaves and twigs in litter that beneficial termites feed on and consequently increase soil humification process and ensure nutrient recycling (Attignon *et al.*, 2005).

I.1.3.3. Agroforestry systems reduce termite incidence and damage

Shaded cocoa agroforestry systems are known to support much higher functional biodiversity that unshaded systems (Clough *et al.*, 2010; Tscharntke *et al.*, 2011). Functionally important groups include insectivorous birds and bats (Sperber *et al.*, 2004; Cassano *et al.*, 2009), parasitoids (Sperber *et al.*, 2004), amphibians and predatory insects like ants (Wanger *et al.*, 2010; Bisseleua *et al.*, 2017) providing biocontrol services. Agroforestry systems also support high diversity of entomopathogenic bacteria and fungi to control herbivorous (Clough *et al.*, 2010). All the aforementioned groups are well known to feed/grow on termites and can be considered as control agents of termites in cocoa agroforestry systems. In particular, ants are excellent agents to control termites. Beard (1973) reported that ants are able to destroy entirely a colony when they attack the nest as observed with *Reticulitermes flavipes*. Ants are highly diversified in cocoa agroforestry systems and help boosting crop yield (Philpott & Armbrecht, 2006; Tadu *et al.*, 2014; Bisseleua *et al.*, 2017). However, Bos *et al.* (2007) suggested that a proper shade management is necessary to optimize activities of predatory insects in agroforestry systems.

I.2. Termite control strategies: knowledge and perspectives

I.2.1. The use of chemicals in termite control: history and lessons learned

In agricultural landscape, the first damage of termites were reported in forestry where termites constitute a major constraint to tree growth and reforestation (Parry, 1959; Harris, 1966). Their control relied almost exclusively on the use of persistent organochlorine (cyclodiene) insecticides during the 20th century (Cowie *et al.*, 1989; Logan *et al.*, 1990; Nyeko & Olubayo, 2005; Rouland-Lefèvre, 2010). The cyclodienes or diene-organochlorine insecticides have been used in huge quantity as soil insecticides (especially chlordane, heptachlor, aldrin and dieldrin) for the control of subterranean termites and soil-borne insects whose immature stages (larvae) feed on the roots of plant (Jayaraj *et al.*, 2016). Cyclodienes are effective long-lasting and economical insecticides for termite control that can protect the crop up to 30 years after field application. Because of their stability and persistence, the use of cyclodienes on crops was restricted and sometimes banned because undesired residues remained beyond the time of harvest. (Okoffo *et al.*, 2016). Their long persistence in soil is responsible of all the bad effects and restriction measures associated to their use, especially against non-target organisms. However, it seems that termites have rapidly developed resistance against them (Cowie *et al.*, 1989; Logan *et al.*, 1990; Wood & Pearce, 1991). To replace cyclodienes, organophosphates (malathion, chlorpyriphos, dichlorvos), carbamates (aldicarb, carbosulfan) and chlorinated phenols (pentachlorophenol) have been brought forward to the market and successes are noted (Wardell, 1987; Rouland-Lefèvre, 2010). For instance, chlorpyriphos is more effective than aldrin for protecting groundnuts against *Odontotermes obesus* and doubles the yield (Logan *et al.*, 1992). In Malawi, carbosulfan reduced mortality due to *Macrotermes*, *Odontotermes* and *Ancistrotermes* by 34% in a eucalyptus plantation (Chilima, 1991). However the new insecticides do not possess the persistence that is important in tropical conditions and termites attacks resurface at short term (Horwood *et al.*, 2010; Rouland-Lefèvre, 2010).

In cocoa, a recent study suggested that farmers rely on carbamates (carbosulfan) to deal with termite damage on trees (Djuideu *et al.*, 2020). The most used products are labelled under trade names Bastion^{TM} and Furadan® and their effectiveness is mitigated. Farmers reported they are not as effective against termites as before. In addition, these products are under restricted use or banned (as it is the case for Furadan®) for field application by approval services due to environmental drawbacks (Nyeko & Olubayo, 2005; Djuideu *et al.*, 2020). Nowadays, there is no highly effective approved chemicals for field application against termites either in cocoa or other cultivated crops. In the meantime, termite damage are getting more and

more important leading farmers to used harmful products to protect their livelihoods (Djuideu *et al.*, 2020).

Another important drawback of pesticide use is their indirect effect human health. Indeed, chemicals can accumulate in edible plant tissues such as leaves, fruits and roots and seriously affect health of consumers (Gilden *et al.*, 2010; Yadav, 2010). Some studies revealed that the increase of the range of cancer formation and neurotoxicity in humankind is strongly related to the presence and quantity of chemical residues in their food (Alavanja *et al.*, 2004). Moreover, a study carried out by Flower *et al.* (2004) alarmed on the cancer risk in childhood after parental exposure to pesticides. This important situation has led major brands of chocolate around the world to encourage and support organic cocoa that is produced without chemical inputs. Organic cocoa receives higher prices compared to non-organic cocoa, and farmers producing it are often incentivized (Armengot *et al.*, 2016; Riedel *et al.*, 2019). Such an ecological cocoa farming yields very low compared to regular cocoa farming because of pest outbreaks and limited control means. Searching effective alternative control means to chemicals is of greatest interest in agricultural research, especially against termites (Nyeko & Olubayo, 2005).

I.2.2. Alternative solutions for termite control

During the last three decades, research on alternative measures in termite control has increased. Various control methods like physical, cultural, indigenous and biological are applied for termite control (Logan *et al.*, 1990; Ambele *et al.*, 2018a; Verma *et al.*, 2018). However, the effectiveness of each method is relatively limited compared to highly effective toxic cyclodienes. Majority of control practices are ineffective and ecologically unsustainable and, above all, do not address the root cause of termite infestation, merely providing a temporary relief to the problem (Mugerwa, 2015).

I.2.2.1. Physical methods

Farmers use several methods aimed at destroying termite mounds in an attempt to reduce termite densities and subsequently mitigate termite damage to tree crops. Physical destruction mainly involves digging out the entire mound using locally available farm tools until the queen and king are reached and removed (Kiwuso *et al.*, 2004; Akutse *et al.*, 2011; Ogedegbe & Ogwu, 2015; Ambele *et al.*, 2018a). As the mound is dug out, the workers and soldiers are exposed to direct sunlight leading to desiccation. However, this method is labor-intensive, and the practice simply provides temporary relief to foraging activity of termites after which their activity is fully restored (Nyeko & Olubayo, 2005; Ambele *et al.*, 2018a). In addition, some

species of termites have developed physiological adaptation to queen death and have the ability to transform some larvae into neotenic larvae capable to produce eggs without achieving their adult stage (Nyeko & Olubayo, 2005). The practice is also only directed towards mature colonies of the mound-building species, and species that do not build epigeal mounds but cause serious damage to tree crops are often overlooked (Sileshi *et al.*, 2009). Heat treatment as a means of mound destruction involves inserting dry wood, grass or car tyres into the mound, setting it on fire and sealing to confine the smoke (Nyeko & Olubayo, 2005; Mugerwa *et al.*, 2011). Others burn plant residues on top of termites mound to suffocate them (Tasida & Gobena, 2013; Djuideu *et al.*, 2020). Burning plant residues in or on termites mound does not give long-lasting results, as it does not kill the entire colony.

I.2.2.2. Cultural and indigenous methods

Farmers have developed indigenous and cultural methods to control termites in farm, allowing them to do not rely exclusively on chemicals. Cultural methods encompass procedures which help to enhance plant vigor or reduce termite abundances with generally good agricultural practices (Logan *et al.*, 1990). Cultural methods include good quality seed selection, appropriate transplantation procedure, water management, crop rotation, cutting off infected branches, weeding, inorganic fertilizer, removal of crop residues and other organic matter in field, intercropping, high density planting, spacing and time of harvesting (Logan *et al.*, 1990; Wood & Pearce, 1991). These practices have however mitigated results and need to be associated to others methods to be really effective (Djuideu *et al.*, 2020). Indigenous methods include plants extracts and excreta used to kill or repel termites out of the farm. Cattle excreta is used in an attempt to reduce termite damage in agricultural fields in Kenya and Uganda (Kiwuso *et al.*, 2004), while farmers in Nigeria, Ghana and Cameroon use goat excreta as well as human excreta and urine for termite control in plantation crops (Akutse *et al.*, 2011; Ogedegbe & Ogwu, 2015; Ambele *et al.*, 2018a). Some researchers have suggested that the mechanism through which dung reduces termite damage is by providing alternative food resources to termites, hence relieving crops from termite attacks or by enhancing proliferation of termite predators that eventually reduces termite foraging activity and also by enhancing soil fertility and thus boosting plants' vigor, making them less vulnerable to termites (Mugerwa, 2015). Plant extracts have a higher effectiveness in termite control compared to excreta. They include plant insecticides, wood ash and other substances produced from plants that are known as toxic or repellent to insects or have antifeedant properties (Logan *et al.*, 1990). These products are commonly heaped around the base of the trunk or within termite mounds. Several families of plants are recognized to affect termite foraging (Leguminosae, Palmae, Meliaceae,

Moraceae, Solanaceae etc…) among which Neem (*Azadirachta indica*) is one of the most used plant species. Saponins are suspected to be responsible for the toxicity of these plants against termites but they have received little rigorous assessment in field (Logan *et al.*, 1990; Djuideu *et al.*, 2020).

I.2.2.3. Biological methods

With the growing realization of hazards and side effects associated with the extensive and indiscriminate use of synthetic chemical insecticides, entomologists have adopted a new concept of pest control, termed as integrated pest management (IPM). This term refers to a system that utilizes all suitable techniques and methods, in an as compatible manner as possible, in order to maintain the pest population at levels below a threshold causing significant economic losses. In this context, the role of biocontrol agents: predators, parasitoids, and microbes, needs no emphasis due to their specificity, effectiveness, and safety to non-target organisms, besides other components in relation to man and biosphere (Verma *et al.*, 2018).

I.2.3. Natural enemies as best weapons for termite control

Various natural enemies have shown the potential for use in biological control of termites and serve as an alternative to broad-spectrum chemical insecticides. Control methods with sugar-based products to attract termite predators in termite-infested fields have been utilized in some parts of Africa to increase the populations of predatory ants associated with reduction in termite population (Figure 4) (Sekamatte, 2001). Nigerian farmers bury dead animals or fish viscera to attract ants which in turn reduce termite attack on cocoa trees (Owolabi & Okunlola, 2015). A few parasitoids of termites are known, but their potential for regulating termite population is poorly explored because of the protected, underground location of many termite species (Culliney & Grace, 2000). Among enemies of termites, the main groups used in attempts of termite control for their proven pathogenicity are fungi (Blackwell & Rossi, 1986; Rath, 2000), mites (Eickwort, 1990; Zhang *et al.*, 1995), nematodes (Wang *et al.*, 2002) and parasitoid flies (Sze *et al.*, 2008).

Figure 4. Matabele ants (*Megaponera analis*) attacking a termite soldier of *Macrotermes* (Photo ©Getty Images, 2020)

Laboratory studies have shown that termite species are highly susceptible to entomopathogenic fungi infestation from many genera including the most commonly studied species, *Metarhizium anisopliae* and *Beauveria bassiana* (Rath, 2000). Nematode infestations due to *Steinernema siamkayai*, *S. pakistanense* and *Heterorhabditis indica*, also induce severe mortality in colonies of both *Reticulitermes flavipes* and *Odontotermis hornei* in laboratory experiments (Razia and Sivaramakrishnan, 2016). Nematodes *Mermis* sp. and *Neosteinernema longicurvicauda* may kill their termite hosts upon emergence (Wang *et al.*, 2002). Although the relationship between mites and termites is mainly phoretic, some mite species such as *Acotyledon formosani* can cause death in weak termite colonies (Phillipsen & Coppel, 1977; Wang *et al.*, 2002). The phoretic instar or deutonymph of *A. formosani* appeared to negatively affect a large laboratory colony of *Coptotermes formosanus* Shiraki by fastening primarily to termite heads and mouthparts, thereby impeding normal feeding (Wang *et al.*, 2002). Parasitoid flies associated with termites is also well documented in literature and the main group of dipterans is Phoridae (Dupont & Pape, 2009). Sze *et al.* (2008) isolated larval instars of *Verticia fasciventris* (Diptera: Calliphoridae) infesting head capsules of *Macrotermes barneyi* and killing its host upon emergence.

I.2.4. Importance of entomopathogenic fungi for termite control

The potential of most biocontrol agents (ants, mites and parasitoid flies) for regulating termite population seems negligible because of the protected, underground location of many termite species (Culliney & Grace, 2000). Only soil-dwelling agents such as fungi seems suitable to termite control (Ambele *et al.*, 2018). Research has been conducted to develop biological technologies for use against economically important termite species, focusing on

Metarhizium anisopliae and *Beauveria bassiana* in Kenya, Uganda and Cameroon (Figure 5) (Maniania *et al.*, 2002; Nyeko & Olubayo, 2005; Ambele *et al.*, 2019). Although both fungi have been shown to be effective against various termite species in the laboratory (Hussain *et al.*, 2010), their success in the field has been extremely limited because of the ability of termites to detect and avoid other infected termites (Rath, 2000; Yanagawa *et al.*, 2008) and because virulent entomopathogenic fungi are repellent to termites (Mburu *et al.*, 2009; Hussain *et al.*, 2010).

Figure 5. Mass deaths in termite colonies due to fungal infestation by *Metarhizium* sp. (Photo ©Professional Pest Manager, 2020)

I.2.5. New technologies of formulation of biopesticides: Attract&Kill approach

Entomopathogenic fungi (EPF) have long retained interest of researcher for termite control studies in laboratory (Rath, 2000). They are one of the best weapons for pest control (Verma *et al.*, 2018) but their application during field experimentation are often difficult due to many environmental and biological factors, e.g. handling problems, low shelf life, poor establishment in soil and high costs due to high dosage per/ha (Przyklenk *et al.*, 2015). In order to address these drawbacks in the application of EPFs, innovative technologies such as Attract&Kill (A&K) Strategy have been developed (Figure 6). The A&K strategy is often recognized as a solution for a better termite control than old chemical-dependent strategy (Lösel *et al.*, 2000; Drosu *et al.*, 2008). This strategy is based on the co-formulation of an attractive substance like CO2-emitting baits (Bernklau *et al.*, 2005) combined with an EPF virulent to the termite pest targeted. Recent studies have proved the capacity of encapsulation of EPFs and baker's yeast (*Saccharomyces cerevisiae*) in Calcium-Alginate matrix, producing small pellets

capable to emit CO2 at a relevant quantity (Przyklenk *et al.*, 2015; Vemmer *et al.*, 2016; Ambele *et al.*, 2019). Encapsulation increases the shelf life of EPF, improves their establishment in soil and reduces the costs of field application (Przyklenk *et al.*, 2015).

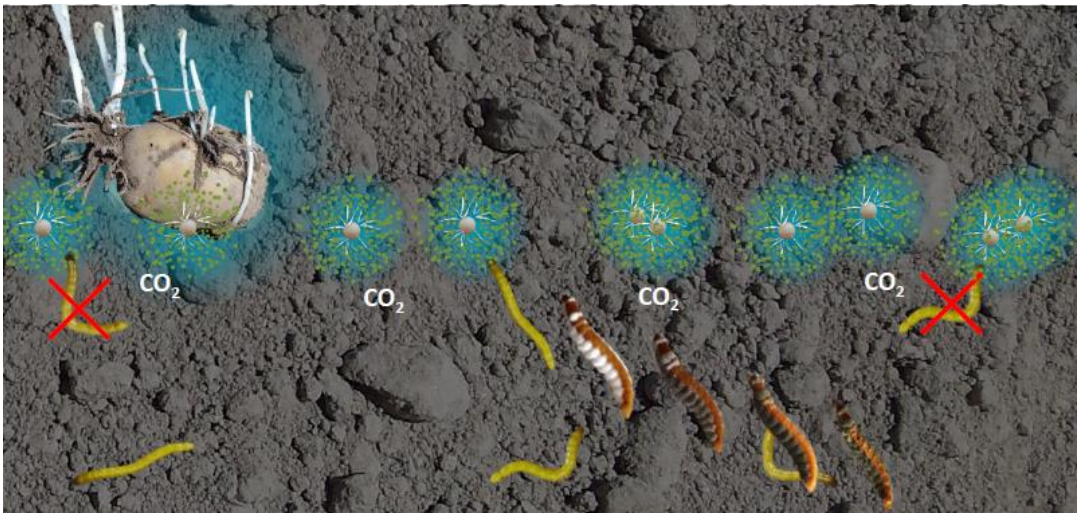


Figure 6. Examples of field application of CO2-emitting capsules against wireworms, belowground pests of potato (Photo ©Anant Patel, 2020)

A recent study carried out by Ambele *et al.* (2019) confirmed a severe mortality in termite colonies directly exposed to sporulating CO2-emitting capsules associated with *Metarhizium brunneum*. However, termites are capable to avoid these capsules when used as baits. According to Hussain *et al*. (2010), termites can detect volatiles emitted by virulent fungi species and then avoid emitting areas. More the fungus species is virulent (e.g. *Beauveria bassiana*, *Metarhizium* spp.) more termite detect volatiles easily. *Isaria fumosorosea*, less virulent than than *B. bassiana* and *Metarhizium* spp., was less detectable by termites and induced equivalent level of mortality during tests (Hussain *et al.*, 2010). In addition, a recent study showed that termites rapidly removed *Metarhizium*'s conidia (spores) from nest mates through intensive allogrooming behaviour, resulting in very low mortality (Liu *et al.*, 2019). This allogrooming behavior is amazingly efficient, managing to remove all fungal spores from the exposed termites' cuticles within 12 hours. Thus exploring naturally-infesting fungi strains with less detectable volatiles may be a good approach to improve the effectiveness of field application of the A&K approach against soil-dwelling termites. The endophytic colonization of EPF could also constitute a another promising approach to bypass this behavioral response of termites to entomophagens (Ambele *et al.*, 2020).

I.3. The use of molecular tools to identify biological control agents

The definition of the concept of "*species*" is quite simple but the accurate assignment of an unidentified specimen to a given species may become somewhat very complex. To do so, several of the components that should be taken into consideration when deciding which technique should be used are numerous and include, but not limited to, speed, accessibility of information, accuracy of the information, and overall cost (Hebert & Gregory, 2005). There has become a great debate/divide within the biological scientific community regarding opinions about which of the methods of specifies identification is better. For the past 250 years, taxonomy has been defined based on morphological structures; the phenotypic characteristics of individual organisms (Hebert & Gregory, 2005). Till today, species identifications based on morphological characters remain strongly reliable as most species can be distinguished from each other on the basis of visual aspects. In addition, this method is very cost-effective, requiring few equipment. However there are major issues in using morphological features to distinguish certain species. One of the issues with morphological systematics is that there are few physical characteristics that are common among major groups of organisms, (e.g., eubacteria and eukaryotes) (Hillis, 1987). For instance, the majority of the species already described, including microbial species, are difficult, or nearly impossible to accurately separate with the naked eye, as observed in cryptic species complex (Savolainen *et al.*, 2005). There are many species that use camouflage, or even mimicry, as a defense mechanism (Park, 2008). This also makes it difficult and often confusing when using morphological features to identify one species from another.

Recently, with the growing understanding of genetics, evolutionary scientists have begun using molecular systematics to identify one species from another (Hebert & Gregory, 2005). Using DNA removes the opinion-based disputes amongst scientists on how to define one feature from the next and provides a stable tool for comparison and segregation among species. By using the genome, and studying specific genes, it makes it relatively easy to compare one individual to another, and to determine how closely related one species is to the next. One gene that is suggested to be used is the CO1 gene, cytosine oxidase 1. This gene has been relatively constrained throughout evolution and so far has been found in every living thing. Mitochondrial DNA is some of the most rapidly evolving DNA, and it has been useful in phylogenetic population studies (Friedheim, 2016). MtCOI and MtCOII sequences have been used for species-level and population-level phylogeny due to a high rate of substitution in the third codon position (also called the wobble position) (Liu & Beckenbach, 1992). The universal primers designed by Folmer *et al.* (1994), LCO 1490 and HCO 2198, with 25 and 26 base pairs (bp) in

length, respectively, are widely used primers in the animal kingdom. A Folmer primer amplifies the first half of the COI gene, which is a gene fragment of length approximately 700 bp. The success rate of the primers in amplifying the COI fragment in highly divergent animal species has been remarkable due to its conserved 3′ ends (Folmer *et al.*, 1994). Insects, including parasitoid flies, are commonly identified using LCO 1490 and HCO 2198 primers.

In the case of fungi identification, the frequent lack of distinctive morphological characters, the preponderance of microscopic species, and the considerable socioeconomic importance of this kingdom reinforce the need for a DNA-based identification system. DNA-based systems for species of fungi have variously used a barcode-like 400- to 600-bp region of the nuclear large ribosomal subunit, the internal transcribed spacer (ITS) cistron, partial β-tubulin A (*Ben*A) gene sequences, or partial elongation factor 1-α (EF-1α) sequences (Seifert *et al.*, 2007). The most common primers used for the identification of entomopathogenic fungi are ITS1 and ITS4, amplifying the 16S rDNA ITS gene from the small ribosomal subunit (White *et al.*, 1990). The potential effectiveness of CO1 in species identification of fungi has not been thoroughly evaluated, but a preliminary study led by Seifert et al. (2007) suggested that MtCO1 gene could also be used for fungi identification although less effective as ITS gene.

CHAPTER II: MATERIALS AND METHODS

II.1. Study sites

The study was conducted in 05 cocoa agroforestry systems in the Centre region of Cameroon from 2018 to 2021 (Figure 7). The Centre region is the main producing basin in Cameroon, contributing to about 40% of total production in 2018 (Lescuyer *et al.*, 2020). These systems presented shade levels ranging from rustic to full sun type (Rice & Greenberg, 2000; Bisseleua *et al.*, 2013). These cocoa agroforestry systems were situated at Boumnyebel, Obala, Talba, Kedia and Bakoa. The altitude varies between 375 and 610 m above sea level. The study areas were located between 4°12' and 4°30' latitude north, and 10°6' and 11°15' longitude east. The Centre region is influenced by a bimodal equatorial Guinean climate with four seasons: a long dry season (mid-November to mid-March); a short rainy season (mid-March to June); a short dry season (July to August) and a long rainy season (September to mid-November) (Suchel, 1988). The temperature is between 22 and 29° C (Santoir & Bopda, 1995). The pH of the soil varies from 4.29 to 5.43 (Kotto-Same *et al.*, 1997; Kanmegne *et al.*, 2006). In each system, 04 cocoa plantations were selected for a total of 20. The plantation size ranged from 1 to 4ha.

Boumnyebel is located in the **Nyong et Kelle** division at approximately 70 km of Yaoundé. Cocoa plantations were located in villages of Si-Manyaï and Pan-Makak. In this locality, cocoa is grown under a very dense cover of shade tree species called as "rustic shade" level. Boumnyebel is characterized by an evergreen forest vegetation surrounding villages. Land is occupied at 70% by pristine forest and 30% by agricultural fields. Cocoa fields constitute of 20% of land and annual crops cover 10% (cocoyam and plantain). Cocoa plantations are very old (> 50 years old in average). The climate is typical of the humid forest zone with more than 2100 mm of annual rainfall regime (Bisseleua *et al.*, 2013). Boumnyebel has ferric oxisols with highly unsaturated acid rocks and exhibit a low agricultural yield. The soil is highly acidic and so requires chemical inputs (Kotto-Same *et al.*, 1997; Kanmegne *et al.*, 2006).

Obala is located in the **Lekie** division at approximately 40 km of Yaoundé. Cocoa plantations were located in villages of Ekabita Essele and Nkolobang. In this locality, cocoa is grown very close to houses with a high variety of fruit trees species under a "heavy shade" level. Obala is considered as an ecotone between the forested zone from the South and the savannah zone from the North. Land is occupied at 5% by secondary forest and 95% by agricultural fields. Cocoa fields constitute of 70% of the land and 25% is cover by mixed annual crop fields known as homegardens (cassava, groundnuts, maize, tomatoes etc…) and

agroforestry trees (citrus, safou, avocado, etc…). Cocoa plantations are old with an average age of 40 years old. The annual rainfall regime is estimated to about 1300 mm (Bisseleua *et al.*, 2013). Obala has ferric oxisols with fairly unsaturated acid rocks, with a well-drained system and good chemicals properties (Kotto-Same *et al.*, 1997; Kanmegne *et al.*, 2006).

Talba is a village of the locality of Mbangassina in the **Mbam et Kim** division. Mbangassina constitutes actually the main cocoa producing sub-basin in the Centre region (Lescuyer *et al.*, 2020). In this village, cocoa is grown in larger plantations under an "intermediate shade" level. Land of Talba is occupied at 25% by pristine forest and 75% of agricultural fields. Cocoa fields occupy 70% of land and 5% is covered by annual crop fields (banana, plantain). Cocoa plantations are ageing, averaging 30 years old. The annual rainfall regime is about 1200 mm (Bisseleua *et al*., 2013). The yellow soils/ferric acrisols are seen in Talba, where soils are less deep, well drained with the topsoil containing over 60 % sand and less than 2 % organic matter (Kotto-Same *et al.*, 1997; Kanmegne *et al.*, 2006).

Bakoa is a village of the district of Bokito, in the **Mbam et Inoubou** division. Bokito constitutes another important cocoa producing sub-basin in the Centre region (Lescuyer *et al.*, 2020). In this locality, young cocoa is grown on modified "full sun-like" savannah agroecosytems. The land in Bakoa is occupied at 5% by secondary forest and 95% by agricultural fields. Cocoa fields occupy 50% of the land, annual crop fields (maize, yams) and agroforestry trees (citrus) cover 25% of land and 5% is allocated to patchy pasture fields. Cocoa plantations are aged between 10 and 15 years old (Bisseleua *et al.*, 2013). The annual rainfall regime is about 1100 mm. Bakoa has yellow soils with characteristics similar to Talba soil (Kotto-Same *et al.*, 1997; Kanmegne *et al.*, 2006).

Kedia is another village of the district of Bokito, in the **Mbam et Inoubou** division. In this locality, cocoa traditionally grown under full sun (as now observed in Bakoa) is now cultivated under a "low shade" level, an innovation of this area characterized by a progressive addition of shade trees in old full sun systems (Jagoret *et al.*, 2012; Bisseleua *et al.*, 2013). Land is occupied at 5% by secondary forest and 95% of agricultural fields. Cocoa fields occupy 65% of land, annual crops (mainly maize) are established on 25% of land and 5% is dedicated to pasture lands. Cocoa plantations are aged from 15 to 20 years old. The annual rainfall regime averages 1050 mm. Kedia has yellow soils with characteristics similar to Talba soil (Kotto-Same *et al.*, 1997; Kanmegne *et al.*, 2006). General features of each agroforestry system are presented in Table I.

The identification of insect specimens sampled during fieldwork and lab trials was carried out at the Entomology Laboratory of IRAD (Yaoundé, Cameroon) under the supervision of Dr NDZANA ABANDA François-Xavier. The fungal culture and morphological identification was carried out at the Regional Laboratory of Biological Control and Applied Microbiology of IRAD (Yaoundé, Cameroon) under the supervision of Dr BEGOUDE Didier. The molecular identification of fungi and flies was done at the Laboratory of Valororization of Symbiotic Microorganisms at Regional Centre of Excellence WAVE (Bingerville, Côte d'Ivoire) under the supersion of Dr LEABO Linda.

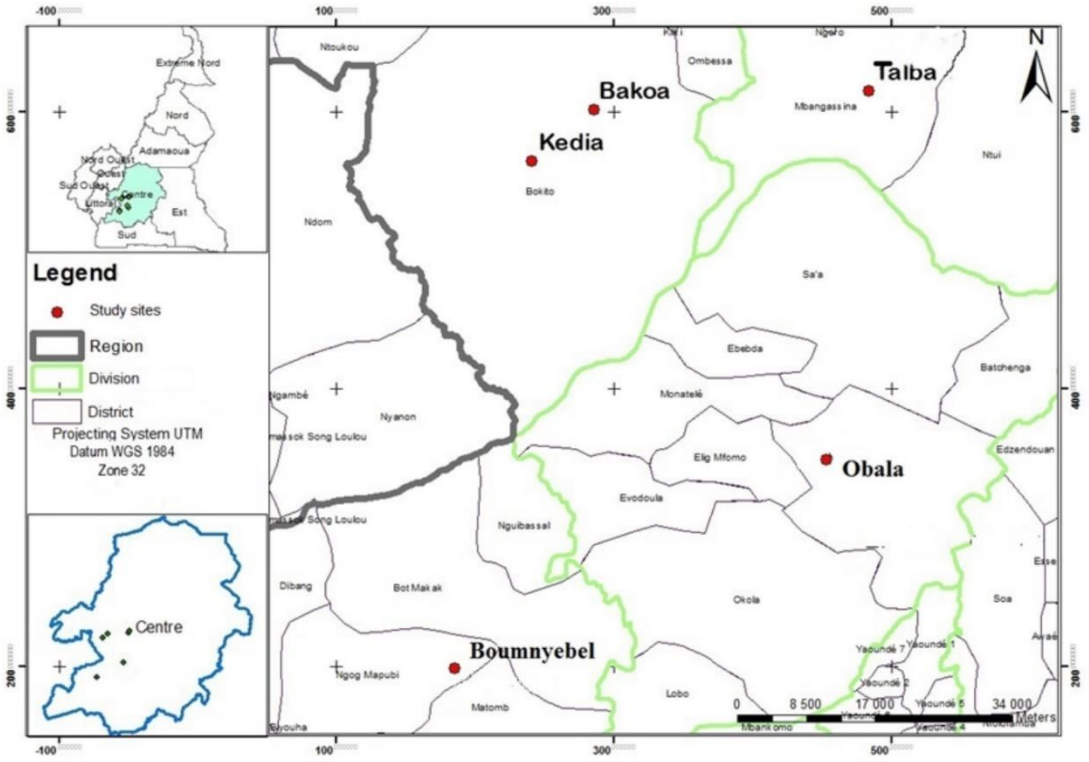


Figure 7. Map of study sites (Ambele *et al*., 2018b)

Table I. General features of cocoa agroforestry systems in studied localities (Djuideu *et al*., 2020)

Variables/System	Rustic	Heavy shade	Intermediate	Low shade	Full sun
Altitude (masl)	402	557	462	459	469
Age (years)	>50	>40	35-40	20-25	15-20
Cocoa density (plans ha^{-1})	1250 ± 28.8	1550 ± 155.45	1075 ± 96.82	1095 ± 69.10	1182.5 ± 80.45
Shade trees (trees ha^{-1})	196.5 ± 15.5	158.3 ± 21.6	65.9 ± 9.15	98.6 ± 31.8	14.3 ± 3.1

II.2. Methodological approach

II.2.1. Experimental design of the study, environmental landscape characterization and climate parameter records

In March 2018, we selected 4 plantations per each Cocoa agroforestry system with sizes varying from 1 to 4 ha. In each plantation, we delimited two plots of 30 m x 30 m (900 m^2). The mean tree height was 6.01 ± 0.28 m and the mean diameter at breast height (DBH) was 0.32 ± 0.04 m. The cocoa planting materials were dominated by the old German and locally produced hybrids varieties. In plantations of rustic and heavy shaded systems, we found mainly the old German cocoa with certain plantations around 100 years old (first cocoa plantations created by Germans). Plantations of intermediate, low shaded and full sun-like systems have mixed cocoa varieties but overall the old German cocoa was the main cultivated variety. The intercropping distance between cocoa stands was 2.5 – 3 m in average.

In every chosen plantation, we collected data on the vegetation characteristics and the number of shade trees species, with unknown tree species given a unique morphospecies number. Scientific and vernacular names (the latter given by local stakeholders) were recorded. Species that could not be identified in the field were identified at the National Herbarium of Cameroon (Yaoundé). We measured shade cover at 10 subpoints for each of the four cardinal directions within a 30 m radius circle using a convex spherical densitometer (R. E. Lemmon Forest Densiometers, USA) and calculated the mean shade cover per circle (Bisseleua *et al.*, 2013).

In this study, we also selected the air temperature and the relative humidity (R. H.) per season as they are the most affected factors by the global warming (Shako, 2015) and the differential shift from a dry season to a wet season gives a good information on the mitigating effect of systems towards climate change. During each sampling season, we recorded air temperature (T°) and R. H. at different points under cocoa canopy (at 2 m height) when the sun was at the zenith (from 12 pm to 2 pm) using a pocket thermo-hygrometer Testo® (Testo S.a.r.l, France).

II.2.2. Assessment of taxonomic and functional diversity of termites on cocoa

II.2.2.1. Termite sampling procedure

From March to September 2018, we sampled termite soldier and worker casts associated with cocoa trees in 40 delimited plots of 30 m x 30 m in 20 farms across the five production systems (Figure 8). We excluded the winged caste because their presence on a cocoa tree do not necessarily imply a foraging activity on that tree (Eggleton *et al.*, 2002a). In each plot, we randomly selected 15 cocoa trees on which we collected termites at two levels: (1) aboveground parts where we sampled termites in galleries (carefully scratched up to 2 m height), in dead branches/woods and in arboreal nests; (2) belowground parts where we sampled termites in

roots through a non-destructive methods by taking 4 soil samples of 12x12x10 cm^3 at the basis of each tree after litter clearance using an auger. We sieved the collected soil samples and extracted termites using forceps. The procedure was replicated in 2 plots per plantation. We also replicated the sampling during two successive seasons: the rainy season (from March to May), and the dry season (from mid-June to August). Collected termite samples were preserved in labelled tubes containing 70% alcohol for an optimal conservation in laboratory where they were sorted per species, counted and identified.

Figure 8. Sampling of termites on cocoa trees (Photo ©Djuideu, 2018). **A)** Delimited plot of 30 m × 30 m, **B)** Holes digging using an auger, **C)** Holes dug around tree collar after litter

clearance, **D**) Manual search and collection of termites from soil samples, **E**) Scratching of galleries along the trunk.

II.2.2.2. Termite identification

We identified the soldier castes to genus and sometimes to species levels using dichotomous keys of Emerson (1928), Bouillon & Mathot (1965) and Sands (1965, 1972, 1998), and through the reference collections of Institut de Recherche Agricole pour le Développement (IRAD) Nkolbisson, Yaoundé (Cameroon). Whenever possible, specimens were identified to species level, and when this proved impossible, unidentified species were added the suffix "sp." to the generic names because many species are not easy to be identified with certainty in some genera (Darlington *et al.*, 2008). The identified specimens were placed in 70% ethanol in 1.5-ml Eppendorf tubes with labels (name of species, locality, plot, name of identifier, date identified) inserted into each tube. The tubes with the specimens are stored in the Zoological Collections of the Entomology Laboratory of IRAD, Nkolbisson, Yaoundé (Cameroon).

The identification of soldierless termites followed the protocol of Ambele *et al*. (2018b). In this protocol, the enteric valves (a reduced proctodeal segment (P2 homolog) that is located between the first proctodeal segment (P1) and the third proctodeal segment (P3) of the digestive tube) were used for identification. The workers were immersed in 70% ethanol and dissected under a dissecting microscope using entomological mandrins. The abdominal integument was removed from each specimen, and the mandrin was used to cut out the section of the gut containing part of the P1, P2 (containing the enteric valve), and P3. The gut section was placed on a microscopic slide in a drop of Berlese mounting liquid, and the organic matter from inside the gut section and muscle tissue from the outer wall of the gut were carefully removed. The gut section was then cut in half and splayed out on another microscope slide in another drop of Berlese mounting liquid. After re-orientating the enteric pads and longitudinal ridges to face upwards on the slide, a drop of Berlese mounting liquid was added, and a cover slip carefully placed on the slide. The slides were then observed under an Omax microscope at a magnification of 40x. The enteric valve structures were compared to previous features using Sands (1972, 1998) or to existing prepared reference slides preparations of enteric valves structures of termite collections stored at IRAD.

II.2.2.3. Functional classification of termites

After identification, we placed termite species into one of four feeding groups, following the classification based on worker character subsets of the right mandible, enteric valve and gut morphology (Constantino, 1992; Eggleton *et al.*, 1996; Donovan *et al.*, 2001). These groups are as follows: group I (wood and grass feeders, lower termites), group II (wood and litter feeders), group III (wood-soil interface feeders) and group IV (true soil feeders). Many species in Group I and II are fungus-growing and cause damage to cocoa (Ackonor, 1997; Vos *et al.*, 2003; Tra-Bi *et al.*, 2015; Ambele *et al.*, 2018b). Group III and IV are soil feeders, and provide important ecosystem services, especially nutrient recycling which are beneficial to crops (Eggleton *et al.*, 1996). The termite species were also classified into pest and non-pest species according to their economic significance as reported by Sands (1973, 1998).

II.2.3. Evaluation of ecosystem services and disservices provided by termites on cocoa trees

II.2.3.1. Evaluation of ecosystem services provided by termites

From termite samples above, we quantified the ecosystem services provided by termites by calculating a weighted humification score (HS) as described by Attignon *et al*. (2005) to quantify the services provided by functional groups of termites to cocoa trees. This index is based on the functional assemblage of termites and quantifies the level of soil fertilization attributed to termite activities. The weighted HS theoretically measures the ecosystem services provided by termite assemblage in farm and was calculated as follows:

$$\mathbf{HS} = \frac{[\sum(\boldsymbol{ni} \times \boldsymbol{fi}]}{\boldsymbol{N}} \qquad [1]$$

where ni is the number of termite encounters in the ith feeding group, fi the corresponding termite feeding group score, ranging from f = 1 for wood and grass feeders (group I) to f = 4 for true soil-feeders (group IV) and N is the total number of termite encounters in soil of a farm. The HS depicts the position of termite species along a gradient of increasing humification of their food substrate (Donovan *et al.*, 2001; Davies *et al.*, 2003), and the weighted HS the position of whole termite assemblages along this gradient. The weighting procedure in this study differs from the one of Davies *et al*. (2003) in which the HS was weighted by number of encounters instead of species number. A weighted HS < 2.5 refer to a community of wood feeders with low fertilization index while a weighted HS > 2.5 refers to a community of soil feeders with high fertilization index.

II.2.3.2. Evaluation of ecosystem disservices provided by termites on cocoa trees

II.2.3.2.1. Assessment of termite pest severity on cocoa trees

In each delimited plot of all the systems, we randomly selected 15 cocoa trees. On each selected tree of the 20 plantations, we sampled termites at two infestation sites, aboveground and belowground (Figure 9). Aboveground, we sampled termites at three different categories of harmfulness: (a) we carefully scratched off a randomly selected gallery (earth run) from the collar to up to 2 m height, representing the first category of damage of aboveground infestation; (b) we then sampled dead branches on trees which represents the second category of harmfulness; and (c) we sampled arboreal nests found on trees as the highest category of harmfulness. A selected cocoa tree showing conspicuous galleries but without termites inside, was considered as a non-infested tree. Belowground, we sampled root-infesting termites using a non-destructive sampling method by taking four soil samples of 12 x 12 x 10 cm^3 in the root system near the collar and covering the entire soil surface surrounding the tree. This sampling method is a cost-effective approach to avoid the killing of cocoa trees through root excavation (Figure 9b) (Boogaard & van Dijk, 2012). The belowground infestation of a tree was then assumed when we found pest species of termites in soil samples around roots. The termite sampling on cocoa trees was replicated in the same plantations during two consecutive seasons as seen above. Ambient temperature and relative humidity significantly shifted from one season to another.

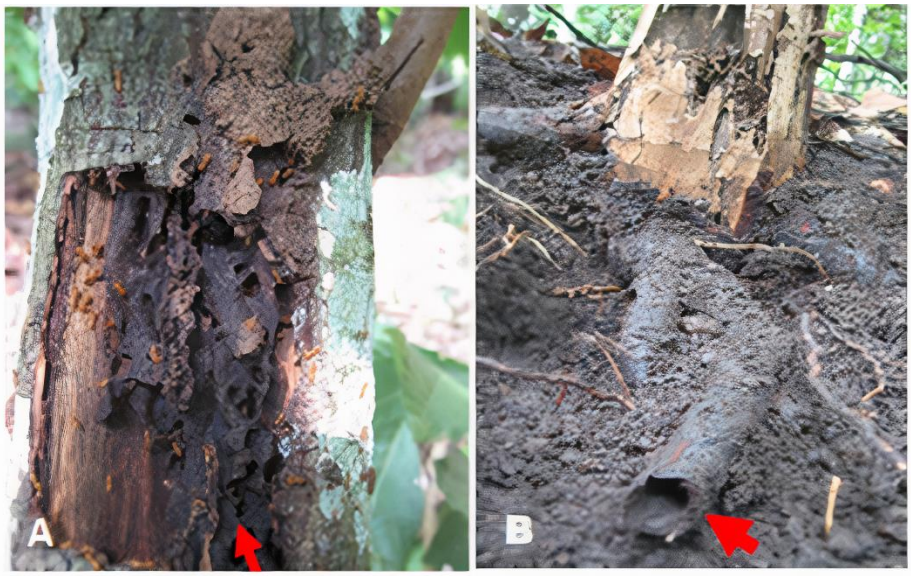


Figure 9. Main types of damage attributed to termite infestation on cocoa trees. a) Aboveground infestation, b) Belowground infestation.

From sampled termites, we only considered worker and soldier casts from species identified as pest following the above protocol, whose presence is attributed to foraging activities. The number of termites sampled galleries and root sampling categories were recorded

for density analysis. We excluded the abundance of termites sampled from dead branches and arboreal nests for density analyses because these abundances strongly depend on the size of branches and nests investigated. The size of branches or nests strongly depends on the age of infestation, old plantations may have bigger nests and branches than younger ones and this may bias our comparison (Table 1). This size also significantly varies from one termite species to another. These aspects may therefore bias the results obtained. A total of 1200 trees were sampled, with 240 per CAFS and 600 per season. The infestation rate per plot (I) was obtained by the quotient of the number of infested trees at a precise sampling level (n_i) by fifteen (total number of trees sampled per plot): $I\ (\%) = (n_i / 15) \times 100$.

II.2.3.2.2. Description of termite damage on cocoa trees and severity assessment

We described and documented the observed disservices provided by termites to cocoa agroforestry systems. These genera were selected on the basis of their infestation levels, their ubiquitous character in all the systems, their infestation site (aboveground vs belowground), as well as their visual damage observable on the cocoa trees (if applicable). The description looked at tree development stage susceptible to pest attacks, damage location on the cocoa stand, infestation signs, visual aspect of damaged tissues (if applicable), progression of infestation and further consequences on plant health. On the basis of the superficial damage on cocoa trees, which were the only accessible damage because belowground damage are difficult to assess without wounding the crop, we selected randomly 15 cocoa trees in each plot per system and we hierarchically classified the severity of damage in 4 visual scales corresponding to chronological damage steps (Figure 10):

- **Primary level**: the cocoa tree does not present any signs of ongoing aerial damage.
- **Secondary level**: the cocoa tree presents a few galleries running along the trunk (less than 4, sometimes visible only at the basis of the trunk) with no visible signs of bark perforation. This level was characteristic of recent infestation by termites.
- **Tertiary level**: the cocoa tree presents numerous and anastomosed galleries running along the tree (from collar to branch ends). The bark is perforated and sometimes earth plates are formed on the trunk in which termites are eating the plant.
- **Quaternary level**: the cocoa tree presents numerous anastomosed galleries along the tree and conspicuous arboreal nest on branches or trunk. The bark is perforated or presents earth plates. Most of the time, the cocoa tree is dying.

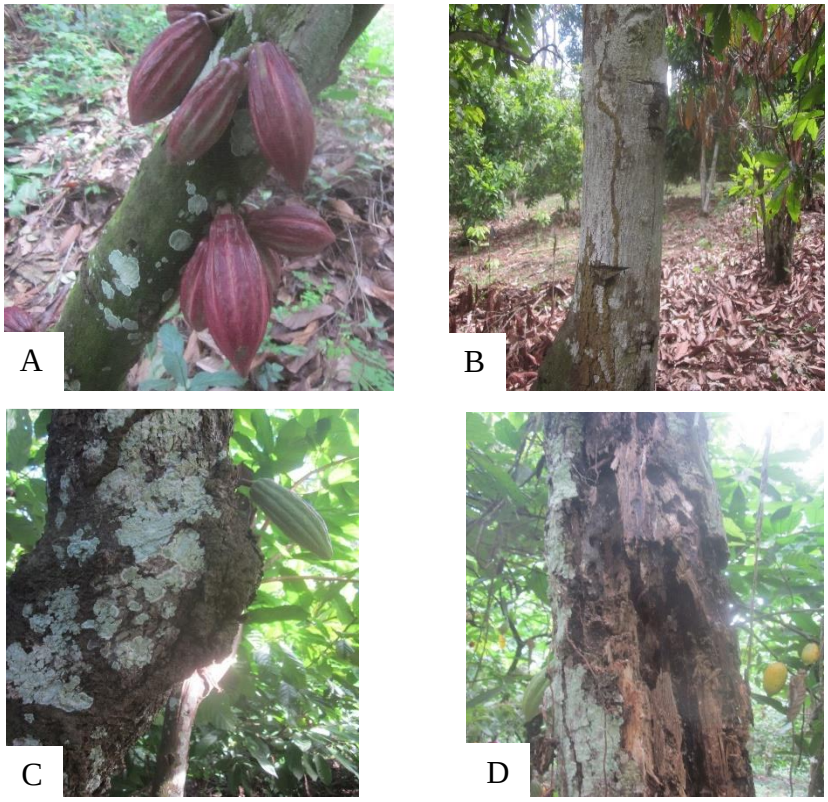


Figure 10. Different damage levels on above parts of cocoa (Photo ©Djuideu, 2018). **A**) Primary, **B**) Secondary, **C**) Tertiary, **D**) Quaternary.

II.2.4. Effect of termite ecosystem disservices on tree development and total yield

II.2.4.1. How termite ecosystem disservices affect tree development

During the main cocoa productive periods (June to September) of years 2018 and 2019, we assessed the effect of termites on cocoa tree development and yield by comparing yield parameters between two tree groups: infested trees vs healthy trees. Infested trees presented signs of ongoing termite attacks while healthy trees were free of any termite attack signs. The signs of termite attacks referred to the presence of common pest species of termites on different tree parts (branches, trunk and roots) with observable damage as described above. We selected randomly 15 cocoa trees per tree group for monitoring. In case of highly or poorly infested plots, i.e. less than 15 healthy or infested trees in the plot respectively, we selected all the cocoa trees available in each tree group respectively.

We also assessed the severity of termite infestation by distinguishing three levels of infestation within infested trees: (1) gallery infested trees with termite galleries covering flower cushions and preventing flowering and pod formation. In 20% of cases galleries are associated with arboreal nests. (2) Root infested trees were characterized by termites feeding on the roots of cocoa trees. (3) Gallery + root infested trees referred to cocoa trees with both gallery and

root infestations (Vos *et al.*, 2003; Tra-Bi *et al.*, 2015). We considered that trees with gallery + root infestation were the most severely infested, followed by root infested trees.

On each selected cocoa tree, we recorded data on tree development (height) and tree vigor (basal area). We measured the height of each tree from the collar to tree crown. The basal area was calculated from the DBH (diameter at breast height) of each tree, measured 50 cm above the ground. Height and basal area of trees are variables closely related to their yield (Lachenaud & Mossu, 1985). We evaluated the flowering of each tree by counting the number of flowers and active flower cushions on trunk and branches of the tree. Cocoa flowers are commonly produced on flower cushions dispersed along trunks and branches and these cushions are essential components of the yield (Frimpong-Anin *et al.*, 2014). We then evaluated the pod formation on each tree by counting the number of pods produced. As pods smaller than 10 cm long are susceptible to physiological wilt (Wood & Lass, 2008), we only counted pods longer than 10 cm, and we marked them to avoid double counts. These pod counts enabled us to calculate the mean number of pods/cocoa tree (Nb Pods).

II.2.4.2. Relationship between termite ecosystem disservices and yield

a) Parameters recording on pods

In each cocoa plot, we harvested and labelled about 20 ripe cocoa pods from the 30 selected trees. About 10 pods were harvested from healthy trees and 10 from infested trees in each plot. A total of 750 pods were harvested. We immediately recorded length and diameter of each pod using an electronic caliper. The length and diameter of each pod were used to calculate the size of the pod following the formula (2). Each pod was then weighted using an electronic balance, opened and we extracted the fresh beans inside the pod. We counted the number of beans in each pod.

$$\mathbf{V}=\frac{\mathbf{1}}{\mathbf{12}}\ \boldsymbol{\pi D^2 L} \qquad [2]$$

With V the volume of the pod, *D* its diameter and *L* its length.

b) Parameters recording on beans

We selected randomly 150 beans from pods harvested in each tree group (healthy vs infested) and per production system. We measured length and width of each bean using an electronic caliper. We then weighted each bean using a precision balance. Afterward, the beans were dried for at least two weeks following the drying method described by Wood & Lass (2008). The dry beans were weighted again using a precision balance and we estimated the water content (WC) of each bean using the equation (3):

$$\mathbf{WC\ (\%) = 100 \times (W_f - W_d) / W_f} \quad [3]$$

with W_f the weight of fresh bean and W_d the weight of dried bean.

The marketable yield (Y) of each tree group was calculated following the equation of Jagoret *et al.* (2017) (4):

$$\mathbf{Y\ (kg.ha^{-1}) = (Nb\ Pods \times Wbeans \times TC) \times KkoDens} \quad [4]$$

where Nb Pods is the mean number of pods/cocoa tree, Wbeans is the mean weight of fresh beans/pod (kg), TC is the marketable cocoa/fresh bean weight transformation coefficient, and KkoDens is the number of all cocoa trees/ha counted in the quadrat. Wbeans depends on the number of beans/pod and the mean fresh bean weight. It was calculated using these two variables we have recorded. We considered a constant value of TC at 0.35, a "conservative" value that is commonly used in hybrid cocoa tree comparative tests (Lachenaud, 1984) to avoid repeating measures. The KkoDens was fixed to 1200 trees per ha for calculations per tree group as it is the recommended tree density per ha (Wood & Lass, 2008). For yield calculation per production system, we used the cocoa density in each delimited plot per system.

We estimated the yield loss induced by termite infestation on cocoa trees following the Walker's protocol of crop loss (Walker, 1983), by comparing calculated yield between the healthy trees and infested trees. The yield loss was estimated following the equation (5). The yield loss was calculated per tree group and per severity of termite infestation.

$$\mathbf{Yield\ loss\ (\%) = 100 \times (Y_H - Y_I) / Y_H} \quad [5]$$

with Y_H the marketable yield from healthy trees and Y_I the marketable yield from infested trees.

II.2.5. Screening of potential biological control agents of termites responsible of disservices in cocoa agroforestry systems

During the cocoa productive period of years 2019 and 2020, the main damaging termite species belonging to genera previously selected for damage description were randomly sampled in each plantation at different levels such as litter, dead wood, soil and cocoa trees. The living samples were transported to the entomology laboratory of IRAD in plastic boxes perforated with small holes on the lip to allow air circulation. Termite samples were therefore divided into three distinct groups to isolate entomopathogenic fungi, parasitoid flies and parasitic mites separately at the same time. The samplings were realized during 2 consecutive seasons as described above.

II.2.5.1. Screening of fungi species associated with termites

II.2.5.1.1. Isolation, and purification of fungi isolates

a) Preparation of termites

The first group of termites transported to in laboratory were separated into 2 subgroups:

- In the first subgroup, we randomly selected 20 individuals per termite species and per farm (when available). The exoskeleton of these insects was first cleaned by spraying fine droplets of alcohol 70% all over termite bodies. After about 10 seconds when the alcohol has dried on termite bodies, we then sprayed alcohol for a second time on their bodies to ensure that the exoskeleton is completely cleared of any external fungi infestation (Humber, 2012). The cleaned living termites were then introduced in a sterilized plastic zip bag and crushed inside the closed bag. The termite crush was conserved in a freezer (-20°C) for at most 48h.
- In the second subgroup, we selected 5 dead individuals per termite species. The cadavers were cleaned in the same way as living termites as described above. The cleaned cadavers were introduced in a sterile petri dish in which we previously added a cotton pad soaked in distilled water. The petri dish was then sealed with a parafilm tape and conserved in darkness in a humid chamber for development of Hypocreales fungi (Humber, 2012) during at most 10 days.

b) Preparation of culture media

We used the Molisch's agar medium for the culture of isolated fungi. This medium is specifically used for entomopathogens (Blackwell *et al.*, 2003). The different components of the culture media (agar, sucrose, peptone, Mg2SO4 and K2PO4) were weighted using a precision balance at the indicated quantities and introduced in bottle with coverlid. We then added 1L of tap water and we mixed it all up by shaking vigorously the bottle. We autoclaved the prepared medium at 121°C during 15 min using a vertical steam sterilizer. After the medium has cooled slightly, it was transported to a FASTER laboratory fume hood where about 15 ml of medium was poured in each sterile petri dish. Before pouring the medium in petri dishes, we amended it with chloramphenicol and streptomycin (dose of 250 mg for 1L of medium each) to prevent any bacterial development in petri dishes and avoid competition with growing fungi (Blackwell *et al.*, 2003). The petri dishes containing the Molisch's agar medium amended with antibiotics were left in the laboratory fume hood for at least 12h to allow the medium to cool completely and condensate.

Composition of Molisch's agar medium for 1L of tap water (Blackwell *et al.*, 2003):

- 15 g agar
- 20 g sucrose
- 10 g peptone
- 0.25 g Mg2SO4
- 0.25 g K2HPO4

c) Inoculation of termite crush and purification of growing fungi isolates

We inoculated termite samples on the Molisch's agar medium under the laboratory fume hood 24 hours after the medium preparation. The termite crush, withdrew from the freezer, was left for some minutes under the fume hood to defrost. We always worked near the flame of the Bunsen burner to avoid aerial fungal contagion. We used a Pasteur pipette to take samples and inoculate on the medium. The pipette was sterilized with fire and alcohol 95% after each inoculation.

For each plastic zip bag, we carefully opened the bag and used the Pasteur pipette to take small quantities of termite crush which were immediately spread out on the top the Molisch's agar medium. A plastic zip bag contain was inoculated on two different petri dishes. Each petri dish was then sealed using a parafilm and labelled per termite species and per system. Concerning termite cadavers, any cadaver presenting fungal growth at its surface was collected using a sterile Pasteur pipette and inoculated on a single petri dish containing Molisch's agar medium. The petri dish was then sealed and labelled as above. After inoculation, the petri dishes were stored in a cool place at ambient temperature and with a photoperiod of 12L/12D.

From the 3rd day after inoculation, we started the purification of fungi isolates growing on culture medium, especially fast growing isolates. The slow growing isolates were progressively retrieved until the 10th day after inoculation. The fungi purification was done under the fume hood and consisted to delicately separate each growing fungal colony into new petri dishes. We used a sterile scalpel to cut a small area of medium around the growing fungal isolate (about 0.25 cm^2) which was aseptically inoculated on a sterile medium. After growth on the new petri dish, if another fungi isolate grew in contact with the purified isolate, we then purified the petri dish again until we have only one fungi isolate in each petri dish. The pure petri dishes were labelled per termite species, per system and per isolate.

II.2.5.1.2. Morphological characterization of fungi isolates

The morphological characterization of fungi isolates followed the method described by Gaddeyya *et al.* (2012). The fungal morphology was studied macroscopically by observing the colony features (color, shape, size and hyphae), and microscopically using a compound microscope with a digital camera where a small portion of the mycelium was slide mounted in a drop of sterilized water. The microscopic features observed were the color and septum of the hyphae, the shape and size of spores, the structure of sporulating formations. We used the recorded features to morphologically identify fungi isolates to the generic/specific level using identification keys of Rieuf (1985), Samson *et al.* (1988), Watanabe (2002) and Humber (2012). After morphological characterization, pieces of media with fungal mycelia were put in Epperndorf tubes containing glycerol 10% and conserved at -20°C for molecular steps.

II.2.5.1.3. Molecular identification of fungi isolates

a) DNA extraction

Fungal cultures were realized on potato dextrose agar (PDA) and sometimes on potato dextrose broth (PDB) liquid medium following the growth effectiveness on PDA. The DNA extraction followed a modified CTAB buffer protocol for plant leaves (Doyle & Doyle, 1990) after mechanistic disruption of fungi cells using sterile glass spheres (Oliveira *et al.*, 2012). After preheating CTAB lysis buffer (2 M Tris HCl at pH 8, 0.7 M NaCl, 0.5 M EDTA, 13.7 mM CTAB and 1% w/v PVP-40) at 65°C in a hot-bath water, 500 µl of CTAB + one sterile glass sphere were introduced in a 2 ml-Eppendorf tube. Under a laminar flow hood, fungi cultures of 2 – 3 week growth were put in tubes containing preheated CTAB + spheres using a sterile scalpel and vortexed a few seconds. We then added 1000 µl of CTAB to the mixture and vortexed again on a Bead Blaster^{TM} 24 for 2 min to crush spores. The crushed mixture was incubated at 65°C in a hot-bath water for 1 hr and gently agitated every 15-20 min. The crushed mixture was then centrifuged at 20,000 rcf during 10 min. We transferred 700 µl of the supernatant to a 1.5 ml-tube, added CIA (chloroform – Isoamyl acid) in proportion 24:1 and we vigorously shook the mixture for 5 min. The mixture was then centrifuged at 20,000 rcf for 10 min. We transferred 500 µl of the supernatant to another 1.5 ml-tube, added again 500 µl of CIA 24:1 and we vigorously shook for 5 min. The mixture was centrifuged again at 20,000 rcf for 10 min. We transferred again 400 µl of the supernatant to a new 1.5 ml-tube, added 400 µl of cold isopropanol and we gently mixed together to allow nucleic acids to precipitate. The mixture was incubated at – 20°C for 30 min and centrifuged at 20,000 rcf for 10 min. We poured the supernatant, added 500 µl of ethanol 70% and left to stand for 20 min to clean the DNA pellets. The mixture was then vortexed for a few seconds and centrifuged at 20 rcf for 10 min.

We carefully poured the supernatant. The formed DNA pellets were air-dried for 2 hrs at room temperature, re-suspended in 50 µl of sterile water and conserved at – 20°C (Figure 11).

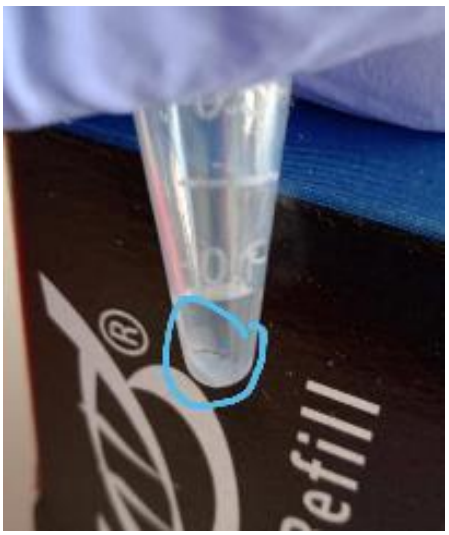


Figure 11. DNA pellet re-suspended in 50 µl of sterile water (Photo ©Djuideu, 2021)

b) DNA amplification through PCR

Molecular identification was achieved by amplification of the internal transcribed spacer region (ITS), using the universal primers ITS1 and ITS4 (White *et al.*, 1990)(White *et al*., 1990). PCR reactions (25µl) comprised 5 µl (50 ng) of genomic DNA, 0.2 µM of each primer (ITS1 and ITS4), 1× GoTaq® Flexi buffer (Promega), 2 mM MgCl2 (Promega), 2 mM dNTP Mix and 1 U GoTaq® DNA polymerase (Promega). Amplifications were carried out in the thermocycler Eppendorf® using a temperature gradient protocol as follows: initial denaturation at 94°C for 3 min, followed by 35 cycles of 0.5 min at 94°C, 0.5 min at 55°C, 1 min at 72°C and a final 10-min extension at 72°C. PCR products were detected by gel-electrophoresis in a 1% TAE-agarose gel, stained with ethidium bromide and visualized under UV light. When PCR did not worked with total DNA, we performed successive dilutions at 1/5th, 1/10th, 1/25th, 1/50th, 1/100th and 1/250th, 1/500th and 1/1000th with unsuccessful samples.

The DNA band length was expected between 500 bp and 600 bp (White *et al.*, 1990). We performed enzymatic digestions in RFLP with PCR products of similar isolate clusters. For each sample, we used 1 µl of each of the 3 digestion enzymes (EcoRI, NdeI and SpeI), 4 µl of enzyme buffer and 5 µl of PCR product in 20 µl of reaction volume. The mix was incubated at 37°C for at least 3 hours in the thermocycler. The incubated mix migrated by gel-electrophoresis in a 2.5% TAE-agarose gel, stained with ethidium bromide and visualized under UV light.

c) DNA sequencing and fungi identification

After obtaining PCR amplifications from our samples, we selected the best amplicons per samples based on the brightness of the bands on electrophoresis gel (in comparison with the DNA ladder 1kb). Amplified fragments were sequenced using both ITS1 and ITS4 primers with a DNA sequencer by Genewiz lab in Germany. After receiving the sequences, we first checked the quality of sequences by analysing the chromatogram curves using software BioEdit 7.2.5 (Hall, 1999) and sequences with good "curves" were kept for next steps (Figure 12). The sequences with acceptable quality level was then trimmed forward and/or backward to clean initiating and/or ending sequences with low quality. The resulting cleaned sequences (length from 400 to 550 bp) were then compared with existing fungal sequences for identification using the NCBI GenBank® database and BLAST algorithm (Meyling, 2007; Oliveira *et al.*, 2012).

After identification of isolates, we grouped fungi species according to their ecological roles in plants and in insects. In plants, we identified three ecological roles of fungi: **pathogens** (inducing pathologies to cultivated and non-cultivated plants), **neutrals** (colonizing the intercellular or intracellular spaces of plants without any advantage or inconvenient from the association), **beneficials** (colonizing plant cells with notable benefits from the association) (Stone *et al.*, 2004; Trillas & Segarra, 2009; Campbell & Johnson, 2013). Regarding insects, we grouped identified fungi into three ecological roles: **entomopahogens** (inducing pathologies to insects leading to death), **saprophytes** (present in insect body but do not inducing any advantageous or negative effect from the association when the insect is alive, but feed on cadavers), **symbionts** (close relationship with insect host in which both parts gain from the association) (Humber, 2012; Blackwell, 2017).

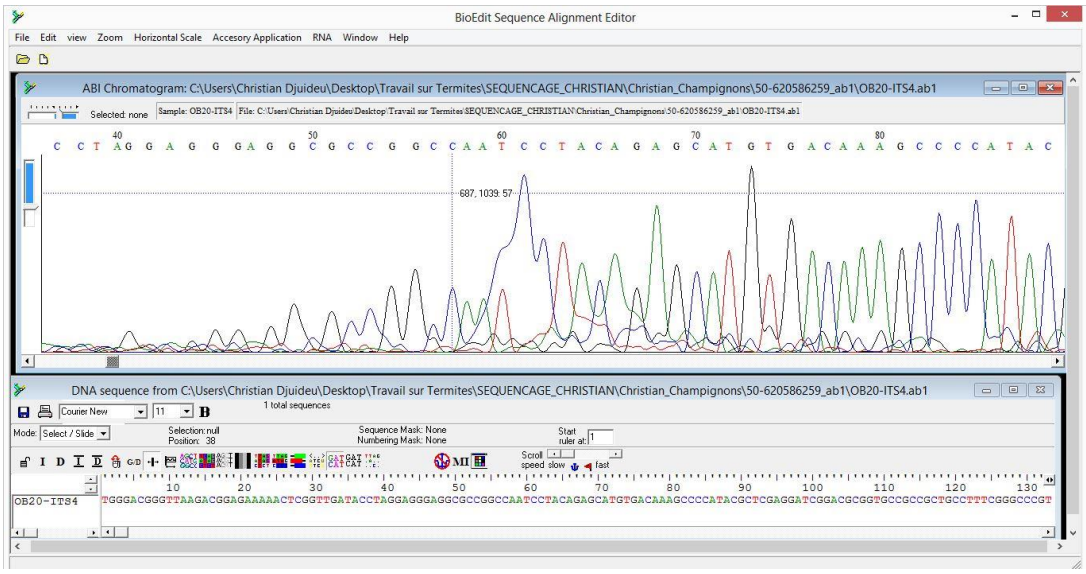


Figure 12. Analysis of sequence chromatogram curves using software BioEdit 7.2.5

II.2.5.2. Screening of parasitic mites associated with termites

a) Isolation of mites from termite colonies

In the second group of termite samples, we first isolated roaming, disconnected or predator mites within the specific colony of termites that we found after breaking a dead wood in a plate or a petri dish. We then selected randomly 100 individuals of termite per species to examine hooked mites under stereo microscope. We assumed that the mites associated with termites will remain on the termite body after the termites will be transferred to the laboratory (Wang *et al.*, 2002). Each individual of termite was examined at five infestation sites on the body which are strongly linked to the pathogenicity of the mites: head capsule, thorax, abdomen, legs and antennas. Where mites were found, we used an entomological needle to delicately disconnect them off the termite body and introduced them in an Eppendorf tube containing 70% alcohol. The samples of mites were then separated per infestation site, termite species and site.

b) Mounting medium and identification of mites

The mounting procedure of mites followed the protocol of Krantz & Walter (2009) consisting of lightening mite cuticle and slide mounting it in a HPVA (Heinze polyvinylic alcohol) medium. For the lightening of mite cuticle, mite specimens were grouped in morphospecies on the basis of their morphological observation under a stereo microscope ×45 and 5 individuals of each morphospecies were introduced in Eppendorf tubes containing lactic acid 85% to reduce mite weight and dissolve soft tissue. Specimens maintained in lactic acid for 1-2 days. After their stay in lactic acid, specimens were then cleaned with distillated water and prepared for mounting.

<u>The HPVA mounting medium composition (Krantz & Walter, 2009)</u>:

- 10g Polyvinylic alcohol
- 100 g Choral hydrate
- 30 ml Glycerol
- 35 ml Lactic acid 85%
- 60 distillated water

For the preparation of the HPVA medium, we introduced 10 g of polyvinylic alcohol crystals in 60 ml distillated water in an Erlen-Meyer. The Erlen-Meyer was brought to hot-water bath and circularly stirred until complete dissolution of crystals. Then, we added 35 ml of lactic acid to the mixture and stirred the whole for a few minutes. While stirring until the

mixture foams, we added 30 ml of glycerol and 100 g of Choral hydrate crystals. We continued to stir the mixture in hot-water bath until dissolution of all crystals. The mixture was cooled and transferred in a glass bottle.

For the slide mounting, we placed 2 specimens belonging to the same morphospecies on a slide, the first on the ventral view and the second in dorsal view. Using a syringe, a drop of the HPVA medium was deposited on the specimens and they were covered with a microscope slide. The identification of mites was realized using dichotomous keys of Krantz & Walter (2009) until the family level following specific characters (Figure 13). For the identification until the species level, we followed the idiosomal chaetotaxy from Griffiths *et al.* (1990), solenidiotaxy from Griffiths (1970) and the terminology of coxisternal setae from Norton (1998). We also used the identification keys of Eraky *et al.* (2019), Sarwar & Ashfaq (2012), Fakeer *et al.* (2014), Sarwar *et al.* (2015) and Klimov & Khaustov (2018).

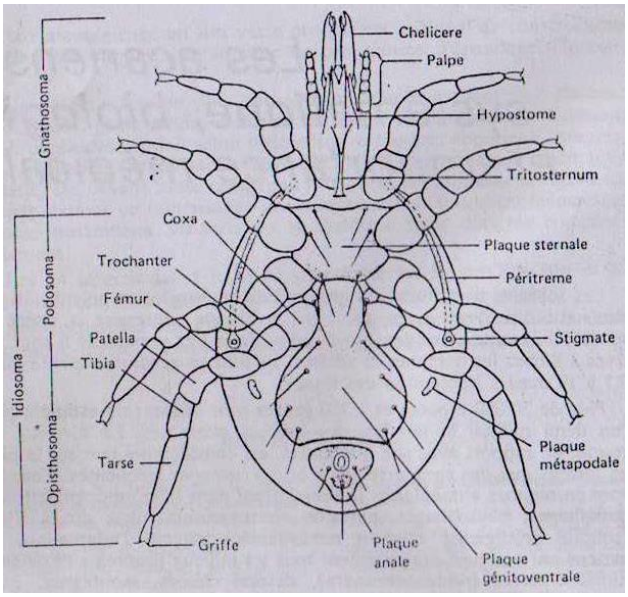


Figure 13. General morphology of mite with diagnosis characters (From Rodhain & Perez, 1985)

II.2.5.3. Screening of parasitoid flies associated with termites

a) Isolation of emerging flies

The third group of termites was isolated in humid chamber to encourage the emergence of parasitoid flies. Two batches of 100 individuals of each termite specie per farm were carefully extracted from wood or nest and placed in a plastic container filled at 2 cm depth of moistened vermiculite + sand mixture (v/v) per batch. We perforated the coverlid of containers and added a fine mesh (0.1 mm diameter) to allow air to circulate meanwhile preventing flies to come out.

Mature larvae leaving their host buried into the vermiculite and pupariate (Sze *et al.*, 2008). The container was verified for emergence every day, and we finely watered it every two days for 20 days to avoid desiccation to kill both termites and flies. When a fly emerged in a container, we soaked a small piece of cotton with etheroxide that we placed at the top of the container for 1 – 2 min to knock out the flying insect without killing it. The fly was then placed in a 5 ml tube containing alcohol 70% and labelled per termite species and date.

b) Identification of emerging flies

❖ **Morphological identification**

The different specimens of flies that emerged from termites were grouped into morpho-species. The identifications were made through observation under a binocular stereo microscope AmScope, mainly based on the morphological characters. The identification key of Delvare & Aberlenc (1989) was used to identify all flies to the family level. For identification to the genus and species levels, we first separated the collected specimens into male and female clusters using key of Mayr (1991) under a stereo microscope. The identification keys of Disney (1994) and Disney *et al.* (2013), as well as the collection of new morphological characters of the structure of the thorax for the classification of Phoridae by Brown *et al.* (2015), were used to identify the genera and species of parasitoid flies.

Figure 14. A parasitoid fly isolated from termites under a binocular stereo microscope for identification (Photo ©Djuideu, 2021)

❖ **Molecular identification**

- **DNA extraction of fly.** The DNA extraction of fly followed a modified protocol of whitefly DNA extraction from Frohlich *et al.* (1999). One individual of each fly sample was placed on a section of parafilm wrapped around a petri dish and grinded in 60 µl (grinded on

10 µl and adding 50 µl) of ice-cold lysis buffer (5 mM Tris-HCl pH 8, 0.5 mM EDTA pH 8, 0.1% Triton X and 1 mg/ml Proteinase K) using a micropestle. The extract was then incubated at 65°C for 15 min and then at 95° for 10 min. We then centrifuged the extract at 13,000 rcf for 5 min to pellet debris at the bottom of the microtube. We transferred the supernatant to a new microtube and stored at – 20°C for future experiments.

- **PCR amplification of fly DNA.** Amplification of the COI barcoding region was performed using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994; Boehme *et al.*, 2010). Amplification was performed in a total reaction volume of 25 µl containing 1 unit/µl of Taq DNA polymerase, 0.4 mM of each primer, 5 µl of 5X Gotaq buffer, 0.2 mM of dNTPs, 0.5% Tween 20 and 2 mM of MgCl2. 5 µl of the DNA extracts were used as template. All PCR amplifications were performed in a thermal cycler. The thermal cycler program was the following: 1 min at 94°C followed by five cycles of 94°C for 1 min, 45°C for 1.5 min, and 72°C for 1.5 min followed by 35 cycles of 94°C for 1 min, 50°C for 1.5 min and 72°C for 1 min with a final extension step of 72°C for 8 min. PCR products were detected by gel-electrophoresis in a 1% TAE-agarose gel, stained with ethidium bromide and visualized under UV light. For unsuccessful DNA amplification in some samples, we re-performed the successive PCR with $1/3^{rd}$, $1/5^{th}$ and $1/10^{th}$ diluted DNA until visualization on gel. The DNA band length was expected at 710 bp (Folmer *et al.*, 1994).

- **DNA sequencing and fly identification.** After obtaining PCR amplifications from our samples, we selected the best amplicons per samples based on the brightness of the bands on electrophoresis gel (in comparison with the DNA ladder 1kb). Amplified fragments were sequenced using both LCO1490 and HCO2198 primers with a DNA sequencer by Genewiz lab in Germany. After receiving the sequences, we first checked the quality of sequences by analyzing the chromatogram curves using software BioEdit 7.2.5 (Hall, 1999) and sequences with good "curves" were kept for next steps. The sequences with acceptable quality level was then trimmed forward and/or backward to clean initiating and/or ending sequences with low quality. The resulting cleaned sequences (length from 400 to 650 bp) were then compared with existing fly sequences as done with fungal sequences.

II.2.6. Data analysis

II.2.6.1. Diversity indexes of termites in relation to shade management and climate

Sampling completeness of termites was evaluated with the first-order jackknife (S_{jack1}) estimator, using EstimateS (Version 9.1.0) (Colwell, 2013). The first-order jackknife estimator is the best estimator of nonparametric species richness (Basualdo, 2017) (6). The sampling completeness was estimated through the ratio of species richness obtained by the theoretical

species richness of the first-order jackknife estimator. To compare species richness between systems, we constructed rarefaction curves by randomly simulating 100 curves based on the initial data from each tree.

$$\mathbf{S_{jack1} = S_{obs} + Q_1\left(\frac{m-1}{m}\right)} \quad [6]$$

S_{Jack1}: Expected species richness; S_{obs}: Observed species richness; Q_1: number of species in only one sample; m: number of samplings carried out in the site.

We computed the Shannon index of diversity *H'* (Colwell & Huston, 1991) (7), the Pielou index of equitability *J* (Shannon & Weaver, 1949) (8) and the Berger-Parker index of dominance *d* (Magurran, 1988) (9) to examine α –diversity of termites communities within system, using package « vegan » in R (R Core Team, 2013). To determine the similarity of termites' communities among systems, we performed an ordination analysis based on the Non-metric Multidimensional Scaling (NMDS) with Bray-Curtis as similarity index to compare the species distribution on cocoa trees between systems. One-way ANOVA was used to compare weighted HS between systems.

$$\boldsymbol{H' = -\sum_{i=1}^{S} P_i log_2 P_i} \quad [7]$$

S: Species richness, Pi: relative abundance of species i. The Shannon index varies from 0 (null diversity) to $\log_2 S$ (maximal diversity), $0 \leq H' \leq \log_2 S$.

$$\mathbf{J = \frac{H'}{H'_{max}}} \quad [8]$$

J: Pielou's equitability index; *H'*: Shannon diversity index; H'_{max}: maximal diversity *H'* for a community with the same species richness.

$$\boldsymbol{d = \frac{N_{max}}{\sum Ni}} \quad [9]$$

d: Berger-Parker's index; N_{max}: abundance of the most abundant species; Ni: abundance of the species i. This index varies from 0 (no dominance in the community) to 1 (total dominance of only one species).

We further used Generalized Linear Models (GLMs) to assess the effect of shade management, air temperature and R. H. on the abundance of pest species of termite using SPSS (version 20). This analytical method is more powerful than frequently used non-parametric tests (i.e. Kruskal-Wallis' test) which are exempt from the normality assumptions of data and errors distribution. GLMs take into account data distributions which follow generally the Poisson distribution like count data, as it is the case in this study (abundance and species richness) (O'Hara & Kotze, 2010). The power of GLMs obtained per each factor was statistically tested

using an Analysis of Variance (ANOVA). When the probability threshold was reached ($P < 0.05$), the Tukey Post hoc test was then performed for pairwise comparison between systems and seasons. Means of shade cover, tree species richness, aboveground and belowground termite species richness were compared between systems using the Kruskal-Wallis' test. When significant differences were found, a Mann-Whitney's test was then used for pairwise comparison between systems. Temperature and relative humidity were compared between seasons using the Student t-test. We also performed a factorial analysis of correspondence to assess termite species preference within agroforestry systems and highlight endemic species.

II.2.6.2. Relationship between disservices provided by termites and shade management

We used the chi-square test (χ^2) to assess the relationship between seasonal variations and the proportion of infested trees in plantations, while the Spearman ρ coefficient was used to assess the relationship between the shade cover and the infestation rate in plot, and between the infestation (presence of termites on a tree) at different sampling categories (galleries, dead branches, arboreal nests and roots). We performed a linear regression model analysis with "termite densities" as dependent variable and "shade cover" as independent variable to assess the effects of shade level on aboveground (galleries) and belowground (roots) termite densities on cocoa trees, independently of localities surveyed. The mean termite density on cocoa trees at each infestation site (belowground vs aboveground) was compared between CAFS using a Kruskal-Wallis' *H* test. When a statistical difference was found at $P < 0.05$, we then computed a Mann-Whitney's *U* test for pairwise comparison between CAFS. The *P*-values obtained were adjusted using the Bonferroni's correction to avoid the error type I induced by multiple comparisons. Statistical analyses were performed using SPSS 20 (SPSS Inc., 2011).

II.2.6.2.1. Dispersion models of termites identified as pests to cocoa agroforestry systems

We used and compared three common methods to describe the dispersion pattern of termites in the study sites:

- **Taylor's Power Law (10)**. This model states that the variance (S^2) of a population is proportional to a fractional power of the arithmetic mean (m): $\boldsymbol{S^2 = am^b}$. m (mean densities of termites per tree) and S^2 (variances) were calculated for trees in each plot for each plantation and season, and the variance-mean relationship was quantified using the Taylor's power law (Taylor, 1961, 1971; Taylor *et al.*, 1978). To estimate a and b, the values of ln (S^2) were regressed against those of ln (m) using the following formula:

$$\boldsymbol{ln\,(S^2) = ln\,(a) + b ln\,(m)} \quad [10]$$

where the parameter *a* is largely a sampling factor related to sample unit size and *b* the slope. The parameter *b* is an index of aggregation that indicates a uniform, random, or aggregated dispersion when $b < 1$, $b = 1$, or $b > 1$, respectively (Southwood, 1978).

- **Nachman Model (11)**. This model describes the relationship between the sample mean and the proportion of non-infested sampling units by the specific insect pest. We determined the goodness-of-fit of our data to this model. It reduces the time required for monitoring by using incidence counts (presence or absence) instead of direct counts. This relationship is between the mean density (*m*) and the proportion p_0 statistical units with no termites:

$$p_0 = \exp(-fm^g) \qquad [11]$$

where *f* is a scale parameter and *g* is a dispersion parameter of the model. If $f = g = 1$, the distribution of individuals is random. The model is linearized with the mean density regressed on p_0 (Nyrop *et al.*, 1989) as follows (12):

$$ln(m) = A + B ln(-ln(p_0)) \qquad [12]$$

where p_0 is the proportion of non-infested trees in each plot. When a stable relationship is observed between p_0 and *m*, the regression curve is used to forecast the values of *m* from p_0.

- **Iwao's Method (13)**. The Iwao's patchiness regression method quantifies the relationship between the mean crowding index (m^*) and the mean density of pest (*m*) using the following formula:

$$m^* = \alpha + \beta m \qquad [13]$$

where m^* was determined as $[m + (S^2/m - 1)]$ (Lloyd, 1967). The intercept (α) is the index of the basic component of a population or basic contagion (where $\alpha <, =,$ and > 0 represent regularity, randomness, and aggregation of populations in spatial patterns, respectively), and the slope (β) is the density contagiousness coefficient interpreted in the same manner as *b* of Taylor's regression ($\beta < 1$, $\beta = 1$, or $\beta > 1$ for a uniform, random, or aggregated dispersion respectively).

II.2.6.2.2. Optimal sampling plans for termites

Based on the sample counts, we simulated the optimal sampling plans for termites using Southwood's sampling program and Green's sequential sampling. These sampling plans were calculated using parameters of Taylor's Power Law, the best fitted model to describe termite dispersion model.

- **Southwood's sampling program (14).** We estimated the optimal sample sizes (*n*) to develop standard sampling plans with precision levels of 0.10, 0.15, and 0.25 for ecological and pest management purposes, respectively, as suggested by Southwood (1978):

$$n = am^{(b-2)}/D^2 \qquad [14]$$

where *a* and *b* are Taylor's power law coefficients, *m* the termite density and *D* is the desired precision. Precision measures the degree of error in making population estimates and was expressed as a proportion standard error of the mean.

- **Green's sequential sampling (15).** We also developed sampling recommendations using Green's formula (Green, 1970). This formula generates the sampling stop line (*Tn*) for a given precision level as follows:

$$Tn = \left[\frac{D^2}{a}\right]^{1/(b-2)} n^{(b-1)/(b-2)} \qquad [15]$$

where *a* and *b* are Taylor's power law coefficients, *D* is the desired precision and *n* the number of cocoa trees as sampling units. T_n refers to a cumulative count of termite density on cocoa trees. This stop line intercepts the curve of expected cumulative density of termites at the point where the sampling should be stopped.

II.2.6.3. Relationship between termite disservices, shade management and yield

We assessed the effect of shade management on yield by comparing yield parameters on cocoa trees, harvested pods and beans between agroforestry systems using a Univariate GLM test (F). To assess the combined effect of shade reduction and infestation on yield, we compared yield parameters between agroforestry systems and infestation using a Multivariate GLM test (F). When significant differences were found at 0.05 threshold, a Tukey HSD post-hoc test was then computed for pairwise comparison between groups. We also compared the yield parameters per infestation level to assess whether a given level has more impact on tree productivity than another a Kruskal-Wallis test (H). When significant differences were found at 0.05 threshold, a Mann-Whitney test was then computed for pairwise comparison between infestation levels. All data were analyzed using SPSS 20 software (SPSS Inc., 2011) and all probabilities were appreciated at 0.05 threshold.

II.2.6.4. Relationship between shade management and biological control agents of termites

II.2.6.4.1. Analysis of parasitism parameters of parasitic mites and parasitoid flies

We determined the parasite rate, the total parasite abundance and the mean parasite density per termite of associated mites in regards to shade management, species of termite and season. We did the same for the parasite rate, the number of emerging flies and the sex ratio of parasitoid flies. We assumed that there was only one fly larvae parasitizing a termite (Sze *et al.*, 2008). A chi-square test (χ^2) was performed to assess differences in parasitism rate between systems, termite species and seasons. A z test for proportions was subsequently applied for pairwise comparisons of parasitism rate between systems, termite species and seasons. We also performed a Kruskal-Wallis test (H) to compare mean parasite density/mean emerging flies between systems and termite species. It was followed by a Mann-Whitney pairwise comparisons to assess differences between systems and termite species, the values of the test were corrected using a Bonferroni's correction formula. A Mann-Whitney test (U) was also performed to compare mean parasite density/mean emerging flies between seasons. A binary logistic regression was performed to assess the odds of termite infestation by mites and parasitoid flies in regards of different shade management, termite species and seasons.

II.2.6.4.2. Diversity and habitat-host preference of parasitic mites and flies

We computed the Shannon index of diversity *H'* (Colwell & Huston, 1991) (7), the Pielou index of equitability *J* (Shannon & Weaver, 1949) (8) and the Berger-Parker index of dominance *d* (Magurran, 1988) (9) to examine α –diversity of parasite and parasitoid communities within agroforestry system, using package « vegan » in R (R Core Team, 2013). The host preference and habitat preference of each species of parasite and parasitoid were examined by performing the correspondence analysis with confidence ellipse using the package « FactoMiner » in R R (R Core Team, 2013). The model of each correspondence analysis was test for significance using a Chi-square test in R.

II.2.6.5. Phylogenetic analysis of fungi and parasitoid flies and evaluation of genetic distances

After cleaning DNA sequences using BioEdit 7.2.5 (Hall, 1999) and retrieving identity of different isolates and specimens in NCBI genebank, we aligned all the sequences in the software MEGA 11 (Tamura *et al.*, 2021). Sequences of close species of each sample were downloaded from NCBI genebank (accessions) and added to the alignment for comparison purposes. The algorithm MUSCLE was applied to sort out optimal alignments across sequences

(Edgar, 2004) and we discarded tips of singular sequences at the beginning and the end of aligned sequences. After DNA alignments, we then compute genetic distance matrix using the number of base differences per sequence and The Jukes-Cantor model. All ambiguous positions were removed for each sequence pair (pairwise deletion option). For the protein coding DNA of fly mitochondrial COI, Codon positions included were 1st+2nd+3rd+Noncoding. The Jukes-Cantor model computed the probability of substitution between 2 sequences and determined at which extent the two sequences can be related in the evolution process (Jukes & Cantor, 1969).

Prior to the construction of phylogenetic tree, we first evaluate the best construction model among all (Jukes-Cantor, Kimura-2-parameter, Tamura-3-parameter, Hasegawa-Kishino-Yano, Tamura-Nei or General Time Reversible), the type of data distribution (Gamma distributed or not) and the presence or not of invariant sites using Modeltest in Mega 11. The best combination of parameters was selected based on the value of BIC and AIC values. The tree was then constructed and rooted to an out-group, selected from its relatedness with the focus group. In the case of fungi phylogeny, we rooted the tree at midpoint. Midpoint rooting calculates tip to tip distances and then places the root halfway between the two longest tips (Hillis *et al.*, 1996). We constructed and compared phylogeny from two types of trees: a cladogram based on the Maximum Likelihood method that expresses phylogenetic relationships between species through analysis of synapomorphies (derived characters shared by different species from a common ancestor); a phenogram based on the Neighbor-joining method that expresses phylogenetic relationships between species through the calculation of interspecific genetic distances (Darlu *et al.*, 2019).

CHAPTER III: RESULTS AND DISCUSSION

III.1. Results

III.1.1. Diversity of termites associated with cocoa and relationships with shade management and climate variability

III.1.1.1. Taxonomic composition of termites associated with cocoa trees

We recorded 54 termite species belonging to three families and nine subfamilies (Table II). In all cocoa agroforestry systems, the family Termitidae was the most diversified with 6 subfamilies and 51 species. The families Kalotermitidae and Rhinotermitidae were represented by one and two species respectively. The main subfamilies recorded were Apicotermitinae (21 species), Macrotermitinae (12 species), Cubitermitinae (8 species) and Termitinae (6 species). The other subfamilies were constituted of 3 or less species. *Microtermes osborni* was the most encountered species recorded on 449 trees (37.4%). The second most encountered species *Microcerotermes progrediens* was found on 170 trees (14.2%) and the third *Ancistrotermes cavithorax* on 127 trees (10.5%).

From the 54 termite species identified, 38 (70.4%) were exclusively sampled in soil at the basis of the cocoa stand and 12 (22.2%) were sampled both on wooden part of the cocoa tree and in the soil at its basis. Only four species (7.4%) – *Neotermes* sp., *Nasutitermes diabolus*, *Microcerotermes fuscotibialis* and *Microcerotermes sylvestrianus* – were found exclusively on the wooden superficial parts of the cocoa tree.

Table II. Termite species composition and number of associated cocoa trees per species and per system

Termite species	Feeding group	Sampling level	Number of trees per system					Total
			Rustic	Heavy shaded	Intermediate	Low shaded	Full sun	
Kalotermitidae Froggatt, 1897								
Kalotermitinae Froggatt, 1897								
Neotermes sp. Holmgren, 1911*	I	W	2	-	-	-	-	2
Rhinotermitidae Froggatt, 1897								
Coptotermitinae Holmgren, 1910								
Coptotermes sjoestedti * Holmgren, 1911	I	S	1	-	-	-	-	1
Rhinotermitinae Froggatt, 1897								
Schedorhinotermes putorius * (Sjöstedt, 1896)	I	W/S	1	2	-	-	-	3
Termitidae Latreille, 1802								
Apicotermitinae Grassé and Noirot, 1955								
Acholotermes epius Sands, 1972	III	S	3	1	1	-	-	5

Acholotermes sp. Sands, 1972	III	S	1	-	-	-	-	1
Adaiphrotermes choanensis (Fuller, 1925)	III	S	16	5	-	-	-	21
Adaiphrotermes cuniculator Sands, 1972	III	S	-	2	-	-	-	2
Aderitotermes cavator Sands, 1972	III	S	1	-	-	-	-	1
Aderitotermes fossor Sands, 1972	III	S	3	-	2	-	1	6
Alyscotermes kilimandjaricus (Sjöstedt, 1907)	III	S	1	-	-	-	-	1
Alyscotermes trestus Sands, 1972	III	S	-	-	-	2	-	2
Amalotermes phaeocephalus Sands, 1972	III	S	-	1	3	-	-	4
Amicotermes galenus Sands, 1972	III	S	2	-	-	-	-	2
Amicotermes dibogi Sands, 1999	III	S	-	-	1	-	-	1
Amicotermes camerunensis Sands, 1999	III	S	1	1	1	-	-	3
Anenteotermes hemerus Sands, 1972	III	W/S	-	1	-	-	-	1
Anenteotermes nanus (Sjöstedt, 1911)	III	S	1	-	-	-	-	1
Anenteotermes polyscolus Sands, 1972	III	S	6	25	4	1	1	37
Apagotermes stolidus Sands, 1972	III	S	-	1	-	-	-	1
Astalotermes empodius Sands, 1972	III	S	2	-	1	-	-	3
Astratotermes aneristus Sands, 1972	III	S	1	-	-	-	-	1
Astratotermes pacatus (Silvestri, 1914)	III	S	-	1	-	-	-	1
Ateuchotermes retifaciens Sands, 1972	III	S	1	8	-	-	-	9
Jugositermes sp. Emerson, 1928	III	S	2	-	-	-	-	2
Cubitermitinae Weidner, 1956								
Basidentitermes malelaensis (Emerson, 1928)	IV	S	4	3	-	-	-	7
Cubitermes antennalis Sjöstedt, 1924	IV	S	1	-	-	-	-	1
Cubitermes bilobatus (Haviland, 1898)	IV	S	2	-	-	-	-	2
Fastigitermes jucundus (Sjöstedt, 1907)	III	S	-	2	-	1	-	3
Nititidermes sp. Emerson, 1960	IV	S	1	-	-	-	-	1
Proboscitermes tubuliferus (Sjöstedt, 1907)	III	S	1	-	-	-	-	1
Procubitermes undulans Schmitz, 1917	III	W/S	1	-	-	-	-	1
Profastigitermes putnami Emerson, 1960	III	S	-	2	-	-	-	2
Macrotermitinae Kemner, 1934								
Ancistrotermes cavithorax * (Sjöstedt, 1899)	II (F)	W/S	22	4	26	11	64	127

Ancistrotermes crucifer * (Sjöstedt, 1897)	II (F)	S	-	-	1	2	-	3
Ancistrotermes wasmanni * *Snyder and Emerson, 1949*	II (F)	S	3	1	5	1	2	12
Macrotermes bellicosus * (Smeathman, 1781)	II (F)	S	-	2	2	1	3	8
Microtermes feæ Silvestri, 1912 *	II (F)	W/S	30	66	9	-	1	106
Microtermes osborni * Emerson, 1928	II (F)	W/S	3	113	132	110	91	449
Microtermes pusillus * Silvestri, 1914	II (F)	W/S	43	8	5	6	3	65
Odontotermes fulleri * (Emerson, 1928)	II (F)	S	-	-	-	1	-	1
Odontotermes mukimbunginis * Sjöstedt, 1924	II (F)	S	-	5	-	2	1	8
Protermes prorepens (Sjöstedt, 1907)	II (F)	S	-	2	4	-	-	6
Pseudacanthotermes militaris * Hagen, 1858	II (F)	W/S	-	8	-	8	9	25
Synacanthotermes heterodon (Sjöstedt, 1899)	II (F)	S	2	-	-	-	-	2
Nasutitermitinae Hare, 1937								
Nasutitermes arborum * (Smeathman, 1781)	II	W/S	-	2	1	28	9	40
Nasutitermes diabolus * (Sjöstedt, 1907)	II	W	1	-	-	-	-	1
Verrucositermes tuberosus Emerson, 1960	III	S	1	-	-	-	-	1
Sphaerotermitinae Engel and Krishna, 2004								
Sphaerotermes sphaerothorax (Sjöstedt, 1911)	II	W/S	1	8	-	1	-	10
Termitinae Latreille, 1802								
Microcerotermes edentatus * Wasmann, 1911	II	W/S	94	11	-	-	-	105
Microcerotermes fuscotibialis * (Sjöstedt, 1896)	II	W	1	-	-	-	-	1
Microcerotermes progrediens * Silvestri, 1914	II	W/S	58	79	33	-	-	170
Microcerotermes silvestrianus * Emerson, 1928	II	W	3	1	-	-	-	4
Pericapritermes sp. Silvestri, 1914	III	S	2	-	-	-	-	2
Promirotermes orthoceps (Emerson, 1928)	III	S	-	1	-	-	-	1
Total trees sampled			**240**	**240**	**240**	**240**	**240**	**1200**

(*) Refers to pest termite species feeding on living plants; (F) refers to fungus-growing termites; W= wood only, S= soil only, W/S= wood and soil.

The rarefaction curves showed significant differences in termite diversity between systems (Figure 15). Rustic systems were the most diversified with 37 species (19 endemic

species to the systems), followed by heavy shaded systems with 29 (6 endemic species to the system), intermediate shade systems with 17 (1 endemic species to the system), low shaded systems with 14 (2 endemic species to the system) and full sun systems were the least diversified (11 species all found in other systems). We observed that five termite species (*Ancistrotermes cavithorax*, *Ancistrotermes wasmanni*, *Anenteotermes polyscolus*, *Microtermes osborni* and *Microtermes pusillus*) from the 54 identified were recorded in all the five cocoa agroforestry systems. Almost all beneficial termite species were strongly linked with rustic systems while main pest species of termites (*M. osborni, A. cavithorax and N. arborum*) preferred low shaded systems (Figure 16).

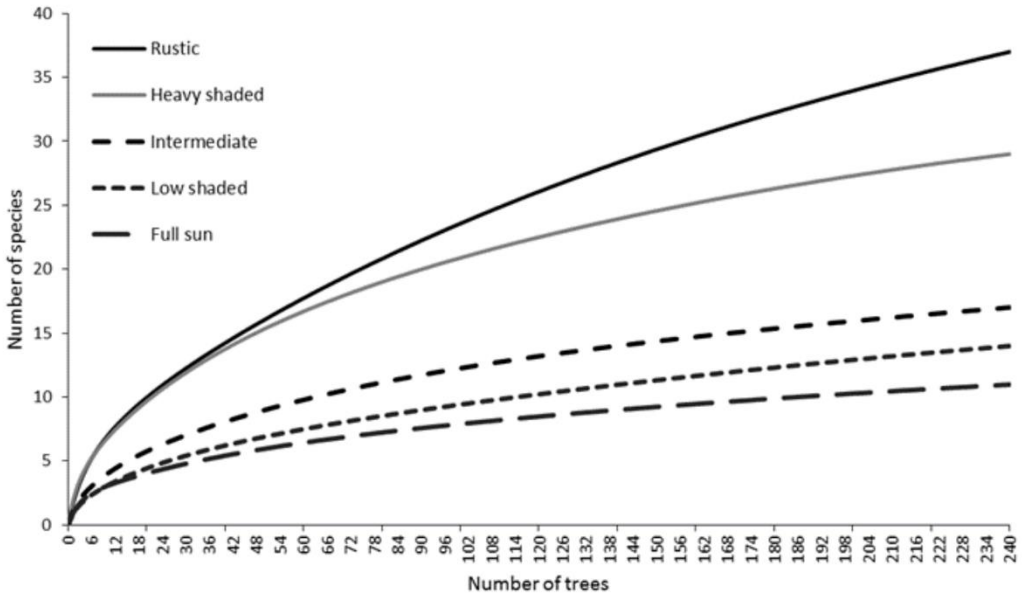


Figure 15. Rarefaction curves of termites sampled on cocoa trees in five cocoa agroforestry systems in southern Cameroon

With exception of the rustic systems, *Microtermes osborni* was the most dominant species in all the systems (found on 113 trees in heavy shaded systems, 132 in intermediate systems, 110 in low shaded systems and 91 in Full sun systems). The rustic systems were dominated by *Microcerotermes edentatus* (found on 94 trees) while *M. osborni* was found on only 3 trees. The value of theoretical richness obtained from the first-order Jackknife showed that the sampling covered a large proportion of termite species in each system (Table III). The diversity indices (Shannon, Equitability and Berger-Parker) showed a progressive reduction of termite diversity and community evenness and an increase of the populations of pest species with the reduction of shade cover (Table III). The rustic systems recorded the highest potential diversity as compare to the other systems based on Jackknife 1 estimator. The ordination analysis

(NMDS) showed high similarities in termite assemblage between low shaded, intermediate and full sun systems (Figure 17).

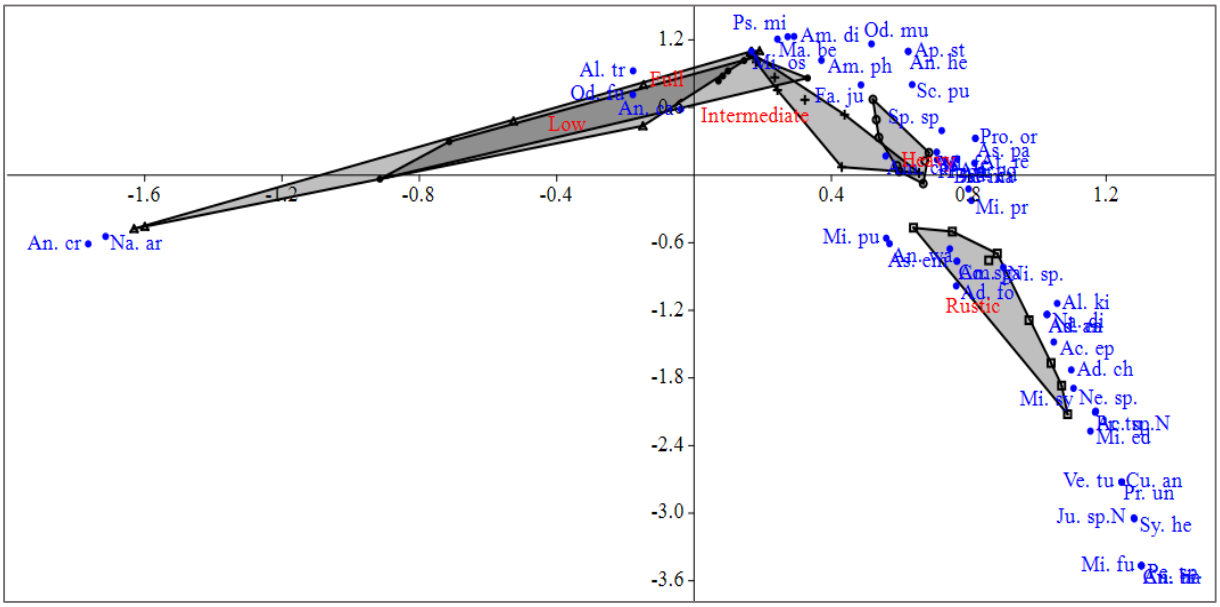


Figure 16. Factorial analysis of correspondence between termite species and AF systems showing endemicity and species preference. **Ac. Ep** *Acholotermes epius*, **Ac. Sp.N** *Acholotermes* sp., **Ad. Ch** *Adaiphrotermes choanensis*, **Ad. Cu** *Adaiphrotermes cuniculator*, **Ad. Ca** *Aderitotermes cavator*, **Ad. Fo** *Aderitotermes fossor*, **Al. ki** *Alyscotermes kilimandjaricus*, **Al. tr** *Alyscotermes trestus*, **Am. Ph** *Amalotermes phaeocephalus*, **Am. Ga** *Amicotermes galenus*, **Am. Di** *Amicotermes dibogi*, **Am. Ca** *Amicotermes camerunensis*, **An. Ca** *Ancistrotermes cavithorax*, **An. Cr** *Ancistrotermes crucifer*, **An. Wa** *Ancistrotermes wasmanni*, **An. He** *Anenteotermes hemerus*, **An. Na** *Anenteotermes nanus*, **An. Po** *Anenteotermes polyscolus*, **Ap. st** *Apagotermes stolidus*, **As. Em** *Astalotermes empodius*, **As. An** *Astratotermes aneristus*, **As. Pa** *Astratotermes pacatus*, **At. Re** *Ateuchotermes retifaciens*, **Ba. Ma** *Basidentitermes maleleansis*, **Co. Sj***Coptotermes sjoestedti*, **Cu. An** *Cubitermes antennalis*, **Cu. Bi** *Cubitermes bilobatus*, **Fa. Ju** *Fastigitermes jucundus*, **Ju. Sp.N** *Jugositermes* sp.**, Ma. Be** *Macrotermes bellicosus*, **Mi. Ed** *Microcerotermes edentatus*, **Mi. Fu** *Microcerotermes fuscotibialis*, **Mi. Pr** *Microcerotermes progrediens*, **Mi. Sy** *Microcerotermes sylvestrianus*, **Mi. Fe** *Microtermes feæ*, **Mi. Os** *Microtermes osborni*, **Mi. Pu** *Microtermes pusillus*, **Na. Ar** *Nasutitermes arborum*, **Na. Di** *Nasutitermes diabolus*, **Ne. Sp.** *Neotermes* sp., **Ni. Sp.** *Niditermes* sp., **Od. Fu** *Odontotermes fulleri*, **Od. Mu** *Odontotermes mukimbuginis*, **Pe. Sp.** *Pericapritermes* sp., **Pr. Tu** *Proboscitermes tubuliferus*, **Pr. Un** *Procubitermes undulans*, **Pr. Pu** *Profastigitermes putnami*, **Pro. Or** *Promirotermes orthoceps*, **Pr. Pr** *Protermes prorepens*, **Ps. Mi** *Pseudacanthotermes militatris*, **Sc. Pu** *Schedorhiotermes putorius*, **Sp. Sp** *Sphaerotermes sphaerothorax*, **Sy. He** *Synacanthotermes heterodon*, **Ve. Tu** *Verrucositermes tuberasus*.

Table III. Diversity indexes and non-parametric species richness estimator of termite communities within AF systems

Diversity indexes	Rustic	Heavy shaded	Intermediate	Low shaded	Full sun
Species richness S	37	29	17	14	11
Pest species (relative %)	13 (35.1%)	12 (41.4%)	9 (52.9%)	10 (71.4%)	9 (81.8%)
Shannon *H*	2.38	2.18	1.56	1.35	1.31
Equitability J	0.66	0.65	0.55	0.51	0.54
Berger-Parker d	0.29	0.31	0.57	0.63	0.49
S_{jack1}	54 (62.6%)	38 (76.3%)	23 (73.9%)	20 (70%)	15 (73.3%)

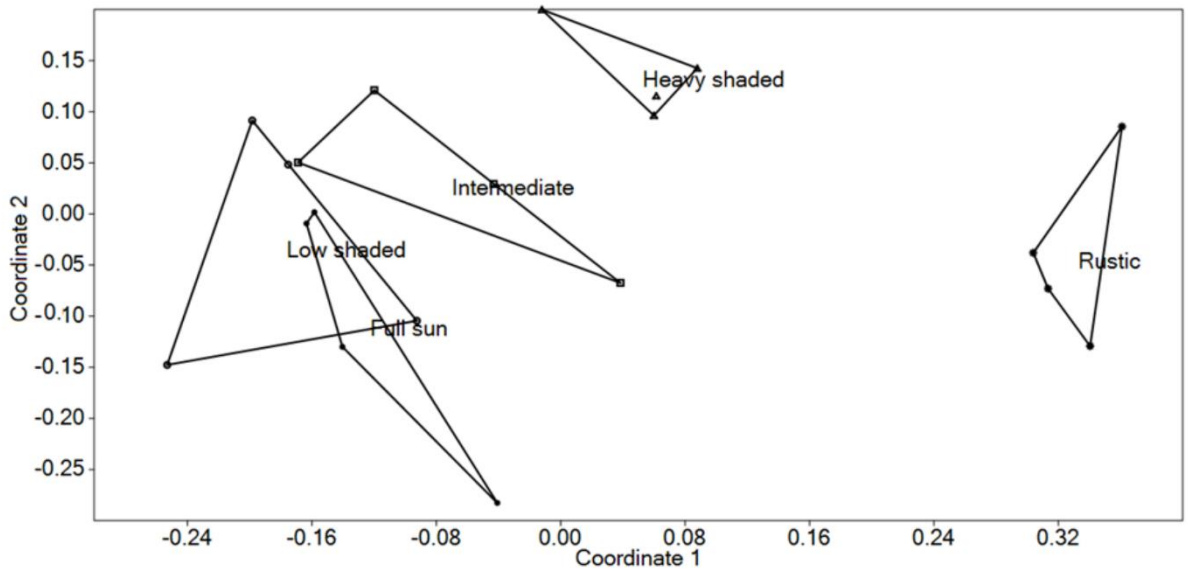


Figure 17. Graphic representation of non-Metric Dimensional Scaling (NMDS) ordination analysis showing similarities in termite assemblage between systems (Stress value = 0.1057)

III.1.1.2. Functional groups of termites and their response to land-use disturbance

We identified 19 termite species as pests of cocoa and 35 as non-pests from the 54 termite species recorded (Table II). We found that pest species of termites were more abundant than non-pest species in all the five systems (Table IV). The most encountered termite species on cocoa trees (*M. osborni*, *M. progrediens* and *A. cavithorax*) were all pests. We also noted that *Procubitermes undulans* and *Anenteotermes hemerus*, species described as soil feeders (non-pests), were causing damage to cocoa trees by building galleries running off the tree trunk. These changes in feeding habits were observed in rustic and heavy shaded systems (Table II). The number of cocoa trees attacked by termites increased in relation with the shade reduction. From the 240 trees sampled per system, rustic systems recorded the lowest number of attacked

trees (104 trees), followed by low shaded systems (144 trees) and full sun systems (145 trees). Heavy shaded (174 trees) and intermediate shade (170 trees) had the highest number of attacked trees.

Table IV. Termite species richness above and belowground per plot sampled in each system

Cocoa AFS	Shade cover (%)	Tree species richness	Aboveground species richness		Belowground species richness	
			Pest	Non-pest	Pest	Non-pest
Rustic	92.54 ± 2.43a	12 ± 1a	2.75 ± 0.32a	0.06 ± 0.05	3.5 ± 0.29ab	2.38 ± 0.45a
Heavy shaded	83.21 ± 1.42b	9 ± 1ab	2.75 ± 0.32a	0.13 ± 0.08	3.81 ± 0.37a	2.19 ± 0.44a
Intermediate	67.63 ± 5.28c	8 ± 1b	0.94 ± 0.19b	0.0 ± 0.0	2.88 ± 0.29ab	0.88 ± 0.22b
Low shaded	55 ± 5.71d	5 ± 1bc	0.38 ± 0.15c	0.0 ± 0.0	2.44 ± 0.32b	0.31 ± 0.15c
Full sun	22.5 ± 2.08e	3 ± 1c	0.19 ± 0.1c	0.0 ± 0.0	2.75 ± 0.73b	0.13 ± 0.12c

Same letters in column show no significant difference between systems based on pairwise Mann-Whitney test.

The group III (humus feeders) was the main feeding group in all systems with 27 species (50% of species) (Table II). The group II (wood and litter feeders) followed with 19 species, mainly pests. Across systems, the proportion of pests (wood feeders) in termite community increased gradually when moving from rustic (32.4% of species) to full sun systems (81.8% of species).

III.1.1.3. Effects of land-use, temperature and relative humidity on pest populations of termites

The GLMs analysis revealed that shade management, ambient temperature and relative humidity are important drivers of termite outbreaks in plantations. Shade management affected pest populations both above and belowground and we observed significant differences in pest density per tree between systems ($F_{GLM} = 8.787$; df= 4; $P < 0.001$). Intermediate (29.20 ± 3.39) and full sun systems (23.08 ± 2.92) showed the highest density of termites per tree, while the lowest density was found in rustic systems (10.08 ± 1.38). The ambient temperature and R.H. affected only belowground pest populations (F_{GLM} (T°) = 8.789; df = 1; $P < 0.001$; F_{GLM} (R. H.) = 8.78; df= 1; $P < 0.001$). Ambient temperature was positively correlated with belowground pest abundances ($r = 0.34$; $P< 0.001$), whereas R.H. was negatively correlated with pest abundances ($r = - 0.41$; $P< 0.002$). Based on the Pearson R correlation test, the R. H. was a more powerful factor on pest populations than ambient temperature and their effects were opposite.

The combined effects of land-use, temperature and R. H. strongly affected belowground pest abundances ($F_{GLM} = 8.77$; df= 7; $P < 0.001$). The dry season (T°= 29.2 ± 0.84; R.H. = 74.15 ± 1.91) was significantly hotter than rainy season (T°= 26.91 ± 0.3; R.H. = 83.95 ± 1.6). We

also noted that seasonality, associated to shifting temperatures, also affected pest abundance. The main pest species of termites were significantly more abundant on cocoa during the dry season than the rainy season in all systems (Figure 19). For instance, the total abundance of *M. osborni* has grown considerably from 1010 individuals during rainy season to up to 3495 individuals during the dry season.

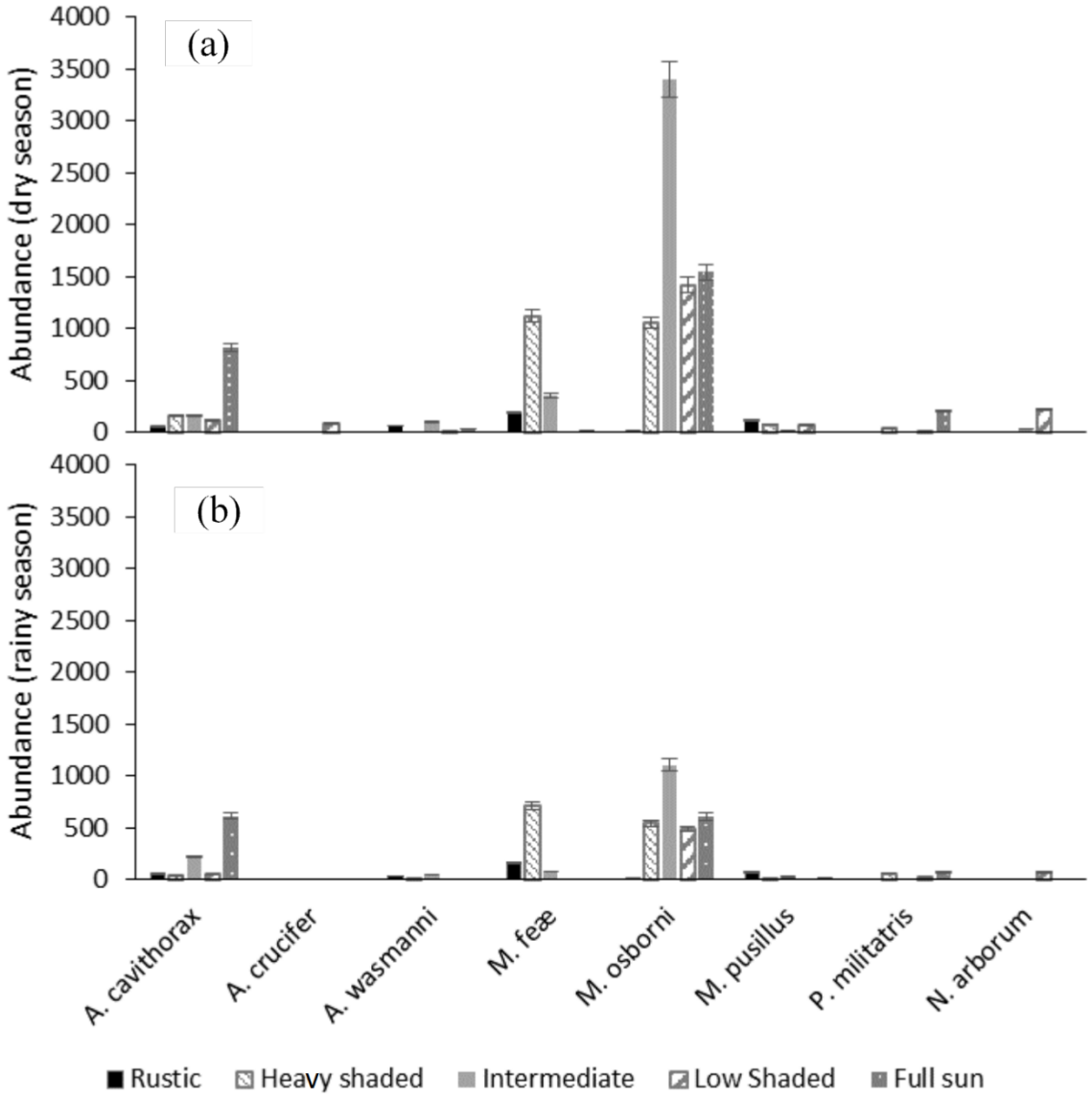


Figure 18. Total abundance per season of main pest termites per shade management. (a) = Dry season, (b) = Rainy season.

III.1.2. Ecosystem services and disservices provided by termites to cocoa trees

III.1.2.1. Ecosystem services provided by termites and consequences of land-use change

The analysis of weighted humification scores (quantified ecosystem service) showed significant differences in fertilization index of termite communities between systems based on

ANOVA (F = 21.9; P < 0.001) (Figure 18). The weighted HS increased gradually in relation to shade cover. The lowest weighted HS was obtained in full sun systems (weighted HS = 2.01), reflecting a poor soil fertilization by termites. The highest weighted HS was obtained in rustic systems (weighted HS= 2.51), reflecting a high fertilization by termites. All beneficial termite species (soil feeders with high fertilization score, i.e. *Cubitermes* spp., *Fastigitermes* sp., *Amicotermes* spp. and *Anenteotermes* spp.) were encountered in shaded systems, and were not sampled in unshaded systems (Table II and IV).

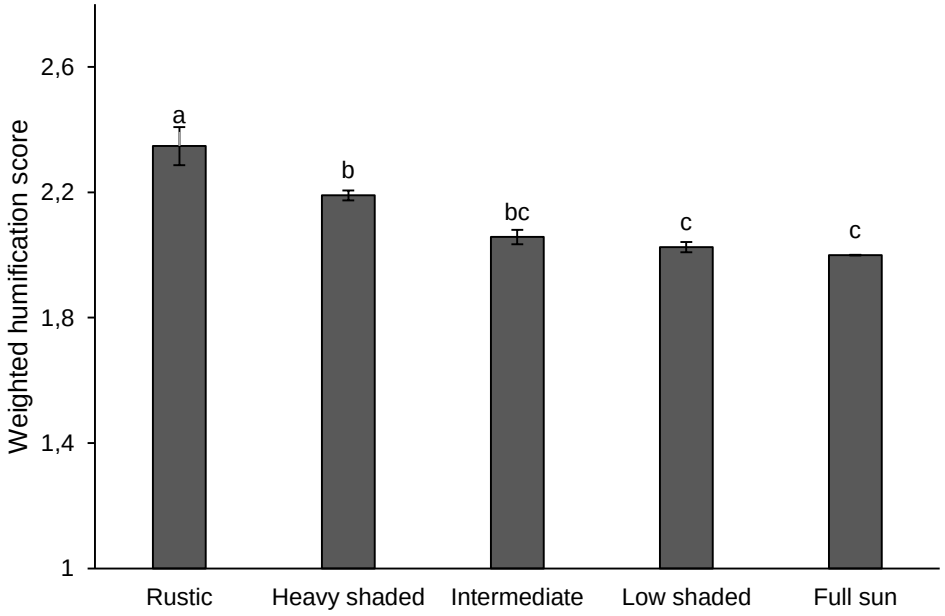


Figure 19. Weighted humification score of termite communities across cocoa agroforestry systems. Same letters between groups show no difference based on Tukey Post-hoc.

III.1.2.1. Ecosystem disservices provided by termites to cocoa

III.1.2.1.1. Termites' infestation in cocoa agroforestry systems and effect of shade management

Of a total of 1200 trees sampled, 870 (73 %) were infested by termites. 138 trees (12 %) were infested only aboveground, 532 trees (44 %) only belowground and 200 (17 %) both above and belowground. Cocoa trees were significantly more infested during the dry season (80 %, 477 trees) than during the rainy season (66 %, 396 trees), both above and belowground (χ^2 = 27.58, *df* = 1, *P* < 0.001).

The highest termite infestation rate was obtained in heavy shaded systems (83 %, 199 trees) followed by rustic shaded systems (79 %, 190 trees), intermediate shade systems (75 %, 180 trees) and low shaded systems (64 %, 154 trees). The full sun-like systems were the least

infested systems (63 %, 150 trees). The highest aboveground infestations were obtained in rustic shaded systems while belowground infestations were preferred in poor shaded systems (Table V). Shade cover was positively and significantly correlated with aboveground infestation (ρ = 0.82, P < 0.001) and it was also negatively and significantly correlated with belowground infestation (ρ = – 0.52, P < 0.05). The correlation table (Table VI) showed that aboveground infestation categories were strongly correlated between them, but no significant connection was found between aboveground and belowground infestation sites.

Table V. Proportion (% ± SE) of infested trees per infestation site in each agroforestry system

Systems	**Shade cover (+ 95% CI)**	**Aboveground**			**Belowground**
		Galleries	Dead branches	Arboreal nests	(roots)
Rustic	92.54a (87.83 – 97.24)	64.6 ± 7.4a	30.0 ± 4.3a	10.0 ± 2.1a	42.9 ± 3.9a
Heavy shaded	83.2b (80.46 – 85.94)	34.2 ± 6.7b	28.8 ± 4.7a	1.6 ± 1.3b	72.5 ± 3.4b
Intermediate	67.6c (57.25 – 77.95)	14.6 ± 4.1c	3.8 ± 1.4c	0.0 ± 0.0b	70.4 ± 5.4bc
Low	55.0d (43.83 – 66.17)	11.3 ± 6.7d	9.2 ± 5.6c	5.4 ± 3.2b	60.0 ± 3.7c
Full sun	22.5e (18.42 – 26.58)	3.8 ± 2.1d	2.9 ± 1.8c	2.1 ± 1.2b	60.4 ± 5.1bc

Same letters in columns show no significant differences between systems based on Mann-Whitney's test.

Table VI. Correlation table of termite infestation on trees per sampling categories based on spearman ρ correlation coefficient values

	Galleries	Dead branches	Arboreal nests
Galleries	1		
Dead branches	0.55**	1	
Arboreal nests	0.36**	0.46**	1
Roots	-0.05	0.01	0.02

**: significant at P < 0.01.

The mean termite densities per tree were 14.8 ± 1.6 SE individuals at aboveground infestation (galleries) and 15.1 ± 1.1 SE individuals at belowground infestation (roots). The linear regression analyses revealed that shade cover has a negative and significant effect on belowground termite densities (y = – 0.13x + 19.28, t = – 3.22, P = 0.003). The shade cover also affected positively aboveground termite densities but significant difference was not found (y = 0.08x + 7.22, t = 0.37, P > 0.05). Significant differences in termite densities were found between systems both aboveground (H = 261.12, P < 0.001) and belowground (H= 85.83, P < 0.001). The highest aboveground densities per tree were obtained in poorly shaded systems with the peak observed in low shaded systems (30.1 ± 6.5 SE individuals) followed by rustic systems (19.1 ± 1.9 SE individuals) (Figure 20). Belowground, the highest termite density per tree was obtained in intermediate systems (23.5 ± 3.1 SE individuals), while the lowest density

was in rustic systems (3.9 ± 0.6 SE individuals) (Figure 20). Shade tree reduction in plantations increases termite density per tree in plot (ρ = – 0.75, P < 0.001).

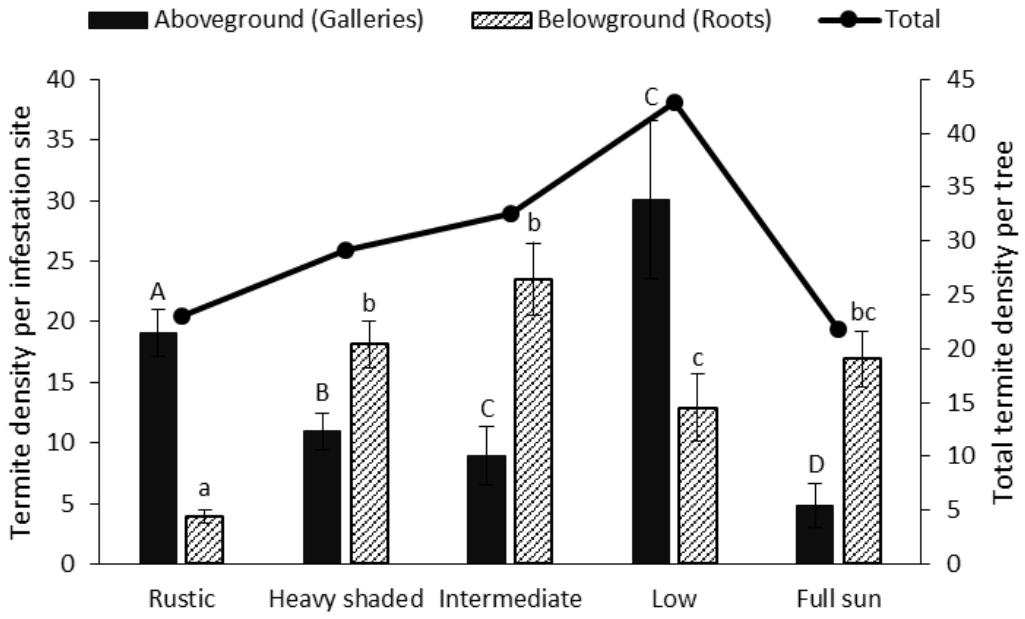


Figure 20. Termite densities (mean number of individuals ± SE) per tree. The bar chart shows termite density in each system per infestation site: aboveground (galleries) and belowground (roots). The line chart shows the total termite density (galleries + roots) per each agroforestry systems. Same letters show no significant differences between systems per infestation site based on Mann-Whitney's test (uppercase for aboveground and lowercase for belowground).

III.1.2.1.2. Dispersion models of termite populations in aboveground and belowground infestation

Taylor's power law confirmed a positive significant relationship between variance (S^2) and mean density (r^2 = 0.93, P < 0.001) (Table VII). When the mean density increases, the variance also increases (Figure 21). Taylor's intercept a was > 0 in aboveground (galleries) and belowground (roots) termite populations (in galleries, t = 10.84, df = 49, P < 0.001; in roots, t = 32.37, df = 79, P < 0.001). The slope b was significantly >1 (in galleries, t = 36.48, df = 49, P < 0.001; in roots, t = 11.48, df = 79, P < 0.001).

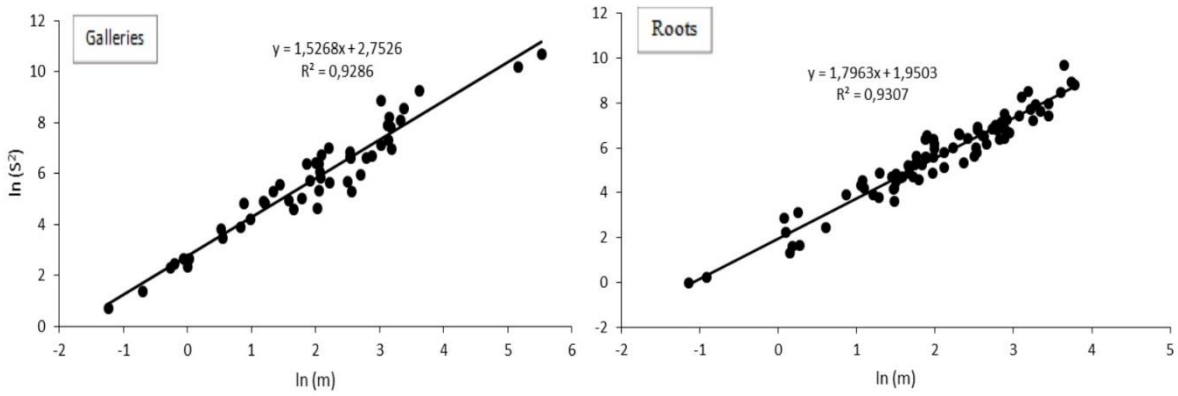


Figure 21. Regression analysis of Taylor's power Law for aboveground (Galleries) and belowground (Roots) termite populations.

Nachman's model provided a fit to the relationship between the proportion of trees without termites (P_0) and the mean density (m) of termites populations at aboveground (r^2 = 0.45, $P < 0.001$) and belowground (r^2 = 0.34, $P < 0.001$) infestation sites (Table VII and Figure 22). The fewer the number of cocoa trees were infested in the plot, the more the termite densities were high on infested trees. However, the coefficient of determination (r^2) was weaker than in the Taylor's model.

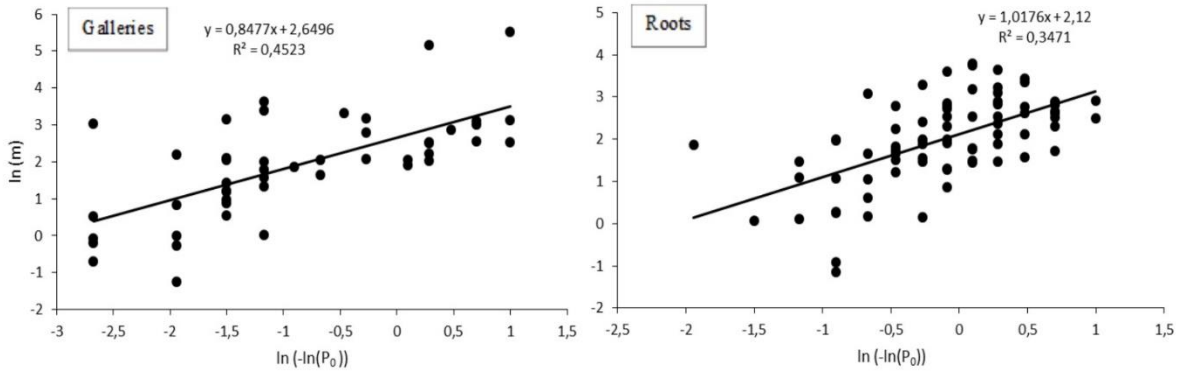


Figure 22. Regression analysis of Nachman's model for aboveground (Galleries) and belowground (Roots) termite populations.

Iwao's patchiness regression revealed that the intercept value (α) was > 0 (t = 4.77, df = 49, $P < 0.001$) in galleries (aboveground) and > 0 (t = 8.23, df = 79, $P < 0.001$) in roots (belowground) (Figure 23 and Table VII), indicating a spatial aggregation pattern of termite populations. Estimates for β, the density contagiousness coefficient, were significantly > 1 (t = 4.77, df = 49, $P < 0.001$ in galleries; t = 8.23, df = 79, $P < 0.001$ in roots).

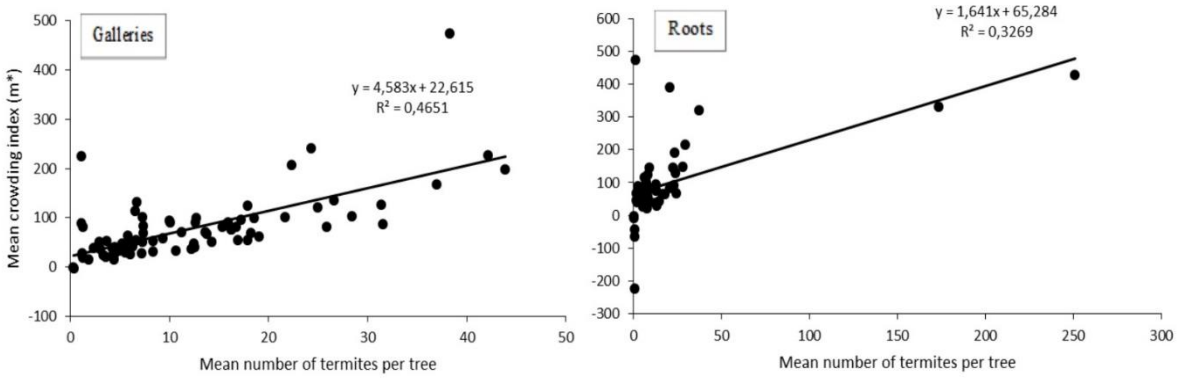


Figure 23. Regression analysis of Iwao's mean crowding index (m*) on mean density (m) for aboveground (Galleries) and belowground (Roots) termite populations.

Table VII. Comparison of model parameters for aboveground and belowground infestation

Model and parameters		Infestation site	
		Aboveground	Belowground
Taylor's ($S^2 = a \cdot m^b$)			
	N	50	80
	$a \pm$ SE	2.75 ± 0.14	1.95 ± 0.13
	$b \pm$ SE	1.52 ± 0.06	1.79 ± 0.05
	r^2	0.93**	0.93**
Nachman's ($P = 1 - \exp(-f \cdot m^g)$)			
	N	47	78
	$f \pm$ SE	2.65 ± 0.19	2.12 ± 0.09
	$g \pm$ SE	0.85 ± 0.14	1.02 ± 0.16
	r^2	0.45**	0.35**
Iwao's ($m^* = \alpha + \beta \cdot m$)			
	N	50	80
	$\alpha \pm$ SE	65.28 ± 15.68	22.61 ± 8.65
	$\beta \pm$ SE	1.64 ± 0.34	4.58 ± 0.56
	r^2	0.33**	0.47**

"**" shows significance at $P < 0.01$.

III.1.2.1.3. Sampling plans for ecological and pest management purposes

Optimal sample sizes for fixed precision levels of 0.10, 0.15, and 0.25 for Southwood's sampling program are presented in Figure 24a. The optimal sample size for a precision of 0.25 ranged from 8 to 62 trees, depending on termite density per tree. For a precision level of 0.10, the sample size ranged from 54 to 384 trees at the same mean interval. At a precision level of 0.25, the number of sample trees needed is 12 trees for a mean density of 14.75 termites aboveground and for a mean density of 15.1 termites belowground. However, if the level of precision was set to 0.10 the number of sample trees required for a mean density of 14.75 and 15.1 became 75 and 76 trees, respectively.

The intersection of stop lines and the curve of expected cumulative density (Figure 24b) indicates the average number of trees required for a fixed precision level for Green's sequential sampling. For the precision level of 0.25, the mean density of 15.1 will require about 10 trees sampled, while it will require about 65 trees for the precision level of 0.10 for the same mean.

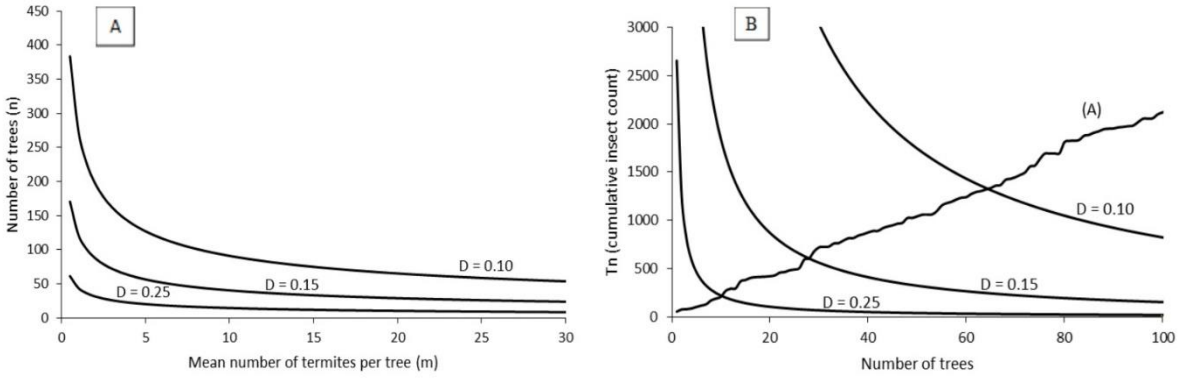


Figure 24. Curves of sampling plans. **A)** Curves of optimal samples size from Taylor's power law model for given precision level of 0.10, 0.15, and 0.25 at different mean density of termites per tree based on Southwood's formula (1978); **B)** Green's sequential sampling stop lines (concaves curves) for estimating densities of termites per tree with three fixed precision level (D = 0.10, D = 0.15 and D = 0.25). The curve (A) represents the expected cumulative number of insects for a mean density of 15.1 termites per tree.

III.1.3. Description ecosystem disservices of key termite genera on cocoa trees and relationships with yield

III.1.3.1. Disservices provided by genus *Microtermes* to cocoa

Damage from *Microtermes* spp. were located almost exclusively belowground (Figure 25). Signs of ongoing attacks on cocoa are unnoticeable and characterized by the presence of active colonies (mushroom comb) in soil around roots. After excavation, we noticed foraging tunnels in some living roots with termite individuals moving inside. Furthermore, they often build small galleries on the trunk but these galleries are only located at the basis of the trunk and measured a few centimeters. The collar of the cocoa tree sometimes presents damaged sections filled with mud. The superficial roots (small roots responsible of water and nutrient absorption) are the preferred plant parts by *Microtermes* spp., but the taproot (big root responsible of the anchorage and stability of the stand) is also often damaged. When the damage are important, the plant becomes unable to absorb enough nutrients and water from the soil and the leaves wilt. The plant may suddenly die in case of intense droughts. *Microtermes* spp. attack all tree development stages but they prefer young trees and seedlings (0-10 years old) where

damage caused are more considerable. The species responsible of damage during this study were *M. osborni*, *M. feæ* and *M. pusillus*.

Figure 25. Damage caused to cocoa by genus *Microtermes*. **A)** Small galleries on the trunk, **B)** root tunneled (red arrow), **C)** attacked young tree with wilting leaves, **D)** Damage on the collar of the tree filled with mud.

III.1.3.2. Disservices provided by genus *Microcerotermes* to cocoa

Microcerotermes' damage are exclusively located aboveground (Figure 26). They build conspicuous and anastomosed galleries going upward from the collar to branch ends. In these galleries, they scratch off/perforate the bark and feed on the inner part of the trunk (sapwood and heartwood) especially on branch ends where the bark is thinner. When the hole is dug on the bark, they infiltrate the inner part of the trunk/branch leading to its death. They also usually build a conspicuous arboreal nest on branches. The older the termite infestation is, the bigger the nest gets. By scratching the bark off and building galleries and nests, *Microcerotermes* spp. hinder the flowering and pod formation by the attacked cocoa tree. In addition, the branches may break under the nest weight in case of heavy storm. The tree usually do not die from *Microcerotermes* infestation (except the case of very severe infestation), but the plant will lose

some producing branches and the yield will be under expectations. *Microcerotermes* spp. mainly attack mature and ageing cocoa (more than 20 years old) trees where the damage caused are more considerable. The species responsible of damage during this study were *M. silvestrianus*, *M. progrediens*, *M. edentatus* and *M. fuscotibialis*.

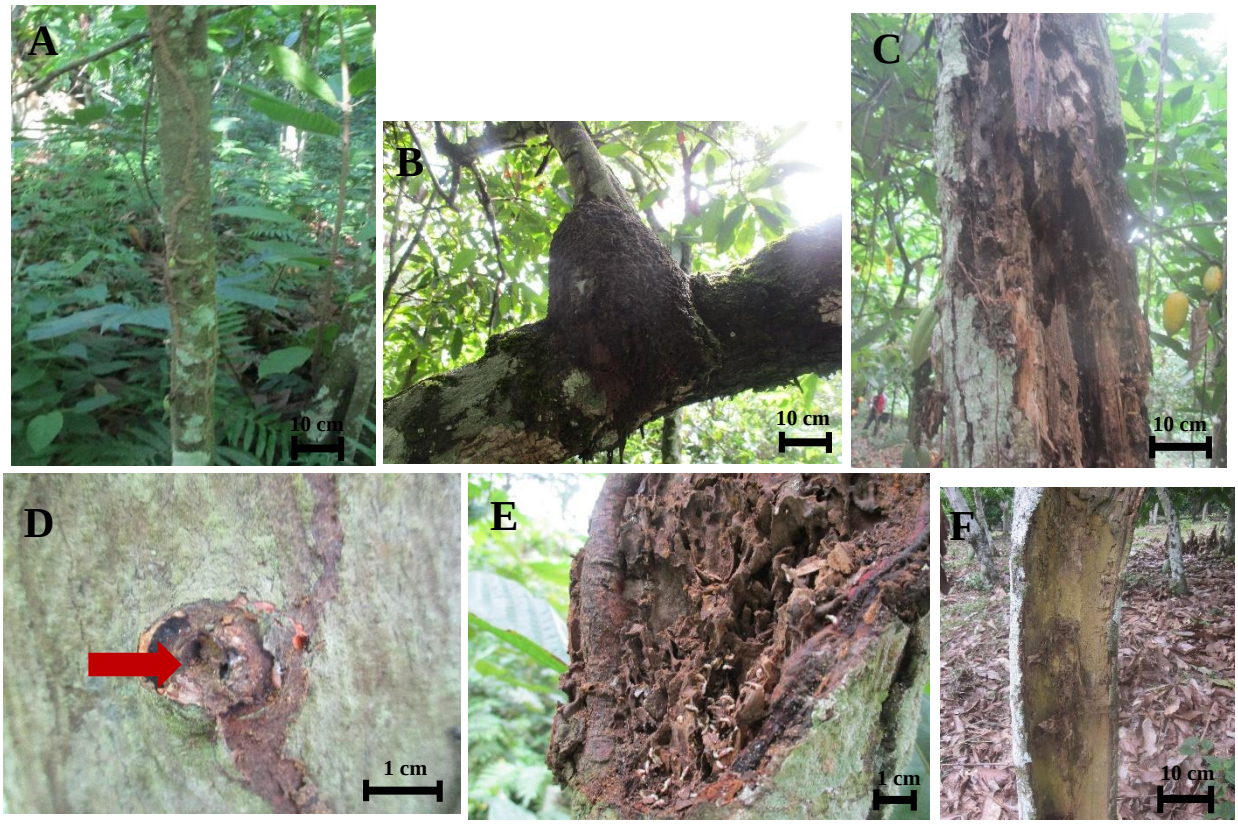


Figure 26. Damage caused to cocoa by genus *Microcerotermes*. **A)** Conspicuous galleries on the trunk, B**)** Arboreal nest on trunk and branch, **C)** extremely damaged trunk, **D)** small holes dug in the bark (red arrow), **E)** inner part attacked, **F)** Aftermath of old termite attacks.

III.1.3.3. Disservices provided by genus *Ancistrotermes* to cocoa

Damage caused by *Ancistrotermes* spp. are mostly belowground but, rarely, they build galleries on the cocoa trunk in which they cause important superficial damage to the tree (Figure 27). These galleries are highly sensible to rainfall and crumble after heavy rains. Belowground, damage are also observable after root excavation with termite individuals tunneling roots. We also noticed some damage at the basis of cocoa trunk where *Ancistrotermes* spp. feed on collar going down in the soil. On roots, damage caused by *Ancistrotermes* spp. are similar to the ones of *Microtermes* spp., but only superficial roots are concerned. When the damage are important, the plant becomes unable to absorb enough nutrients and water from the soil and the leaves get dry. *Ancistrotermes* spp. attack all tree development stages but they prefer young trees and

seedlings (0-10 years old) where damage are more important. The species responsible of damage during this study were *A. cavithorax*, *A. crucifer* and *A. wasmanni*.

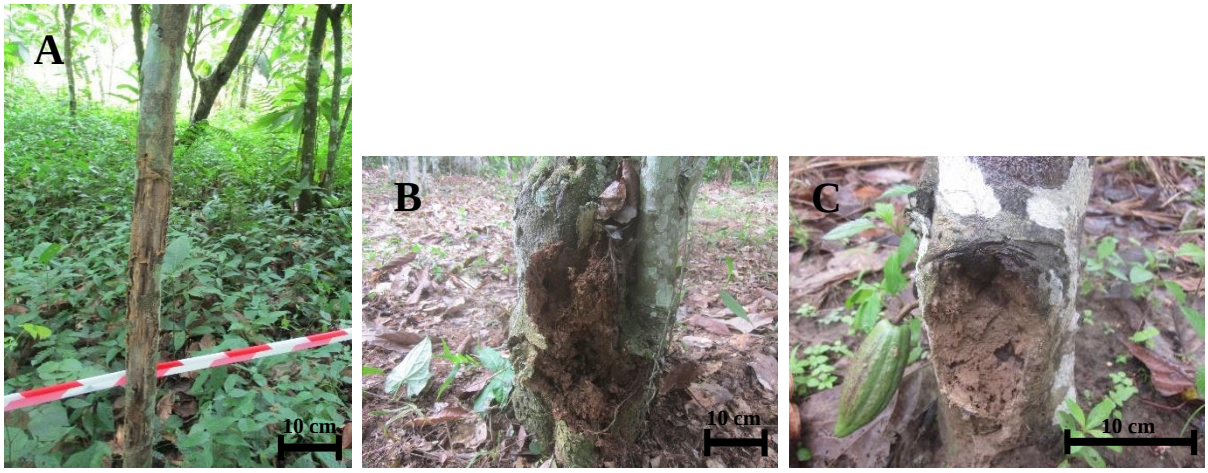


Figure 27. Damage caused to cocoa by genus *Ancistrotermes*. **A)** Aboveground damage on the trunk, **B)** old damage on the collar with inner part completely gnawed, **C)** recently attacked collar filled with mud.

III.1.3.4. Disservices provided by genus *Nasutitermes* to cocoa

Damage of genus *Nasutitermes* are located almost exclusively aboveground (Figure 28). They build conspicuous galleries along the cocoa trunk from collar to branch ends in which they cause important superficial damage to the tree. Some damage were also noticed on cocoa pods where termites fed on external cocoa husk without perforation. Their damage on the trunk and branches are similar to that of genus *Microcerotermes*. They usually build conspicuous arboreal nests on trunk and branches, however smaller than *Microcerotermes*' ones. *Nasutitermes* spp. attack mainly young mature trees (15 – 20 years old). The species responsible of damage during this study were *N. arborum* (mainly) and *N. diabolus*.

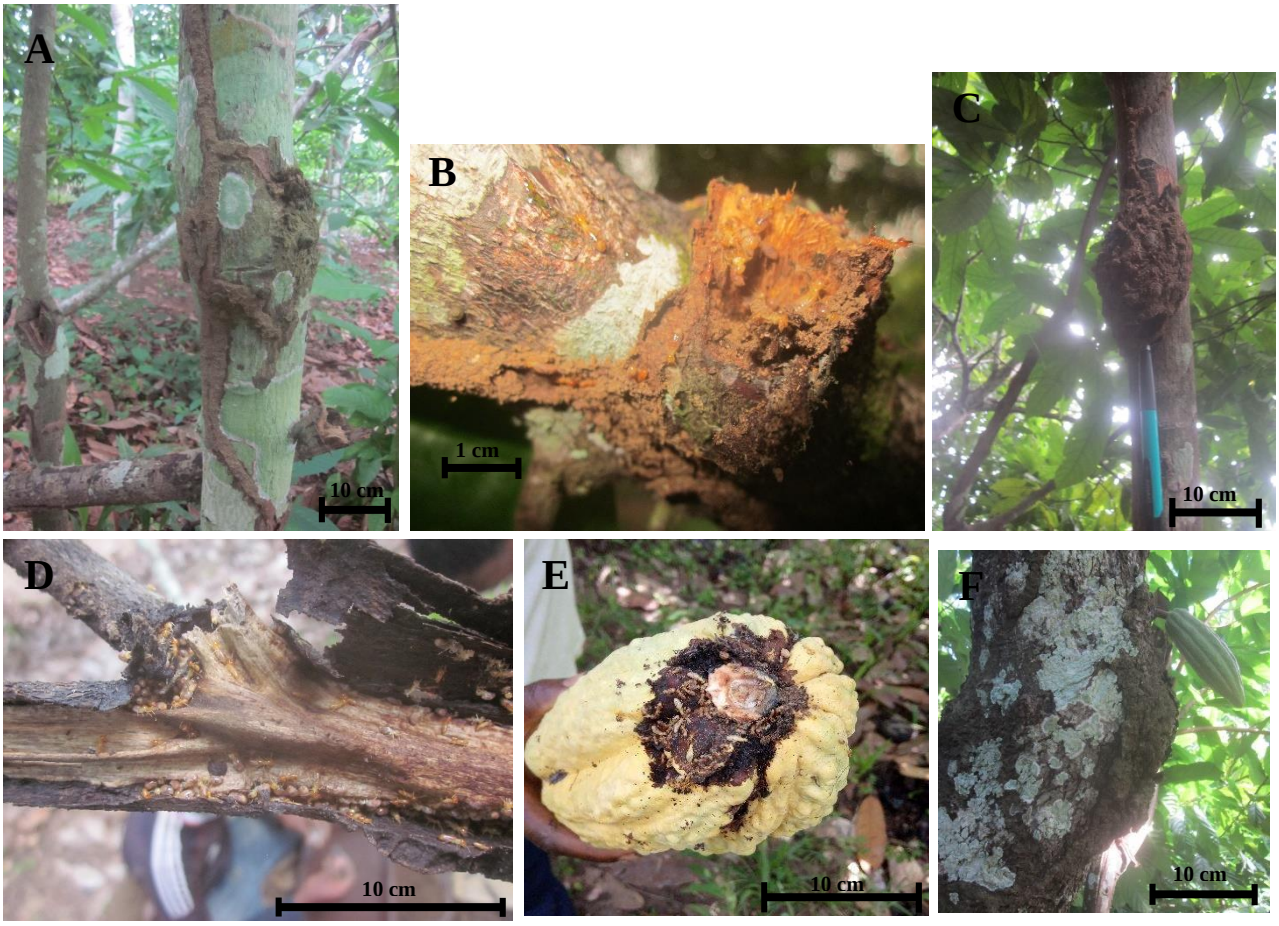


Figure 28. Damage caused to cocoa by genus *Nasutitermes.* **A)** Conspicuous galleries on the trunk, **B)** inner part of a branch attacked, **C)** arboreal nest, **D)** dead branch termite damage below the bark, **E)** Pod attacked, **F)** Basis of young pod attacked.

III.1.3.5. Disservices provided by genus *Odontotermes* to cocoa

Damage of genus *Odontotermes* are mainly located belowground and are similar to that of *Ancistrotermes*. Like *Ancistrotermes*, they sometimes build aboveground galleries highly sensible to rainfall and that crumble after heavy rains. Belowground, *Odontotermes* species damage the roots of seedlings after transplanting in farm. They also build aboveground nests near cocoa trees that cover the basal part of the trunk and prevent any pod formation (Figure 29). In that nest, they feed on the roots of attacked cocoa trees. The species responsible of damage during this study were *O. fulleri* and *O. mukimbunginis*.

Figure 29. Damage caused to cocoa by genus *Odontotermes* with superficial nest around the tree.

III.1.3.6. Severity of disservices provided by termites on cocoa trees and relationship with shade management

All the four levels of damage have been recorded in all the production systems (Figure 30). The primary level (healthy trees) was the most dominant damage level across all systems except in rustic systems where the secondary level was the most abundant (35% of trees). The highest proportions of primary damage level was observed in intermediate systems (66%) followed by heavy shaded systems (65%). These systems showed poor proportions of high damage levels (respectively 1.5% and 1.3% for tertiary level, 1.3% and 2.5% for quaternary level). Poorly shaded systems were the most highly damaged by termites, especially low shaded systems where we noted 25% of quaternary damage level and 15% of tertiary damage level. The full sun systems followed with 20% of tertiary damage level and 10% of quaternary damage level.

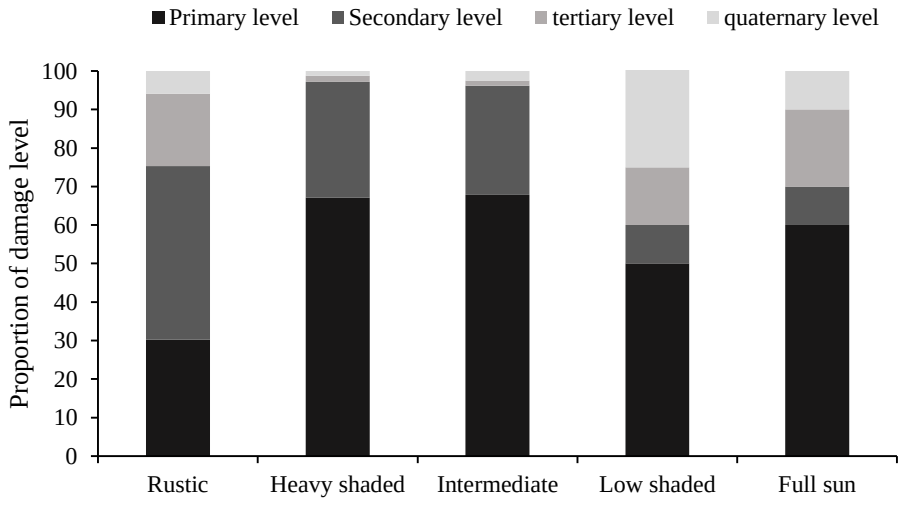


Figure 30: Distribution of superficial damage level of termites on cocoa trees per agroforestry system

III.1.3.7. Relationships between ecosystem disservices provided by termites and cocoa yield

III.1.3.7.1. Relationship between termite disservices and cocoa tree vigor and development

We sampled a total of 1701 cocoa trees across the five agroforestry systems. Cocoa tree basal area (BA) and height differed significantly between systems (BA, $F = 36.2$, $P < 0.001$; height, $F = 47.2$, $P < 0.001$). Cocoa trees in heavy shaded systems were significantly taller than those in poorly shaded systems (Table VIII). The tallest trees were found in rustic systems and the smallest in full sun systems (Table VIII). Cocoa trees with the largest BA was also found in intermediate systems (159 cm^2), followed by rustic systems (104 cm^2). Termite infestation affected cocoa tree development in all systems studied (Figure 31A and 31B; Table IX). Healthy trees (h= 4.15 m) were significantly taller than infested trees (h= 3.42 m), but no significance was found between tree groups regarding the basal area. When termite infestation was severe, cocoa tree vigor (BA) and development (height) was even more affected with the lowest scores obtained (Table IX).

III.1.3.7.2. Relationship between termite disservices and cocoa flowering and pod formation

Cocoa tree flowering differed significantly across production systems (nb of flowers, $F = 2.33$, $P = 0.06$; nb of flower cushions, $F = 2.27$, $P = 0.08$) (Table VIII; Figure 31C and 31D). During the sampling periods, cocoa trees in intermediate and heavy shaded systems produced significantly less flowers than trees in other systems. The flowering did not differ significantly between rustic and full sun systems. Termite infestation however contributed to reduce the flowering on cocoa trees, with infested trees producing less flowers on fewer flower cushions than healthy trees (Table IX). The more termite infestation was severe the more the tree flowering was affected (Table IX).

We also noted that the pod formation differed significantly between production systems ($F = 2.77$, $P = 0.01$) (Table VIII; Figure 31E). The highest number of pods per cocoa tree was recorded in intermediate systems (20 pods per tree) followed by low shaded systems (14 pods per tree) and rustic systems (13 pods per tree). Heavy shaded systems recorded the lowest pod production per tree (8 pods per tree). Termite infestation significantly reduced the pod formation of cocoa trees (Table IX; Figure 31E). The most severely infested trees (gallery + root infested trees) produced the lowest number of pods (8 pods per tree). Termites contributed to reduce pod formation by 34%, 18% and 48% in case of gallery, root and severe infestations

respectively. Termites also reduced pod formation on cocoa trees whatever their age, with trees aged beyond 30 years old the most affected (Figure 32).

Table VIII. Cocoa tree development, flowering and pod formation across cocoa agroforestry systems

System	Height (m)	BA (dm^2)	Nb flowers	Nb flower cushions	Nb pods
Rustic	4.53 ± 0.1a	101.2 ± 4.7a	32.24 ± 3.9a	31.9 ± 2.2a	12.8 ± 0.9a
Heavy shaded	4.14 ± 0.1a	69.7 ± 4.82b	2.72 ± 0.5b	14.5 ± 0.9b	7.88 ± 0.6b
Intermediate	4.27 ± 0.1a	155.2 ± 5.8c	4.86 ± 0.8b	21.6 ± 1.04c	19.9 ± 1.0c
Low shaded	3.71 ± 1.2b	73.17 ± 3.4b	24.9 ± 4.3a	25.2 ± 1.4cd	14.3 ± 0.8a
Full sun	3.11 ± 1.2c	53.08 ± 2.88d	38.8 ± 5.1a	27.6 ± 1.31ad	10.6 ± 0.6ab

*BA: Basal area of cocoa trees.

Same letters in column show no significant difference between groups based on Tukey HSD post-hoc test.

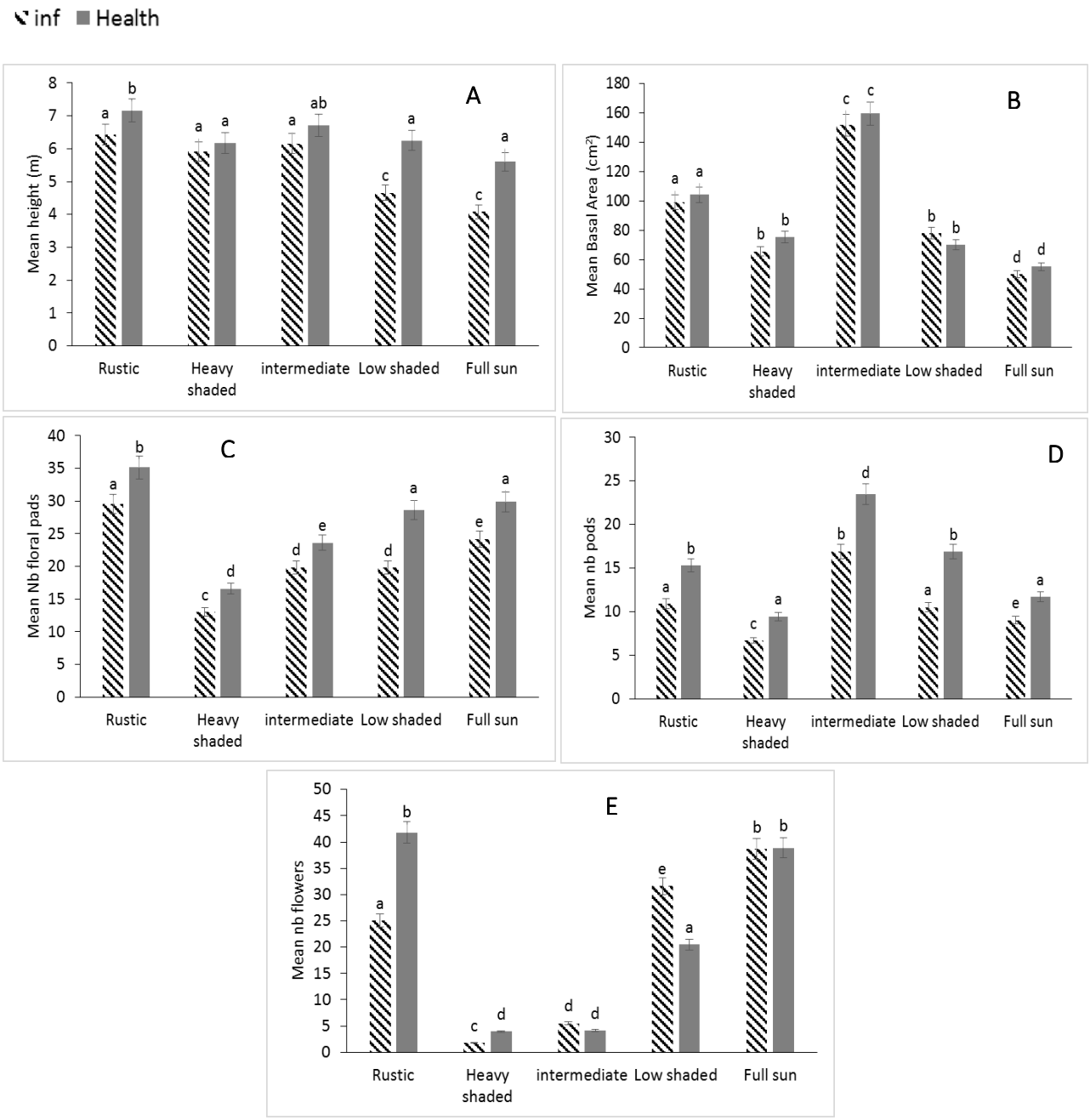


Figure 31. Comparison of cocoa tree development, flowering and pod formation between infested (inf) and healthy (health) trees across agroforestry systems. **A**) Height of the crown, **B**) Basal area, **C**) number of flowers produced, **D**) number of active flower cushions and **E**) number of pods produced (length ≥ 10 cm) per cocoa stand. Same letters show no significant difference between groups based on Tukey HSD post-hoc test.

Table IX. Cocoa tree development, flowering and pod formation per severity of termite infestation on trees

Infestation groups	Height (m)	BA (cm^2)	Nb flowers	Nb floral cushions	Nb pods
Healthy trees	5.7 ± 1.7a	90.4 ± 3.3a	22.6 ± 2.6a	27.0 ± 0.9a	15.3 ± 0.6a
Gallery infested trees	4.13 ± 0.5b	93.1 ± 4.9a	16.7 ± 2.6a	21.4 ± 1.7b	9.86 ± 0.8bc
Root infested trees	4.03 ± 0.1b	92.8 ± 5.1a	22.7 ± 3.3a	22.2 ± 1.2b	12.5 ± 0.8b
Gallery + root infested trees	3.8 ± 0.5b	82.5 ± 7.4a	6.75 ± 1.7b	16.5 ± 2.1b	7.93 ± 1.1c

*DBH: Diameter at breast height

Same letters in column show no significant difference between groups based on Tukey HSD post-hoc test.

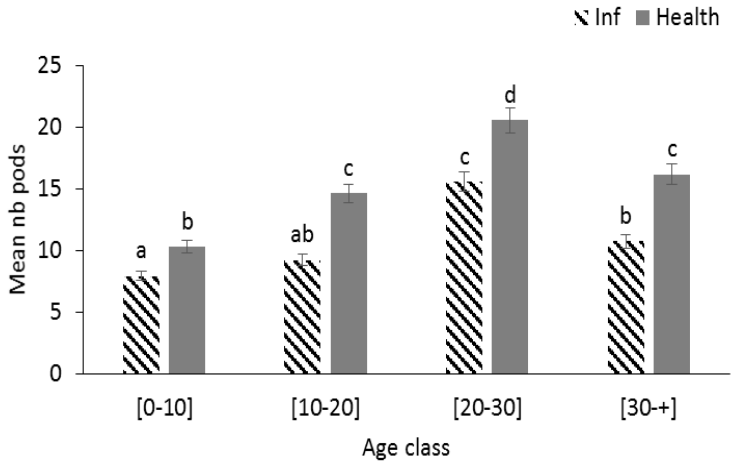


Figure 32. Termite impact on pods produced per age class of cocoa stand. Same letters show no significant difference between groups based on Tukey HSD post-hoc test.

III.1.3.7.3. Relationship between termite disservices and pod harvests on cocoa trees

We noted significant differences in volume, weight and number of beans in harvested cocoa pods across production systems (Table X). The biggest ripe pods were harvested in low shaded systems (460 cm^3), followed by intermediate systems (382 cm^3). The smallest pods (304 cm^3) were harvested in heavy shaded systems. The heaviest pods were also harvested in low shaded and full sun systems. In contrary, the number of beans per pod was higher in heavily shaded systems than in poorly shaded systems (Table X). Heavy shaded systems recorded the highest number of beans (45 beans per pod) and full sun systems the lowest (38 beans per pod). Termites did not significantly affect the volume and the weight of harvested pods on trees but termites reduced pod weight by about 6.5%. In addition, we noted significant differences in number of beans between infestation groups. Pods harvested on healthy trees contained

significantly more beans (45 beans per pod) than pods of infested trees (38 beans per pod) (U= 94421.5, P < 0.001) (Table XI).

Table X. Comparison of harvested pods parameters among agroforestry systems

Systems	Volume (cm^3)	Weight (g)	Number of beans
Rustic	343.3 ± 15.1a	578.46 ± 18.25a	44.81 ± 1.22a
Heavy shaded	304.3 ± 14.8a	500.38 ± 24.8b	45.19 ± 0.75a
Intermediate	381.7 ± 30.4ab	573.64 ± 31.8a	44.03 ± 0.97a
Low shaded	459.6 ± 33.5b	709.7 ± 42.6c	38.43 ± 1.42b
Full sun	377.7 ± 32.1ab	585.75 ± 45.9a	37.8 ± 1.93b

Same letters in column show no significant difference between groups based on Tukey HSD post-hoc test.

Table XI. Comparison of harvested pods parameters between infested and healthy trees

Tree group	Volume (cm^3)	Weight (g)	Number of beans
Healthy trees	383.23 ± 14.8a	615.27 ± 21.9a	44.81 ± 1.22a
Infested tress	374.48 ± 22.8a	575.83 ± 25.1a	37.8 ± 1.93b

Same letters in column show no significant difference between groups based on Mann-Whitney pairwise test.

III.1.3.7.4. Relationship between termite disservices and bean formation and marketable yield

The production systems strongly affected the bean formation and the consequent marketable yield (Table XII; Figure 33). The fresh beans harvested from ripe pods were significantly longer in heavily shaded systems than poorly shaded systems. The longest beans were recorded in rustic systems (2.55 cm) and the shortest in full sun systems (2.43 cm). The largest beans were also recorded in rustic systems (1.38 cm) and the lowest in low shaded systems (1.27 cm) (Table 5). Although low shaded systems recorded the heaviest fresh beans (2.65 g per bean) among the production systems, no significant differences were noted between systems. After the beans have dried, we noted that beans in intermediate systems were significantly lighter than beans in other systems (F = 3.11, P = 0.015) (Table XII). The heaviest beans after drying were recorded in low shaded systems (1.21 g per bean). The water content was significantly more important in heavy shaded systems than poorly shaded systems. Regarding the marketable yield, intermediate systems were the most productive (about 1535 Kg ha^{-1}), followed by rustic systems (about 1166 Kg ha^{-1}) and low shaded systems (about 1124 Kg ha^{-1}) (Figure 33).

Termite infestation strongly affected the bean formation in pods (Table XIII). Fresh beans from healthy trees were significantly longer (U = 224877, P < 0.001) and larger (U = 219261.5, P < 0.001) than fresh beans from infested trees. Both fresh and dry beans from healthy trees were also heavier than beans from infested trees (U= 185580; P < 0.001). Termites reduced bean weight by 3.5% and 7.4% in fresh beans and dry beans respectively. We noted that the water content was significantly more important in beans from healthy trees than in beans from infested trees. The marketable yield obtained from healthy trees was significantly higher than yield from infested trees (Table XIV). Healthy trees yielded about 1118 Kg ha^{-1}, meanwhile severely infested trees (gallery + root infested trees) yielded significantly less with about 503 Kg ha^{-1}. The yield loss induced by termite infestation was about 44% for gallery infested trees, 29% for root infested trees and 55% for gallery + rood infested trees (Table XIV). Termites contributed to yield decrease in all production systems but the most important yield gap was obtained in low shaded systems with up to 47% of yield loss (Figure 33).

Table XII. Comparison of cocoa beans parameters across cocoa agroforestry systems

Systems	**Fresh beans**			**Dry beans weight (g)**	**Water content**
	Length (cm)	Width (cm)	Weight (g)		
Rustic	2.55 ± 0.01a	1.38 ± 0.01a	2.33 ± 0.02a	1.16 ± 0.02ab	0.46 ± 0.01ab
Heavy shaded	2.48 ± 0.02b	1.38 ± 0.01a	2.47 ± 0.02a	1.16 ± 0.02ab	0.49 ± 0.01a
Intermediate	2.44 ± 0.02c	1.29 ± 0.01b	2.29 ± 0.03a	1.11 ± 0.02b	0.42 ± 0.01b
Low shaded	2.43 ± 0.02c	1.27 ± 0.01b	2.65 ± 0.46a	1.21 ± 0.02a	0.37 ± 0.01c
Full sun	2.43 ± 0.01c	1.34 ± 0.01a	2.01 ± 0.03a	1.18 ± 0.02ab	0.31 ± 0.01d

Same letters in column show no significant difference between groups based on Tukey HSD post-hoc test.

Table XIII. Comparison of cocoa beans parameters between infested and healthy cocoa trees

Groups	**Fresh beans**			**Dry beans weight (g)**	**Water content (%)**
	Length (cm)	Width (cm)	Weight (g)		
Healthy trees	2.51 ± 0.01a	1.36 ± 0.01a	2.39 ± 0.02a	1.21 ± 0.01a	0.43 ± 0.01a
Infested trees	2.42 ± 0.1b	1.31 ± 0.01b	2.31 ± 0.18b	1.12 ± 0.01b	0.39 ± 0.01b

Same letters in column show no significant difference between groups based on Mann-Whitney pairwise test.

Table XIV. Yield loss induced to marketable cocoa yield per severity of termite infestation on trees

Groups	Nb pods	Wbeans (kg)	Marketable cocoa yield (kg.ha^{-1})	Yield loss
Healthy trees	15.3 ± 0.6a	0.174	1118.12 ± 43.8a	-
Gallery infested trees	9.86 ± 0.8bc	0.151	625.32 ± 50.73b	44.07
Root infested trees	12.5 ± 0.8b	0.151	792.76 ± 51.1b	29.10
Gallery + root infested trees	7.93 ± 1.1c	0.151	502.92 ± 66.9c	55.02

Same letters in column show no significant difference between groups based on Mann-Whitney pairwise test.

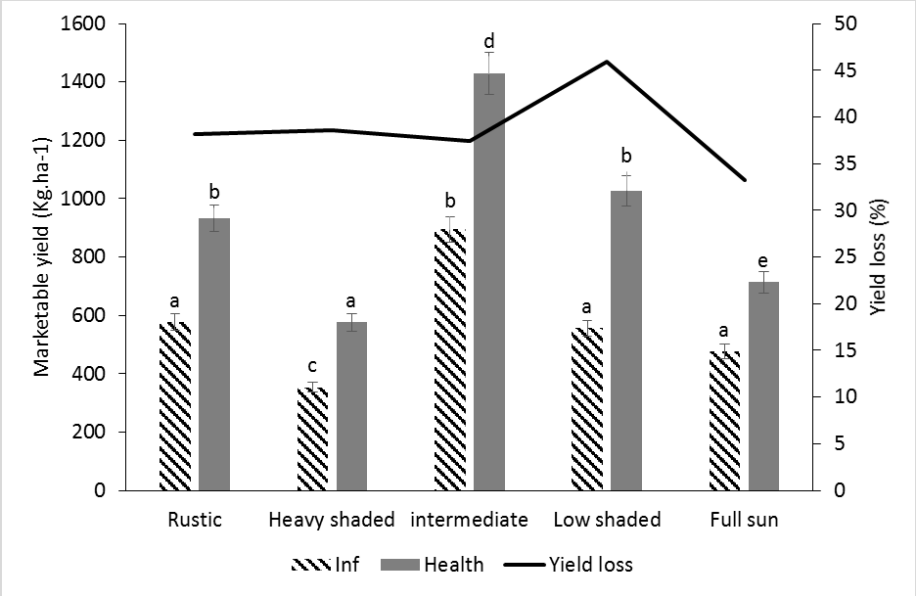


Figure 33. Yield loss induced by termite infestation across production systems. Same letters show no significant difference between groups based on Tukey HSD post-hoc test.

III.1.4. Diversity of biological control agents screened on termites, parasitism parameters and phylogeny

III.1.4.1. Diversity of fungi species associated with termites and phylogenetic analysis

III.1.4.1.1. Inventory of fungi species isolated on termites, functional groups and effect of shade management on their distribution

From the visualization of PCR products under UV light, we identified amplification bands of fungi ITS segment between 500 and 600 bp (Figure 34). Based on the molecular identification, we have recorded 26 fungi species belonging to 13 genera, 9 Families, 7 Orders, 4 Classes and 2 phyla (Table XV). The Phylum Ascomycota (3 Classes, 5 Orders, 7 Families, 11 genera and 24 species) was most diversified than the Phylum of Basidiomycota (1 Class, 2 Orders, 2 Families, 2 genera and 2 species). The Class Eurotiomycetes was the richest class

with 11 fungi species followed by the Classes Saccharomycetes (8 species) and Sordariomycetes (5 species). The class Agaricomycetes was constituted of only 2 fungi species. At the Order level, the Eurotiales were the richest order with 11 fungi species, followed by Saccharomyctales (8 species) and Hypocreales (3 species). All the remaining Orders were constituted of single species. At the family level, the Trichocomaceae was the richest family with 11 species, followed by Family Saccharomycetaceae (8 species). All the other Families were constituted of single species. The genus *Aspergillus* was the most diversified with 8 species followed by *Candida* (3 species). All other genera comprised two or less species.

From the identified fungi, most of them (12 fungi species, 46.15%) were known as pathogens to plants. Beneficial fungi to plants counted for 34.62% (9 species) and neutral fungi represented 19.23% (5 species) (Table XV). Regarding their association with insects, most identified fungi species (38.46%) were known as saprophytes (10 fungi species). Fungi known as entomopathogens and symbionts of insects represented 30.77% each (8 species each).

Across agroforestry systems, the most diversified systems were rustic and intermediate systems with 10 fungi species each. They were followed by heavy shaded systems with 9 fungi species (Table XV). Poorly shaded systems recorded fewer species (7 and 6 species respectively in low shaded and full sun systems). In rustic systems, fungi community was dominated by members of Class Sordariomycetes, mostly entompathogens (50% of species). In heavy shaded systems and low shaded systems, fungi species belonging to Class Eurotiomycetes were the more represented, mostly plant pathogens and saprophytes (66.67% and 71.43% of species respectively). In intermediate systems and full sun systems, fungi species in Class Saccharomycetes dominated the community, mainly insect symbionts (50% of species each). Among fungi species, only *Candida parapsilosis* was ubiquitous in all systems surveyed. *Aspergillus sydowii* was also found in all systems except full sun systems. Regarding habitat specificity, *Penicillium paxilli*, *Cordyceps tenuipes*, *Trichoderma harzianum*, *Scedosporium boydii* and *Hypoxylon* sp. were only found in rustic systems. *Aspergillus fumigatus* was only found in heavy shaded systems. *Aspergillus carneus*, *Aspergillus protuberus*, *Candida duobushaemulonis*, *Meyerozyma carpophila*, *Starmerella etchellsii* and Saccharomycetaceae sp.1 were only found in intermediate systems. *Aspergillus tamarii* and Saccharomycetaceae sp.2 were only found in low shaded systems; and *Candida orthopsilosis* and *Meyerozyma caribbica* were uniquely found in full sun systems.

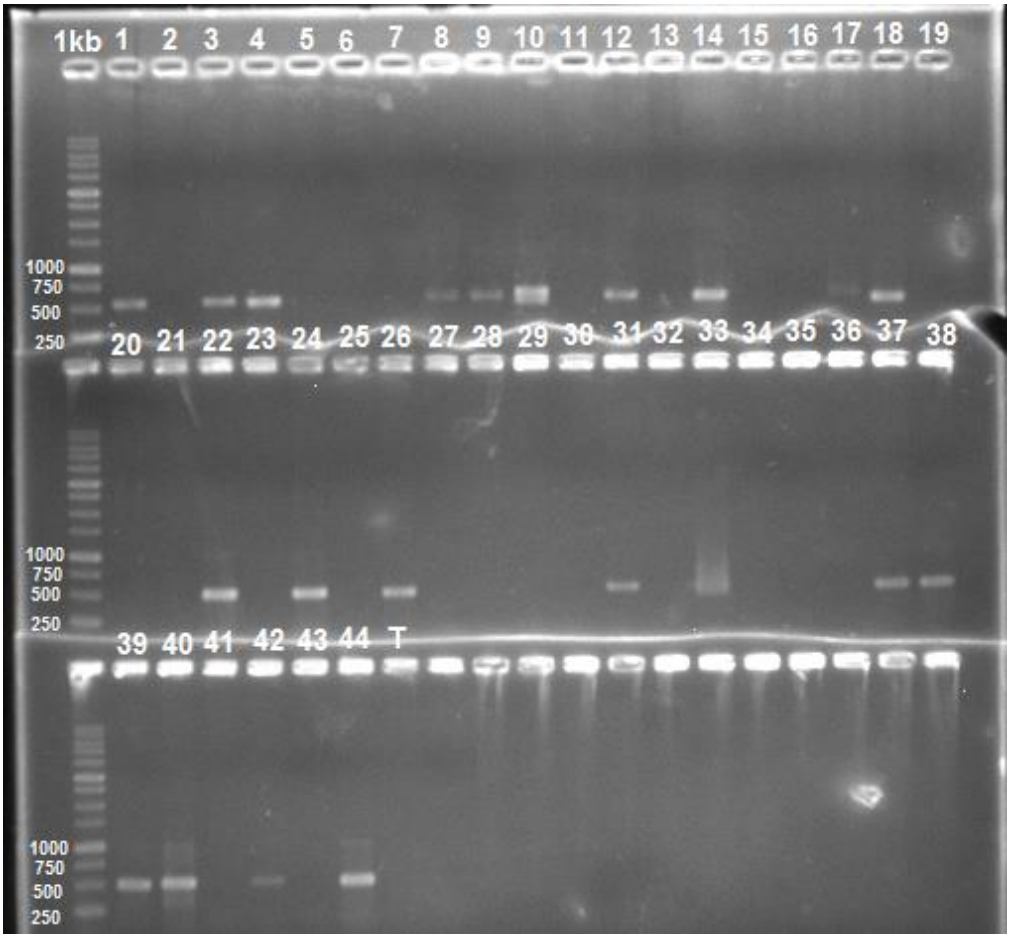


Figure 34. PCR amplification bands of fungi ITS segment using DNA dilution at 1/5th. Numbers refer to different fungi samples and T is the negative control. 1kb is the DNA ladder.

Table XV. Inventory of fungi species isolated from termites in different agroforestry systems with ecological roles in plants and insects

Fungi species	Ecological role		Rustic	Heavy shaded	Intermediate	Low shaded	Full sun
	Plant	Insect					
ASCOMYCOTA							
Eurotiomycetes **Eurotiales** **Trichocomaceae**							
Aspergillus carneus (Tieghem) Blochwitz 1933	Pa	Sa			+		
Aspergillus flavus Link, 1809	Pa	En		+		+	
Aspergillus fumigatus Fresen., 1863	Pa	En		+			
Aspergillus niger (van Tieghem, 1867)	Pa	En		+		+	
Aspergillus nomiae (Kurtzman, Horn & Hesseltine (1987)	Pa	En			+		+
Aspergillus protuberus (Muntañola-Cvetkovic (1968)	Pa	Sa			+		
Aspergillus sydowii (Bainier & Sartory) Thom and Church (1926)	Pa	Sa	+	+	+	+	

Aspergillus tamarii Kita (1913)	Pa	Sa					+	
Penicillium citrinum Thom, 1910	N	En		+			+	
Penicillium paxilli Bainier, 1907	N	En	+					
Talaromyces purpureogenus Samson, Yilmaz, Houbraken, Spierenburg, Seifert, Peterson, Varga & Frisvad, 2011	Pa	Sa	+	+				
Saccharomycetes								
Saccharomycetales								
Saccharomycetaceae								
Candida duobushaemulonis Bueno, Kolecka, Alastr.-Izq., Gómez-López, Cuenc.-Estr. & Boekhout, 2012	B	S			+			
Candida orthopsilosis (Ashford) Langeron & Talice, 1932	B	S						+
Candida parapsilosis (Ashford) Langeron & Talice, 1932	B	S	+	+	+	+	+	
Meyerozyma caribbica (Vaughan-Mart., Kurtzman, Mey. & O'Neill) Kurtzman & Suzuki 2010	B	S						+
Meyerozyma carpophila (Phaff and Mill.) Yurkov and Péter, 2017	B	S			+			
Starmerella etchellsii (Lodder & Kreger-van Rij) S.A. Mey. & Yarrow, 1978	B	S			+			
Saccharomycetaceae sp.1	B	S			+			
Saccharomycetaceae sp.2	B	S					+	
Sordariomycetes								
Hypocreales								
Cordycipitaceae								
Cordyceps tenuipes (Peck) Kepler, B. Shrestha & Spatafora 2017	N	En	+					
Hypocreaceae								
Trichoderma harzianum Pers. 1794	B	Sa	+					
Nectriaceae								
Fusarium oxysporum Schltdl., 1824	N	En	+	+				
Microascales								
Microascaceae								
Scedosporium boydii (Shear) Gilgado, Gené, Cano & Guarro, 2008	N	Sa	+					+
Xyriales								
Hypoxylaceae								
Hypoxylon sp. Bull., 1791	Pa	Sa	+					
BASIDIOMYCOTA								
Agaricomycetes								
Corticales								
Corticiaceae								
Corticiaceae sp.	Pa	Sa	+	+				

Polyporales **Phanerochaetaceae** *Phanerochaete chrysosporium* (Burds.) Hjortstam & Ryvarden, 2010	Pa	Sa			+		+
Total number of species	-	-	10	9	10	7	6

In ecological role: Pa = pathogen, N = neutral, B = beneficial, En = entomopathogen, S = symbiont, Sa = Saprophyte. The symbol "+" refers to the presence of the fungi species in the given environment.

III.1.4.1.2. Relationship between fungi composition and termite host group

During this study, all termite groups displayed fungal growth, but the species richness of fungi per termite group varied from one group to another (Table XVI). The termite groups recording the richest fungi community were *Microcerotermes* spp. with 13 fungi species followed by *Microtermes* spp. (10 species). *Nasutitermes* sp. recorded 6 fungi species, *Odontotermes* spp. 5 fungi species, and *Ancistrotermes* spp. 4 fungi species. In regard of isolation techniques, we have obtained 6 fungi species from termite cadavers while crushed termites recorded 24 fungi species. Among termite groups, all crushed termites displayed fungal growth but *Odontotermes* spp. cadavers did not record fungal growth on culture medium. In fungi community from crushed termites per group, the highest species richness was obtained in *Microcerotermes* spp. (12 species), followed by *Microtermes* spp. (9 species). In fungi community from termite cadavers, *Microcerotermes* spp. also recorded the highest fungi richness (3 species) followed by *Microtermes* spp. and *Nasutitermes* sp. (2 species each).

Table XVI. Distribution of identified fungi species per termite host and isolation technique (cadaver vs crush termite isolation) in cocoa agroforestry systems

Fungi species	***Ancistrotermes* spp.**		***Microcerotermes* spp.**		***Microtermes* spp.**		***Nasutitermes* sp.**		***Odontotermes* spp.**	
	Cad	**Crush**	**Cad**	**Crush**	**Cad**	**Crush**	**Cad**	**Crush**	**Cad**	**Crush**
Aspergillus carneus						+				
Aspergillus flavus					+					
Aspergillus fumigatus				+						
Aspergillus niger				+		+				
Aspergillus nomiae				+			+			
Aspergillus protuberus				+						
Aspergillus sydowii			+	+	+	+				
Aspergillus tamarii								+		
Candida duobushaemulonis				+						
Candida orthopsilosis								+		

Candida parapsilosis		+	+	+		+		+		+
Cordyceps tenuipes				+						
Corticiaceae sp.				+						
Fusarium oxysporum										+
Hypoxylon sp.										+
Meyerozyma caribbica								+		
Meyerozyma carpophila		+				+				
Penicillium citrinum			+			+	+			
Penicillium paxilli				+						
Phanerochaete chrysosporium				+		+				
Saccharomycetaceae sp.1						+				
Saccharomycetaceae sp.2						+				
Scedosporium boydii		+								+
Starmerella etchellsii	+									
Talaromyces purpureogenus				+						
Trichoderma harzianum										+

Total number of species	1	3	3	12	2	9	2	4	0	5
Total number of species per group	4		13		10		6		5	

Cad = termite cadavers, Crush = crushed termites. The symbol "+" refers to the presence of the fungi species in the given host and isolation technique.

III.1.4.1.3. Phylogenetic trees of fungi species and genetic distances

We have compared evolutionary distances between fungi species isolated on termites during this study and to assess their level of relatedness and speciation process. The pairwise genetic distances between fungi species based on the Jukes-Cantor model ranged from 0 to 1.18. The shortest genetic distances were found between *Aspergillus carneus* and *A. protiberus* (JC index = 0), between *A. carneus* and *A. sydowii* (JC index = 0.005), between *A. flavus* and *A. tamari* (JC index = 0.03) and between *A. nomiae* and *A. flavus* (JC index = 0.04). The longest genetic distances were observed between *Meyerozyma carpophila* and Saccharomycetes sp.2 (JC index = 1.13), between *Starmerella etchellsii* and *A. nomiae* (JC index = 1.18) and between *M. caribbica* and Corticiaceae sp. (JC index = 1.19). The pairwise genetic distances between fungi species based on the number of base differences ranged from 0 to 257 bp. The smallest differences were found between *A. carneus* and *A protuberus* (d= 0 bp), between *A. carneus* and *A. sydowii* (d = 2bp) and between *A. sydowii* and *A. protuberus* (d = 2 bp). The largest

differences were found between *M. caribbica* and Corticiaceae sp. (d = 257 bp), between *M. carpophila* and *Scedosporium boydii* (d = 242 bp) and between *A. tamari* and *M. carpophila* (d = 239 bp).

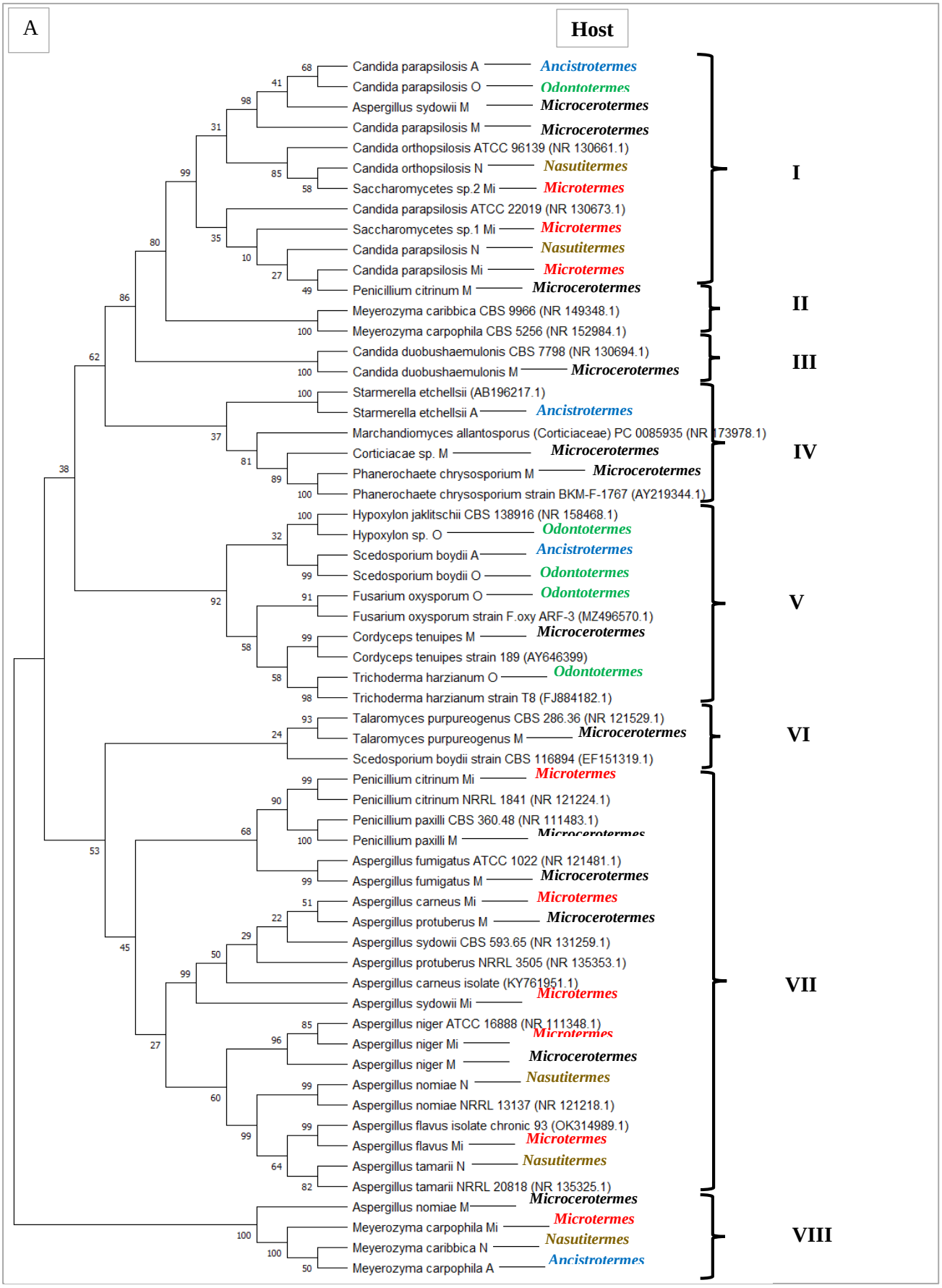
When studying the evolutionary history of fungi isolated from each termite group using a cladogram (Maximum livelihood's tree), we distinguished 8 lineages (Figure 35A). The lineage **I** with a strong bootstrap node (bootstrap = 99) comprised 6 fungi species mainly belonging to the Class Saccharomycetes but we also noted a certain level of relatedness between these Saccharomycetes species and strains of *M. sydowii* and *Penicillium citrinum* both isolated from *Microcerotermes* spp. The lineage **I** also revealed relatedness between unidentified Saccharomycetes sp.1 and *C. parapsilosis* and between the unidentified fungus Saccharomycetes sp.2 and *C. orthopsilosis*. The lineage **II** with a strong bootstrap node (bootstrap = 100) comprised 2 species of genus *Meyerozyma* (*M. caribbica* and *M. carpophila*) taken from genebank accessions. The lineage **III** with a strong bootstrap node (bootstrap = 100) was that of *C. duobushaemulonis* and showed relatedness between the strain isolated on termites and the accession from genebank. The lineage **IV** with a weak bootstrap node (bootstrap = 37) comprised 4 fungi species in 2 branches: the branch of *Starmerella etchellsii* (Class of Saccharomycetes) (bootstrap = 100) and the branch of Basidiomycota (bootstrap = 81). This lineage also revealed a close relatedness between the unidentified fungus Corticiaceae sp. and the accession NR173978.1 (*Marchandiomyces allantosporus*, family Corticiaceae). The lineage **V** with a strong bootstrap node (bootstrap = 92) comprised 6 fungi species all belonging to the Class of Sordariomycetes. In this lineage, all species perfectly matched its related accession in NCBI genebank and we noted a close relatedness between the unidentified fungus *Hypoxylon* sp. isolated from termites with the accession NR158468.1 (*H. jaklitschii*) from genebank. The lineage **VI** with a weak bootstrap node (bootstrap = 24) comprised 2 fungi species: *Talaromyces purpureogenus* (Class of Eurotiomyces) and the accession of *S. boydii* (Class of Sordariomycetes), showing a certain level of relatedness between these two species from different classes. The lineage **VII** with a weak bootstrap node (bootstrap = 27) was the largest lineage with 10 fungi species all belonging to the Class Eurotiomycetes and genera *Penicillium* and *Aspergillus*. In this lineage, 3 branches were differentiated: the branch of *Penicillium* spp. + *A. fumigatus* (bootstrap = 68), the branch of *A. carneus* + *A. protuberus* + *A. sydowii* (bootstrap = 99) and the branch of *A.niger* + *A. nomiae* + *A. flavus* + *A. tamari* (bootstrap = 60). All fungi species in this lineage were closely related to their corresponding accession in genebank. The last lineage, lineage **VIII**, with a strong bootstrap node (bootstrap = 100) was

that of *Meyerozyma* spp. (Class of Saccharomycetes) isolated from termites which were evolutionary distant from corresponding accessions from genebank.

When studying the evolutionary history of fungi isolated from each termite group using a phenogram (Neighbor-Joining's tree), we distinguished 12 lineages (Figure 35B). The lineage **I** with a weak bootstrap node (bootstrap = 48) comprised 4 fungi species mainly belonging to the Class of Saccharomycetes. This lineage showed close relatedness between *C. parapsilosis*, Saccharomycetes sp.1, *P. citrinum* and *A. sydowii* both isolated from *Microcerotermes* spp.. The lineage **II** with a weak bootstrap node (bootstrap = 40) comprised 2 fungi species and showed a certain relatedness between the unidentified fungus species Saccharomycetes sp.2 and *C. orthopsilosis*. The lineage **III** with a strong bootstrap node (bootstrap = 100) was that of *Meyerozyma* accessions (*M. caribbica* and *M. carpophila*) taken in genebank. The lineage **IV** with a strong bootstrap node (bootstrap = 91) was that of *C. duobushaemulonis* and showed evolutionary proximity between the fungi strain isolated from termites and the corresponding accession in genebank. The lineage **V** with a strong bootstrap node (bootstrap = 97) was that of *Hypoxylon* spp. and showed a close relatedness between *Hypoxylon* sp. isolated on termites and the accession NR158468.1 (*H. jaklitschii*) from genebank. The lineage **VI** with a weak bootstrap node (bootstrap = 21) comprised 4 fungi species and was divided in 2 branches: the branch of *S. etchellsii* + Corticiaceae sp and the branch of M. *allantosporus* (family Corticiaceae) + *P. chrysosporium*. This lineage showed that, in contrary to the cladogram, the unidentified fungus Corticiaceae was evolutionarily closer to *S. etchellsii* (Class of Saccharomycetes) than to M. *allantosporus* (family Corticiaceae) in the phenogram. The lineage **VII** with a weak bootstrap node (bootstrap = 24) comprised 4 fungi species all belonging to Class Sordariomycetes. In this lineage, all species perfectly matched its related accession in NCBI genebank with strong bootstrap nodes. The lineage **VIII** with a strong bootstrap node (bootstrap = 84) was that of *P. citrinum* and showed relatedness between the strain isolated on termites and the corresponding accession in genebank. The lineage **IX** with a very weak bootstrap node (bootstrap = 12) comprised 6 fungi species mainly belonging to Class of Eurotiomycetes. In this lineage, all the species perfectly matched their corresponding accessions in genebank except *S. boydii* (Class Sordariomycetes) which was found evolutionarily close to *A. niger* (Accession NR 111348.1). The lineage **X** with a strong bootstrap node (bootstrap = 83) comprised 3 fungi species all belonging to genus *Aspergillus* (*A. niger, A. sydowii* and *A. carneus*). The lineage **XI** with a weak bootstrap node (bootstrap = 19) comprised 2 fungi species (*T. purporeogenus* and *P. paxilli*). Each fungi species in this lineage was closely related to its corresponding accession in genebank. The last lineage, lineage

XII, with a strong bootstrap node (bootstrap = 99) was that of *Meyerozyma* spp. (Class of Saccharomycetes) isolated from termites which were evolutionary distant from corresponding accessions from genebank.

The BLAST results for unidentified fungi species showed that Corticiaceae sp. had 97.99% similarity with unidentified Corticiaceae sp. P10542EM1CC586 in the NCBI database and sampled in Panama. Saccharomycetes sp.1 had 94.54% similarity with *C. parapsilosis* and Saccharomycetes sp.2 had 94.41 similarity with *C. orthopsilosis*.


A
Host
Candida parapsilosis A
Ancistrotermes
Candida parapsilosis O
Odontotermes
Aspergillus sydowii M
Microcerotermes
Candida parapsilosis M
Microcerotermes
Candida orthopsilosis ATCC 96139 (NR 130661.1)
Candida orthopsilosis N
Nasutitermes
Saccharomycetes sp.2 Mi
Microtermes
Candida parapsilosis ATCC 22019 (NR 130673.1)
Saccharomycetes sp.1 Mi
Microtermes
Candida parapsilosis N
Nasutitermes
Candida parapsilosis Mi
Microtermes
Penicillium citrinum M
Microcerotermes
Meyerozyma caribbica CBS 9966 (NR 149348.1)
Meyerozyma carpophila CBS 5256 (NR 152984.1)
Candida duobushaemulonis CBS 7798 (NR 130694.1)
Candida duobushaemulonis M
Microcerotermes
Starmerella etchellsii (AB196217.1)
Starmerella etchellsii A
Ancistrotermes
Marchandiomyces allantosporus (Corticiaceae) PC 0085935 (NR 173978.1)
Corticiacae sp. M
Microcerotermes
Phanerochaete chrysosporium M
Microcerotermes
Phanerochaete chrysosporium strain BKM-F-1767 (AY219344.1)
Hypoxylon jaklitschii CBS 138916 (NR 158468.1)
Hypoxylon sp. O
Odontotermes
Scedosporium boydii A
Ancistrotermes
Scedosporium boydii O
Odontotermes
Fusarium oxysporum O
Odontotermes
Fusarium oxysporum strain F.oxy ARF-3 (MZ496570.1)
Cordyceps tenuipes M
Microcerotermes
Cordyceps tenuipes strain 189 (AY646399)
Trichoderma harzianum O
Odontotermes
Trichoderma harzianum strain T8 (FJ884182.1)
Talaromyces purpureogenus CBS 286.36 (NR 121529.1)
Talaromyces purpureogenus M
Microcerotermes
Scedosporium boydii strain CBS 116894 (EF151319.1)
Penicillium citrinum Mi
Microtermes
Penicillium citrinum NRRL 1841 (NR 121224.1)
Penicillium paxilli CBS 360.48 (NR 111483.1)
Penicillium paxilli M
Aspergillus fumigatus ATCC 1022 (NR 121481.1)
Aspergillus fumigatus M
Microcerotermes
Aspergillus carneus Mi
Microtermes
Aspergillus protuberus M
Microcerotermes
Aspergillus sydowii CBS 593.65 (NR 131259.1)
Aspergillus protuberus NRRL 3505 (NR 135353.1)
Aspergillus carneus isolate (KY761951.1)
Aspergillus sydowii Mi
Microtermes
Aspergillus niger ATCC 16888 (NR 111348.1)
Aspergillus niger Mi
Aspergillus niger M
Microcerotermes
Aspergillus nomiae N
Nasutitermes
Aspergillus nomiae NRRL 13137 (NR 121218.1)
Aspergillus flavus isolate chronic 93 (OK314989.1)
Aspergillus flavus Mi
Microtermes
Aspergillus tamarii N
Nasutitermes
Aspergillus tamarii NRRL 20818 (NR 135325.1)
Aspergillus nomiae M
Microcerotermes
Meyerozyma carpophila Mi
Microtermes
Meyerozyma caribbica N
Nasutitermes
Meyerozyma carpophila A
Ancistrotermes
I
II
III
IV
V
VI
VII
VIII

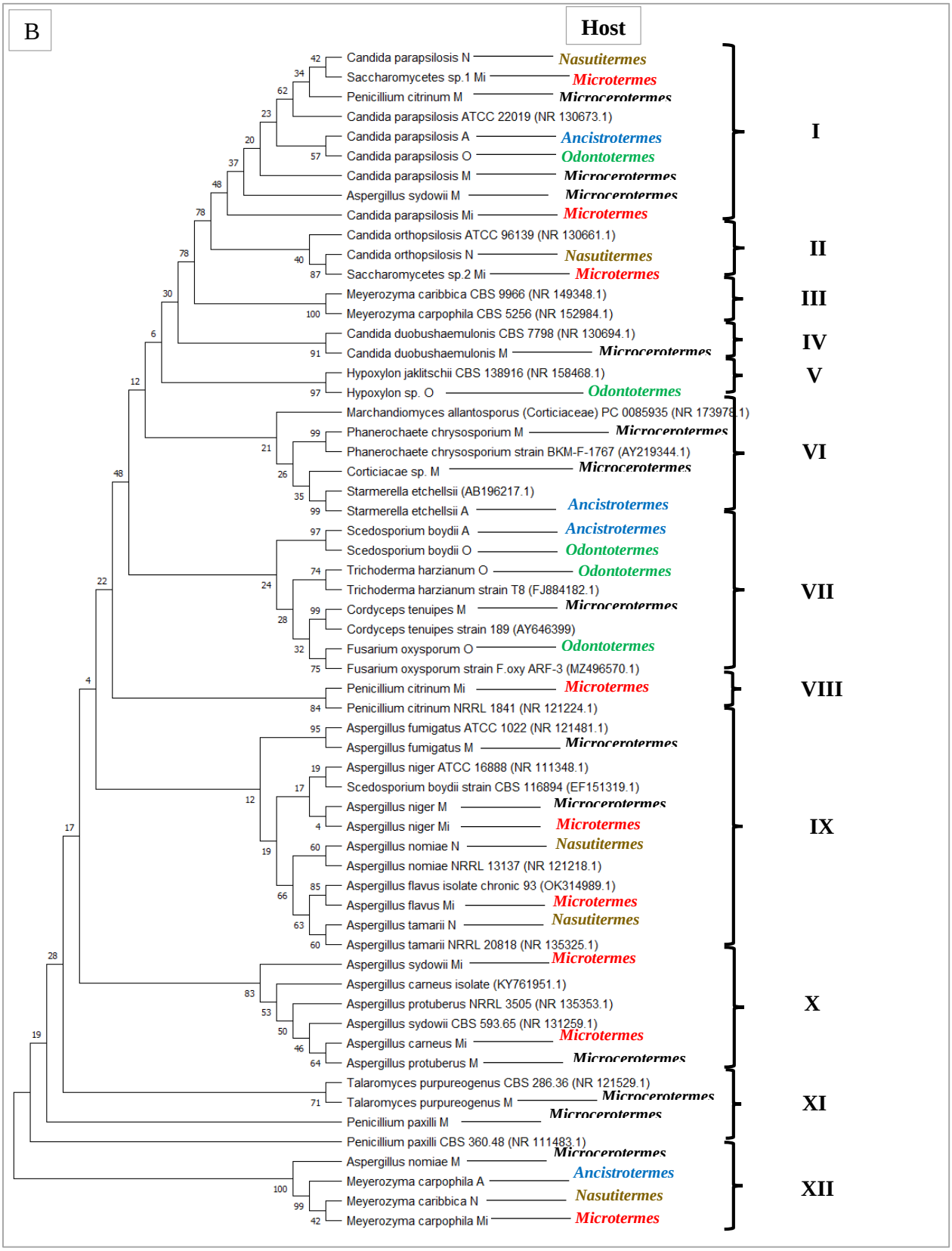


Figure 35. Phylogenetic trees built from sequences of fungi species isolated on termites and supplemented with sequences of related fungi species from NCBI genebank. Trees were rooted at midpoint. **A**= Maximum likelihood's tree built using the Kimura-2-parameter model +

Gamma distributed patterns (K2+G, BIC = 27778.063), **B**= Neighbor-Joining method's tree built using distances from the Kimura-2-parameter model + Gamma distributed patterns (K2 +G). All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 919 positions in the final dataset.

III.1.4.2. Diversity and parasitism parameters of parasitic mites screened on termites

III.1.4.2.1. Parasitism parameters of mites associated with termites and relationships with shade management, seasonality and termite host groups

In total, we examined 6,430 termites of which 129 were parasitized by mites (2.02 % of parasitism rate). The total parasite abundance was 145 mites and the mean density was 0.02 mite/termite. The number of termites examined and the parameters of mites varied from a system to another, from a termite species to another and from a season to another. Regarding production systems (Table XVII), the highest number of termites investigated was obtained in heavy shaded systems (1775 individuals) followed by intermediate systems (1590 individuals). Full sun systems recorded the lowest number of termites examined (610 individuals). The number of termites examined strongly depended on the availability of each termite species per system. Heavy shaded systems also recorded the highest number of termites infested (78), parasitism rate (4.39%), total parasite abundance (91 mites) and mean parasite density per termite (0.05 ± 0.01 mite/termite). Rustic and low shaded systems followed with 23 and 18 infested termites respectively (1.87% and 1.47% of parasitism rate respectively). In full sun systems, we did not record any infested termites. Significant differences were found between systems concerning parasitism rate ($\chi^2 = 81.23$, $P < 0.001$) and parasite density ($H = 81.66$, $P < 0.001$).

Regarding termite species (Table XVIII), *Microcerotermes* spp. recorded the highest number of termites examined (2975 individuals) followed by *Microtermes* spp. (1675 individuals). *Odontotermes* spp. recorded the lowest number of termites examined (475 individuals). *Microcerotermes* spp. also recorded the highest number of infested termites (79 individuals), parasitism rate (2.66%), total parasite abundance (87 mites) and mean parasite density per termite (0.03 ± 0.01 mite/termite). *Microtermes* spp. and *Nasutitermes* sp. followed with 24 and 14 termites infested respectively, although *Nasutitermes* sp. had a higher parasitism rate (2.12%) than *Microtermes* spp. (1.43%). *Odontotermes* spp. showed the lowest number of infested termites (3 individuals) and parasitism rate (0.63%). The lowest mean parasite density per termite was observed in *Nasutitermes* sp. (0.01 ± 0.01). Significant differences were found between termite species concerning parasitism rate ($\chi^2 = 15.01$, $P = 0.005$) and parasite density

(H = 14.93, P = 0.005). Regarding the seasonal variation (Table XIX), rainy seasons showed higher values in terms of termites examined, termites infested and total parasite abundance compared to the dry season. We observed statistically higher values of parasitism rate (χ^2 = 18.59, P < 0.001) and parasite density (U = 5.13e+06, P < 0.001) during the rainy season (2.66% and 0.03 ± 0.01 mites/termites respectively) than during the dry season (1.13% and 0.03 ± 0.01 mites/termites respectively).

The binary logistic regression analysis showed no significant effect of shade management on termite infestation by mites, but it however revealed that increase of shade cover highly increases the odds of termite infestation. The termite species considered and the season significantly affected the odds of termite infestation (Table XX). The aboveground termite species, *Nasutitermes* sp. (OR = 12.12, P < 0.001) and *Microcerotermes* spp. (OR = 4.67, P = 0.009), were significantly more susceptible to termite infestation than belowground termite species. We also noticed that termite infestation by mites had significantly more chances to happen during rainy season than dry season (OR = 2.08, P < 0.001).

Table XVII. Parasitism parameters of parasitic mites screened on termites per agroforestry systems. Parasite density per termite is expressed in mean ± SE.

Parameters	**Agroforestry systems**					**Total**
	Rustic	Heavy shaded	Intermediate	Low shaded	Full sun	
Nb of termites examined	1230	1775	1590	1225	610	6430
Nb of infested termites	23	78	10	18	0	129
Parasitism rate (%)	1.87a	4.39b	0.63b	1.47c	0d	2.01
Total parasite abundance	25	91	10	19	0	145
Parasite density per termite	0.02 ± 0.01A	0.05 ± 0.01B	0.01 ± 0.01CD	0.02 ± 0.01AC	0.0 ± 0.0D	0.02 ± 0.01

Same minor letters in rows show no significant differences in parasitism rate between systems based on z test for proportions. Same capital letters in rows show no significant differences in parasite density per termite between systems based on Mann-Whitney pairwise comparisons.

Table XVIII. Parasitism parameters of parasitic mites screened on termites per key termite species. Parasite density per termite is expressed in mean ± SE.

Parameters	Termite species					Total
	Ancistrotermes spp.	*Microcerotermes* spp.	*Microtermes* spp.	*Nasutitermes* sp.	*Odontotermes* spp.	
Nb of termites examined	645	2975	1675	660	475	6430
Nb of infested termites	9	79	24	14	3	129
Parasitism rate (%)	1.40abc	2.66a	1.43bc	2.12ab	0.63c	2.01
Total parasite abundance	10	87	29	14	5	145
Parasite density per termite	0.02 ± 0.01AB	0.03 ± 0.01A	0.02 ± 0.01B	0.01 ± 0.01AB	0.02 ± 0.01B	0.02 ± 0.01

Same minor letters in rows show no significant differences in parasitism rate between systems based on z test for proportions. Same capital letters in rows show no significant differences in parasite density per termite between systems based on Mann-Whitney pairwise comparisons.

Table XIX. Parasitism parameters of parasitic mites screened on termites per season. Parasite density per termite is expressed in mean ± SE.

Parameters	Season		Total
	Rainy	Dry	
Nb of termites examined	3690	2740	6430
Nb of infested termites	98	31	129
Parasitism rate (%)	2.66a	1.13b	2.01
Total parasite abundance	112	33	145
Parasite density per termite	0.03 ± 0.01A	0.01 ± 0.01B	0.02 ± 0.01

Same minor letters in rows show no significant differences in parasitism rate between systems based on z test for proportions. Same capital letters in rows show no significant differences in parasite density per termite between systems based on Mann-Whitney pairwise comparisons.

Table XX. Binary logistic regression of the presence of mites on termite exoskeleton per shade management, termite species and seasons. Significant factors are highlighted in bold.

Factors	Odds ratio	Pr(>|z|)	CI low (2.5%)	CI high (97.5%)
Shade management				
Rustic	8.46e+06	0.97	12.36	5.67e+78
Heavy shaded	2.11e+07	0.97	30.77	1.41e+79
Intermediate	2.86e+06	0.97	4.17	1.91e+78
Low shaded	3.72e+06	0.97	5.43	2.49e+78
Full sun	1		-	-
Termite species				
Ancistrotermes spp.	2.51	0.17	1.61	6.05
Microcerotermes spp.	**4.67**	**0.009**	**4.82**	**2.86**
Microtermes spp.	2.37	0.16	1.17	1.37
Nasutitermes sp.	**12.12**	**< 0.001**	**6.69**	**21.01**
Odontotermes spp.	1		-	-
Season				
Rainy season	**2.08**	**< 0.001**	**1.39**	**2.72**
Dry season	1		-	-

CI: confidence interval of the odds ratio

III.1.4.2.2. Inventory of parasitic mites screened on termites, locations and relationship with shade management

In total we recorded 145 individuals of mites associated to termites, all belonging to Super-Order Acariforme, two Orders, three Families and 19 species (Table XXI) (Appendix 1). The individuals obtained were all deutonymphs (second larval development stage) and all occasional parasites on termites. The Order Sarcoptiforme (Astigmata) was the most represented both numerically (126 individuals) and in species richness (15 mite species) in the unique Family Acaridae. The Order Trombidiforme (Prostigmata) recorded 19 individuals in two Families and 4 mite species (Pygmephoridae and Scutacaridae). The anchoring position of mites on termite bodies covered almost all body parts (Figure 36). The preferred regions were legs (44%) and head capsule (42%).

The most diversified systems were heavy shaded systems with 11 species, followed by low shaded systems (8 species) and intermediate systems (7 species). In terms of abundance, heavy shaded systems recorded the highest value (91 individuals), followed by rustic systems (25 individuals) and low shaded systems (19 individuals). Acaridae gen.1 sp.1 was the most abundant species on termite samples across systems (35 individuals) followed by Acaridae gen.3 sp. (21 individuals) and *Acotyledon* sp. (19 individuals) (Table XXI). No species was found ubiquitous across all systems, species like Acaridae gen.1 sp.1, Acaridae gen.2 sp.1 and

Acaridae gen.3 sp. were found in at most 3 different systems. Within each system, Acaridae gen.1 sp.1 was the most abundant parasitic mite in heavily shaded systems (10 individuals in rustic and 24 in heavy shaded), Acaridae gen.3 sp. was the most abundant in intermediate systems (3 individuals) and *Caloglyphus* sp.3 was the most abundant in low shaded systems (9 individuals). We did not get parasitic mites from full sun systems.

Table XXI. Mite taxonomic composition and abundance across agroforestry systems

Mite species	**Rustic**	**Heavy shaded**	**Intermediate**	**Low shaded**	**Full sun**	**Total**
Order **Sarcoptiforme**						
Family **Acaridae**						
Acaridae gen.1 sp.1	10	24	1	-	-	35
Acaridae gen.1 sp.2	5	-	2	-	-	7
Acaridae gen.1 sp.3	9	-	-	-	-	9
Acaridae gen.1 sp.4	-	-	1	-	-	1
Acaridae gen.1 sp.5	1	1	-	-	-	2
Acaridae gen.2 sp.1	-	8	1	2	-	11
Acaridae gen.2 sp.2	-	2	1	-	-	3
Acaridae gen.2 sp.3	-	-	-	1	-	1
Acaridae gen.3 sp.	-	17	3	1	-	21
Acaridae gen.4 sp.	-	-	-	2	-	2
Acotyledon sp.	-	19	-	-	-	19
Caloglyphus sp.1	-	-	-	1	-	1
Caloglyphus sp.2	-	-	1	-	-	1
Caloglyphus sp.3	-	2	-	9	-	11
Caloglyphus sp.4	-	2	-	-	-	2
Order **Trombidiforme**						
Family **Pygmephoridae**						
Pygmephoridae sp.	-	9	-	-	-	9
Family **Scutacaridae**						
Coronipes sp.	-	4	-	-	-	4
Scutacarus sp.1	-	3	-	2	-	5
Scutacarus sp.2	-	-	-	1	-	1
Total	25	91	10	19	0	145

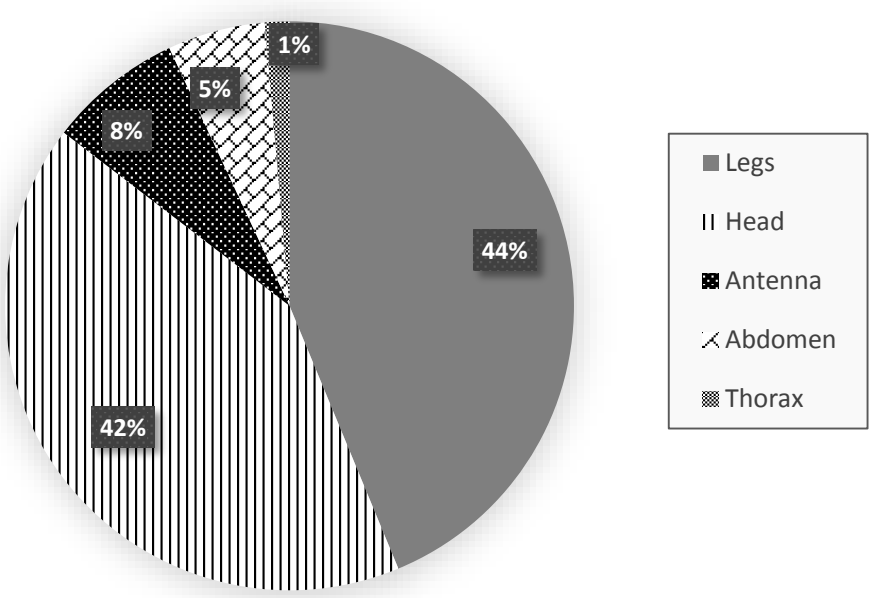


Figure 36. Frequency distribution of mites on the anchoring locations on termites

Diversity indexes showed that heavy shaded (H' = 1.99, J = 0.83) and intermediate systems (H'= 1.83, J= 0.94) are the most diversified systems in terms of mite composition and community structure, followed by low shaded systems (H' = 1.69, J = 0.81) (Table XXII). The Berger-Parker's index revealed a poor dominance of mite species among communities per system (d < 0.5), the highest value being recorded in low shaded systems (d = 0.47). The correspondence analysis of mite species per system revealed significant habitat preference among species (χ^2 = 219.02, df = 54, P < 0.001), with 3 clusters differentiated (Figure 37). The first cluster "rustic" encompasses species Acaridae gen.1 sp.2 and Acaridae gen.1 sp.3 which prefer rustic shaded systems, the second cluster "low" encompasses species Acaridae gen.2 sp.3, Acaridae gen.4 sp., *Caloglyphus* sp.1, *Caloglyphus* sp.3 and *Scutacarus* sp.2 favoring poorly shaded systems. The third cluster "moderate" gathering all the remaining mite species which prefer intermediate to moderate shaded systems.

Table XXII. Diversity indexes of mites associated to termites per cocoa agroforestry systems

Diversity indexes	Rustic	Heavy shaded	Intermediate	Low shaded	Full sun
Species richness S	4	11	7	8	-
Shannon H	1.19	1.99	1.83	1.69	-
Equitability J	0.85	0.83	0.94	0.81	-
Berger-Parker d	0.4	0.26	0.3	0.47	-

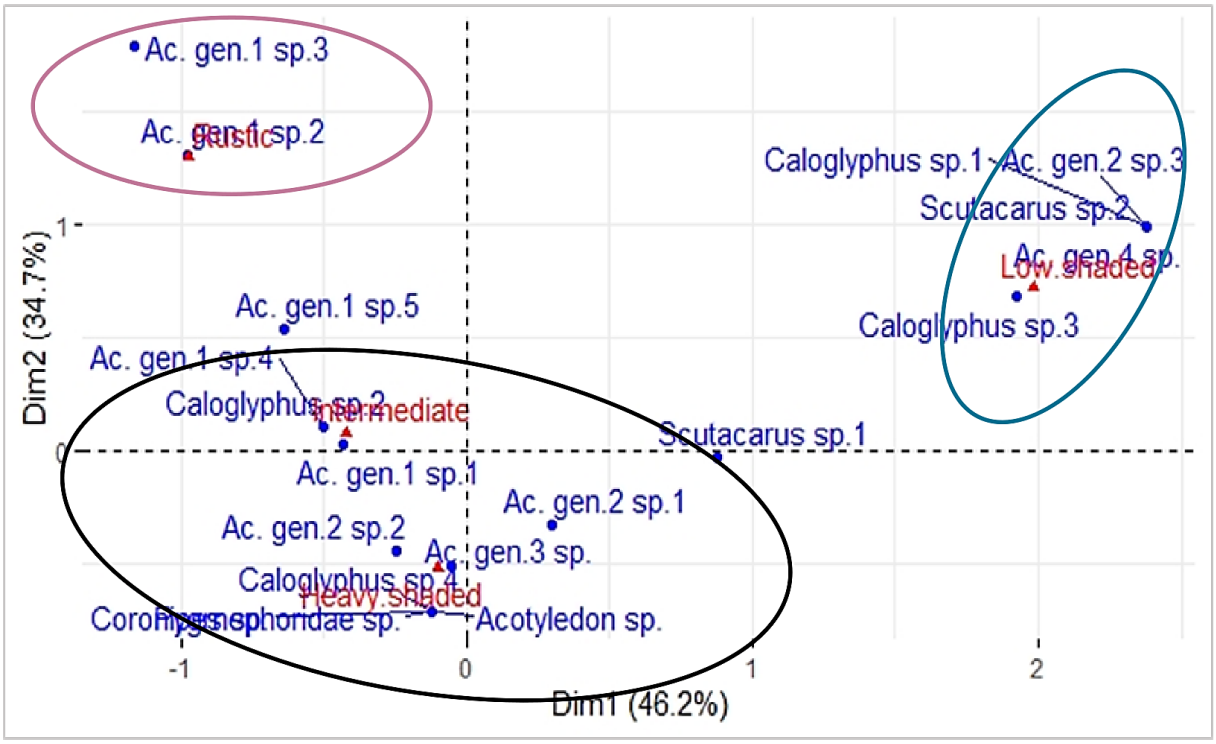


Figure 37. Correspondence analysis of mite species per system (χ^2 = 219.02, df = 54, P < 0.001). Pink ellipse = cluster "rustic", blue ellipse = cluster "low", black ellipse = cluster "moderate".

III.1.4.2.3. Relationship between parasitic mite composition and termite host group and seasonality

During this study, all termite species surveyed were associated with mites, but the species richness of mites per termite group varied from one group to another. The group with the richest mite community was *Microcerotermes* spp. (11 species) followed by *Ancistrotermes* spp. (7 species), *Microtermes* spp. (6 species), *Nasutitermes* sp. (5 species). *Odontotermes* spp. recorded the poorest mite community with 2 species. When comparing the species richness of mites between seasons, we noted that their communities were very similar with a slight advantage of rainy season (14 species) over dry season (12 species). The analysis of mite preference among termite pest species and season revealed a significant difference between mite species (χ^2 = 332.85, P < 0.001) (Figure 38). Among termite species, three clusters were differentiated: the first cluster "aboveground" gather species *Caloglyphus* sp.3, *Scutacarus* sp.1, *Scutacarus* sp.2, *Coronipes* sp. and most Acaridae species which preferred aboveground termite species (*Nasutitermes* sp. and *Microcerotermes* sp.); the second cluster "belowground" grouping species Acaridae gen.2 sp.3, Acaridae gen. sp.4, *Caloglyphus* sp.1, *Caloglyphus* sp.4 and Pygmephoridae sp. which favored belowground termite species (*Ancistrotermes* spp. and

Odontotermes spp.); the third cluster "*Microtermes*" that encompasses species *Acotyledon* sp. and *Caloglyphus* sp.2 which preferred *Microtermes* species. Regarding season, both dry and rainy seasons shared mite species from the same cluster "aboveground".

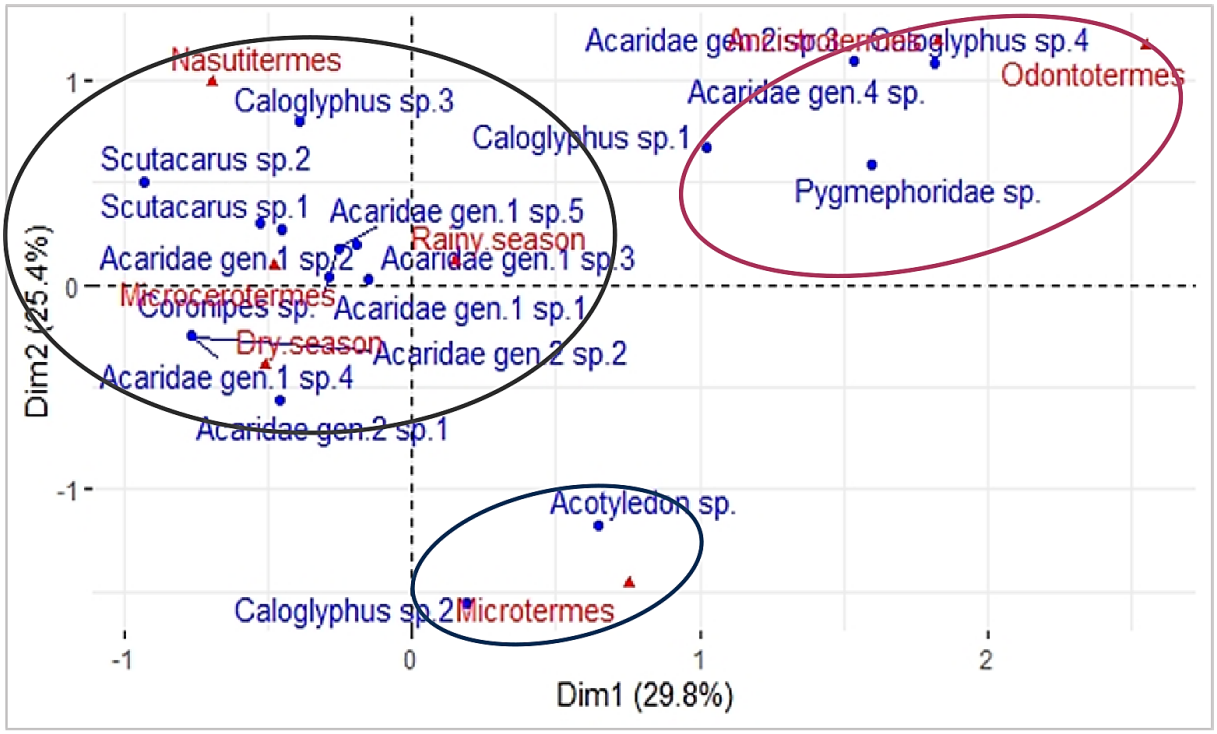


Figure 38. Correspondence analysis of mite species per termite group (χ^2 = 332.85, P < 0.001). Black ellipse = cluster "aboveground", pink ellipse = cluster "belowground", blue ellipse = cluster "*Microtermes*".

III.1.4.3. Diversity and parasitism parameters of parasitoid flies associated to termites

III.1.4.3.1. Parasitism parameters of parasitoid flies of termites and relationship with shade management, termite host groups and seasonality

We reared a total of 32,845 termites in boxes and we obtained 78 emerging flies for a low parasite rate of 0.24%, assuming that each fly emerged from a single termite. The mean number of emerging flies per rearing box was 0.23 ± 0.01. The sex ratio was 2.25 with male flies (54 individuals) more than twice as numerous as female flies (24 individuals). The parameters of emerging flies varied from a system to another, from a termite species reared to another and from a season to another. Regarding agroforestry systems (Table XXIII), heavy shaded systems recorded the highest number of termites reared in box (8500 individuals), followed by rustic systems (6400 individuals) and intermediate systems (6360 individuals). The highest parasite rates were obtained in intermediate systems (0.57%, 36 flies) and in heavy shaded (0.41%, 35 flies). We found significant differences in parasite rate between systems (χ^2

= 85.21, P < 0.001). The highest numbers of emerging flies per box were also obtained in intermediate systems (0.55 ± 0.04 flies) and in heavy shaded systems (0.41 ± 0.01 flies). Full sun systems did not record any emerging fly. We found significant differences in number of emerging flies per box between systems (H = 79.83, P < 0.001).

Regarding termite groups (Table XXIV), *Microcerotermes* spp. recorded the highest number of termites reared in box (9697 individuals), followed by *Microtermes* spp. (8200 individuals) and *Ancistrotermes* spp. (7708 individuals). We found significant differences in parasite rate between termite species (χ^2 = 93.92, P < 0.001). The highest parasite rate was obtained on *Odontotermes* spp. (0.78%, 43 emerging flies), followed by *Microcerotermes* spp. (0.29%, 28 emerging flies). *Nasutitermes* sp. and *Ancistrotermes* spp. were not parasitized and did not recorded any emerging fly. We also found significant differences in mean number of emerging flies per box between termite species (H = 32.13, P < 0.001). The highest numbers of emerging flies were observed in boxes containing *Odontotermes* spp. (0.69 ± 0.40 emerging flies per box), followed by *Microcerotermes* spp. (0.29 ± 0.05 emerging flies per box) and *Microtermes* spp. (0.09 ± 0.03 emerging flies per box). The sex ratio of emerging flies varied from a termite species to another. Belowground termite species (*Microtermes* spp. and *Odontotermes* spp.) were more infested by male flies while aboveground species (*Microcerotermes* spp.) were more infested by female flies. Seasonally, we also noted differences in parameters between dry and rainy season (Table XXV). The rainy season recorded a higher number of termites reared in boxes but samples from dry season recorded a higher number of emerging flies although no difference was found in parasite rate between seasons (χ^2 = 1.21, P = 0.27). However, the number of emerging flies per box was significantly higher in dry season than in rainy season (U = 16650, P < 0.001). Concerning the sex ratio of emerging flies, rainy season recorded more female emerging flies while dry season recorded mainly male emerging flies.

The binary logistic regression analysis (Table XXVI) showed no significant effects of shade management and termite species on fly emergence in boxes. However, heavily shaded systems have greater odds of fly emergence than poorly shaded systems. In addition, aboveground termite species are susceptible to fly infestation meanwhile odds show that belowground termite species avoid fly infestation. Seasonally, we noticed that termite infestation by parasitoid had significantly more chances to happen during rainy season than dry season (OR = 3.58, P = 0.006).

Table XXIII. Parasitism parameters of parasitoid flies screened on termites per agroforestry systems. The number of emerging flies per box is expressed in mean ± SE.

Parameters	**Agroforestry systems**					**Total**
	Rustic	Heavy shaded	Intermediate	Low shaded	Full sun	
Nb of termites reared in boxes	6400	8500	6360	6040	5545	32845
Nb emerging flies	4	35	36	3	0	78
Parasite rate (%)	0.06a	0.41b	0.57b	0.05a	0c	0.24
Nb of Emerging flies per box	0.06 ± 0.04A	0.41 ± 0.05B	0.55 ± 0.38A	0.05 ± 0.03A	0.0 ± 0.0A	0.23 ± 0.01
Nb of males (m)	1	15	35	3	0	54
Nb of females (f)	3	20	1	0	0	24
Sex ratio (m/f)	0.33	0.75	35	-	-	2.25

Same minor letters in rows show no significant differences in parasitism rate between systems based on z test for proportions. Same capital letters in rows show no significant differences in number of emerging flies per box between systems based on Mann-Whitney pairwise comparisons.

Table XXIV. Parasitism parameters of parasitoid flies screened on termites per key termite species. The number of emerging flies per box is expressed in mean ± SE.

Parameters	**Termite groups**					**Total**
	Ancistrotermes spp.	*Microcerotermes* spp.	*Microtermes* spp.	*Nasutitermes* sp.	*Odontotermes* spp.	
Nb of termites reared in boxes	7708	9697	8200	1719	5521	32845
Nb emerging flies	0	28	7	0	43	78
Parasite rate (%)	0a	0.29b	0.09a	0a	0.78c	0.24
Nb of emerging flies per box	0.0 ± 0.0A	0.29 ± 0.05B	0.09 ± 0.03AC	0.0 ± 0.0AC	0.69 ± 0.40BC	0.23 ± 0.01
Nb of males (m)	0	12	4	0	38	54
Nb of females (f)	0	16	3	0	5	24
Sex ratio (m/f)	-	0.75	1.33	-	7.6	2.25

Same minor letters in rows show no significant differences in parasitism rate between systems based on z test for proportions. Same capital letters in rows show no significant differences in number of emerging flies per box between systems based on Mann-Whitney pairwise comparisons.

Table XXV. Parasitism parameters of parasitoid flies screened on termites per seasons. The number of emerging flies per box is expressed in mean ± SE.

Parameters	Season		Total
	Rainy	Dry	
Nb of termites reared in boxes	17208	15637	32845
Nb emerging flies	36	42	78
Parasite rate (%)	0.06a	0.41a	0.24
Nb of emerging flies per box	0.20 ± 0.01A	0.26 ± 0.02B	0.23 ± 0.01
Nb of males (m)	15	39	54
Nb of females (f)	21	3	24
Sex ratio (m/f)	0.71	13	2.25

Same minor letters in rows show no significant differences in parasitism rate between systems based on z test for proportions. Same capital letters in rows show no significant differences in number of emerging flies per box between systems based on Mann-Whitney pairwise comparisons.

Table XXVI. Binary logistic regression of the emergence of parasitoid flies in rearing boxes by shade management, termite species and seasons. Significant factors are highlighted in bold.

Factors	Odds ratio	Pr(>|z|)	CI low (2.5%)	CI high (97.5%)
Shade management				
Rustic	3.44e+07	0.99	0.00	2.9e+292
Heavy shaded	4.96e+08	0.99	0.00	2.9e+300
Intermediate	3.41e+07	0.99	0.00	3.2e+285
Low shaded	4.68e+07	0.99	0.00	3.9e+191
Full sun	1		-	-
Termite species				
Ancistrotermes spp.	0.001	0.99	0.00	1.55e+23
Microcerotermes spp.	2.70	0.06	0.98	7.92
Microtermes spp.	0.70	0.57	0.20	2.38
Nasutitermes sp.	0.001	0.99	0.00	5.21e+64
Odontotermes spp.	1			
Season				
Rainy season	**3.58**	**0.006**	**1.48**	**9.32**
Dry season	1		-	-

III.1.4.3.1. Inventory of parasitoid flies screened on termites and relationship with shade management

From the visualization of PCR products under UV light, we identified amplification bands of fly mitochondrial COI segment at 710 bp (Figure 39). From morphological and molecular identification, we have recorded in total 78 individuals of emerging flies from termite boxes all belonging to the family Phoridae, 8 genera and 14 species (Table XXVII). *Megaselia* was the richest genus with 6 species followed by *Apocephalus* with 2 species. All the other

genera were represented with only one species. The community of parasitoid flies was numerically dominated by *Melaloncha* sp. (36 individuals), *Megaselia* sp.1 (19 individuals) and *Megaselia scalaris* (8 individuals).

The most diversified agroforestry system was heavy shaded system with 12 species followed by rustic system (3 species), intermediate and low shaded systems (2 species each) (Table XXVII). In full sun systems, we did not recorded emergence of parasitoid flies. In terms of abundance, intermediate systems recorded the highest value with 36 individuals closely followed by heavy shaded systems (35 individuals). Flies were poorly represented in rustic and low shaded systems (4 and 3 individuals respectively). No fly species was found ubiquitous across all systems. *Megaselia scalaris* was the only species found in 3 different systems (rustic, heavy shaded and low shaded systems). *Melaloncha* sp. and *Megaselia* sp.1 were found in only two distinct systems. Within systems, *Megaselia scalaris* was the most abundant fly species in rustic and low shaded systems (2 individuals each); *Melaloncha* sp. (35 individuals) was the main species in intermediate systems and *Megaselia* sp.1 was the most abundant in heavy shaded systems with 18 individuals.

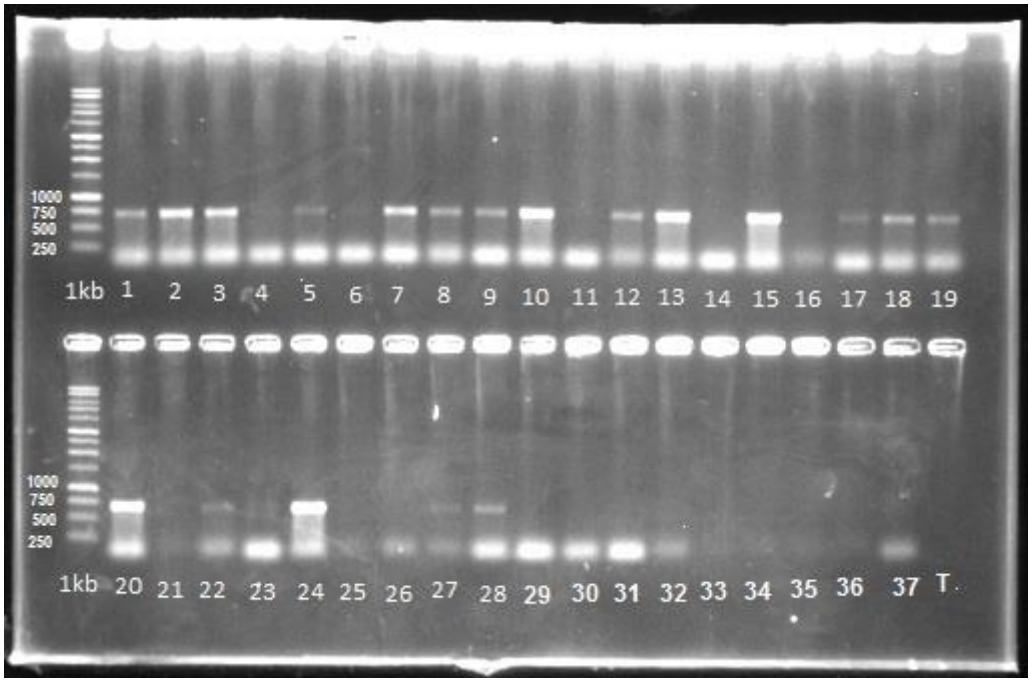


Figure 39. PCR amplification bands of fly mitochondrial COI segment visualized under UV light. Numbers refer to different fungi samples and T is the negative control. 1kb is the DNA ladder. Expected band at 710 bp.

Table XXVII. Parasitoid fly taxonomic composition and abundance across agroforestry systems

Species of flies	Rustic	Heavy shaded	Intermediate	Low shaded	Full sun	Total
Aenictacantha sp.	-	1	-	-	-	1
Apocephalus sp.1	-	2	-	-	-	2
Apocephalus sp.2	1	1	-	-	-	2
Commoptera sp.	-	1	-	-	-	1
Megaselia infraposita	-	1	-	-	-	1
Megaselia nigrescens	-	1	-	-	-	1
Megaselia scalaris	2	4	-	2	-	8
Megaselia sp.1	1	18	-	-	-	19
Megaselia sp.2	-	2	-	-	-	2
Megaselia sp.3	-	2	-	-	-	2
Melaloncha sp.	-	-	35	1	-	36
Paurophora sp.	-	1	-	-	-	1
Stethopathusa sp.	-	-	1	-	-	1
Triphleba sp.	-	1	-	-	-	1
Total	**4**	**35**	**36**	**3**	**0**	**78**

Diversity indexes showed that heavy shaded systems (H' = 1.79, J = 0.65) were the most diversified in terms of parasitoid fly composition, followed by rustic systems (H' = 1.04, J = 0.95) (Table XXVIII). Intermediate systems were the least diversified systems (H' = 0.13, J = 0.18) and the Berger-Parker's index (d = 0.97) showed that these systems hugely dominated by one species (*Melaloncha* sp.). A high dominance was also recorded in low shaded systems with Berger-Parker's d = 0.67. The correspondence analysis of fly species per system revealed significant habitat preference among species (χ^2 = 102.46, P < 0.001), with 3 clusters differentiated (Figure 40). The first cluster "Extreme" gathers together species *Megaselia scalaris* and *Apocephalus* sp.2 that preferred the opposite extreme systems with a notable advantage for rustic systems. The second cluster "Intermediate" encompasses species *Melaloncha* sp. and *Stethopathusa* sp. that have a preference for intermediate shading systems; and the third cluster "Heavy shaded" that grouped 10 species among which *Megaselia* spp., *Paurophora* sp. and *Commoptera* sp.. All these species had a strong preference for heavy shaded systems.

Table XXVIII. Diversity indexes of parasitoid flies of termites per cocoa agroforestry systems

Diversity indexes	Rustic	Heavy shaded	Intermediate	Low shaded	Full sun
Species richness S	3	12	2	2	-
Shannon H'	1.04	1.79	0.13	0.64	-
Equitability J	0.95	0.65	0.18	0.92	-
Berger-Parker d	0.5	0.31	0.97	0.67	-

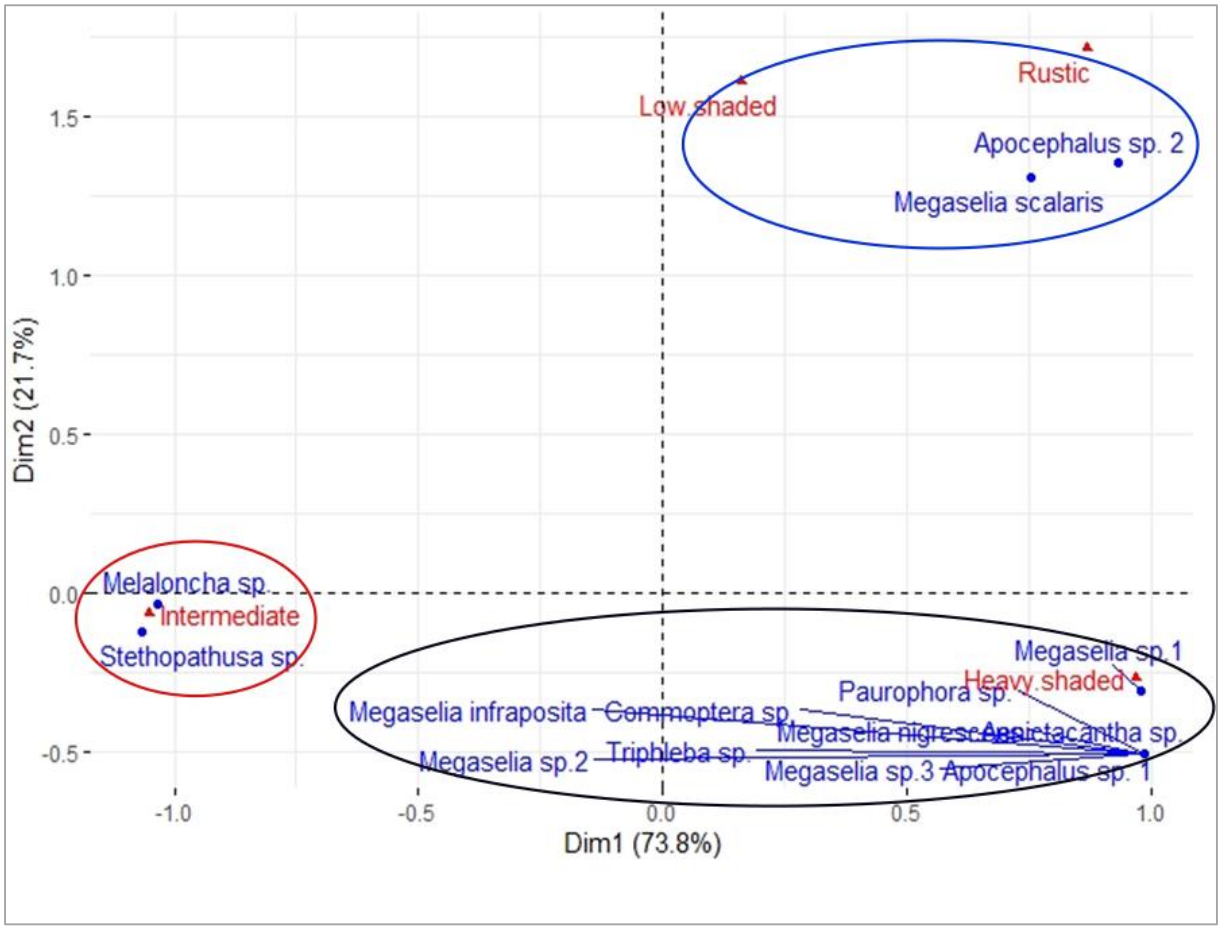


Figure 40. Correspondence analysis of parasitoid fly species per agroforestry system ($\chi^2 = 102.46$, $P < 0.001$). Black ellipse = cluster "Heavy shaded", red ellipse = cluster "Intermediate", blue ellipse = cluster "Extreme".

III.1.4.3.2. Relationships between parasitoid fly composition and termite host group and seasonality

During this study, 3 termite groups out of 5 surveyed were parasitized by flies (*Microcerotermes* spp., *Microtermes* spp. and *Odontotermes* spp.), and the species richness of flies per termite group varied from one group to another. The group recording the richest fly community was *Microcerotermes* spp. (11 species) followed by *Odontotermes* spp. (7 species)

and *Microtermes* spp. (6 species). When comparing the species richness of parasitoid flies between seasons, we noted that the fly community from rainy season (11 species) was more than twice as rich as the fly community from dry season (5 species). The analysis of parasitoid fly preference among termite pest species and season revealed a significant difference between fly species (χ^2 = 144.13, P < 0.001) (Figure 41). Among parasitized termite groups, three clusters were differentiated: the first cluster "Aboveground" comprised 9 fly species that preferred the aboveground *Microcerotermes* spp. as host among which *Megaselia* sp.1 was numerically the most important; the second cluster "*Odontotermes*" comprised species *Melaloncha* sp. and *Stethopathusa* sp. which had a strong preference towards *Odontotermes* spp. as host; and the third group "*Microtermes*" encompassed species *Megaselia scalaris*, *Megaselia* sp.3 and *Apocephalus* sp.1 which showed a notable preference towards *Microtermes* spp. as host.

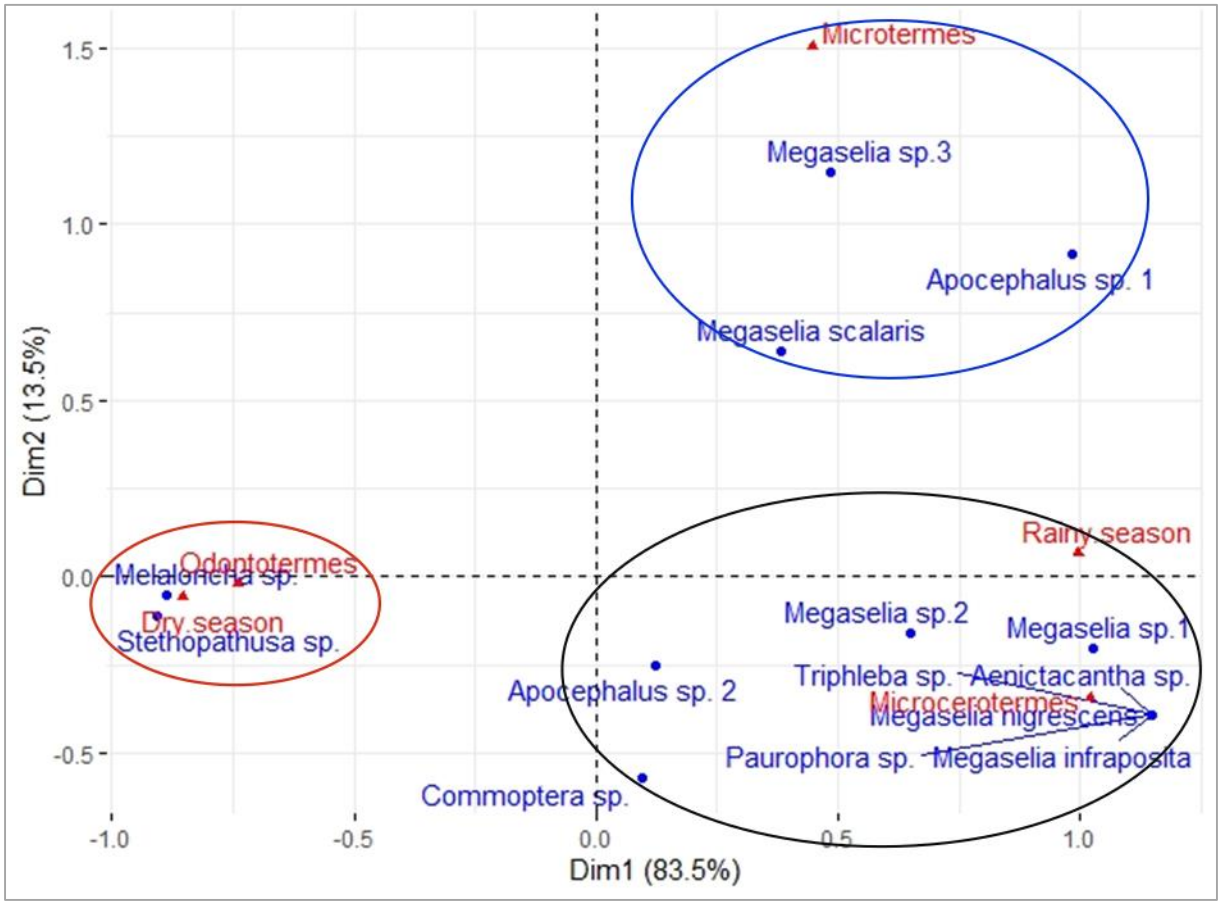


Figure 41. Correspondence analysis of mite species per termite group (χ^2 = 144.13, P < 0.001). Black ellipse = cluster "Aboveground", red ellipse = cluster "*Odontotermes*", blue ellipse = cluster "*Microtermes*".

III.1.4.3.3. Phylogenetic trees of *Megaselia* species and genetic distances

We have compared evolutionary distances between unidentified *Megaselia* species and other well-known *Megaselia* spp. to have a close view on their identity (through their level of relatedness) and relatedness. The pairwise genetic distances between *Megaselia* species based on the Jukes-Cantor model varied from 0.02 to 0.18. The shortest genetic distances were observed between *M. scalaris*-PH2 and the accession KX832638.1 (*M. scalaris*) with JC index 0.02, between *M. scalaris*-PH2 and *Megaselia* sp.2 (JC index = 0.05), between *Megaselia* sp.3 and the accession KX774941.1 (*M. zonata*) (JC index = 0.08), and between *Megaselia* sp.1 and the accession KX774941.1 (*M. zonata*) (JC index = 0.10). The longest distance was found between Megaselia sp.1 and Megaselia sp.2 (JC index = 0.18). The pairwise genetic distances between *Megaselia* species based on the number of base differences showed differences ranging from 9 to 93 bp. The smallest difference was found between *M. scalaris*-PH2 and the accession KX832638.1 (*M. scalaris*) (d = 9 bp) and the largest between Megaselia sp.2 and the accession KP693328.1 (*M. hibernans*) (d = 93 bp).

The study of the evolutionary history of *Megaselia* species using a cladogram (Maximum livelihood's tree) (Figure 42A) revealed that three lineages are differentiated the genus with all weak bootstrap node values. The lineage **I** (bootstrap = 14) comprised 6 species and showed a relatedness between *M. spiracularis* (Accession JQ941752.1) and *Megaselia* sp.1. The lineage **II** (bootstrap = 14) comprised 5 species and showed relatedness between species *Megaselia* sp.2 and *M. scalaris* (Accession KX832638.1), and between species *Megaselia* sp.3 and *M. zonata* (Accession KX774941.1). The lineage **III** (bootstrap = 29) comprised 3 species (*M. hiberans*, *M. lucifrons* and *M. subnitida*).

The same *Megaselia* phylogeny studied using a phenogram (Neighbor-Joining's tree) (Figure 42B) distinguished 4 lineages. The lineage **I** with a weak bootstrap node (bootstrap = 15) comprised 4 species and showed relatedness between species *Megaselia* sp.1 and *M. spiracularis* (Accession JQ941752.1) and between species *Megaselia* sp.3 and *M. zonata* (Accession KX774941.1). The lineage **II** with a weak bootstrap node (bootstrap = 30) comprised 3 species (*M. hiberans*, *M. lucifrons* and *M. subnitida*). The lineage **III** with an average bootstrap node (bootstrap = 66) comprised 4 species (*M. bifida*, *M. hilaris*, *M. clemonsi* and *M. lata*). The lineage IV with a strong bootstrap node (bootstrap = 93) comprised 2 species and showed relatedness between species *M. scalaris* (Accession KX832638.1) and *Megaselia* sp.2.

The NCBI BLAST results for unidentified *Megaselia* species showed that *Megaselia* sp.1 had 96.74% similarity with an unidentified Phoridae sp. BIOUG15337-A12 in the NCBI database and sampled in Pakistan. *Megaselia* sp.2 had 96.32% similarity with *M. scalaris* in the NCBI database and *Megaselia* sp.3 had 92.97% similarity with *M. zonata* in the NCBI database.

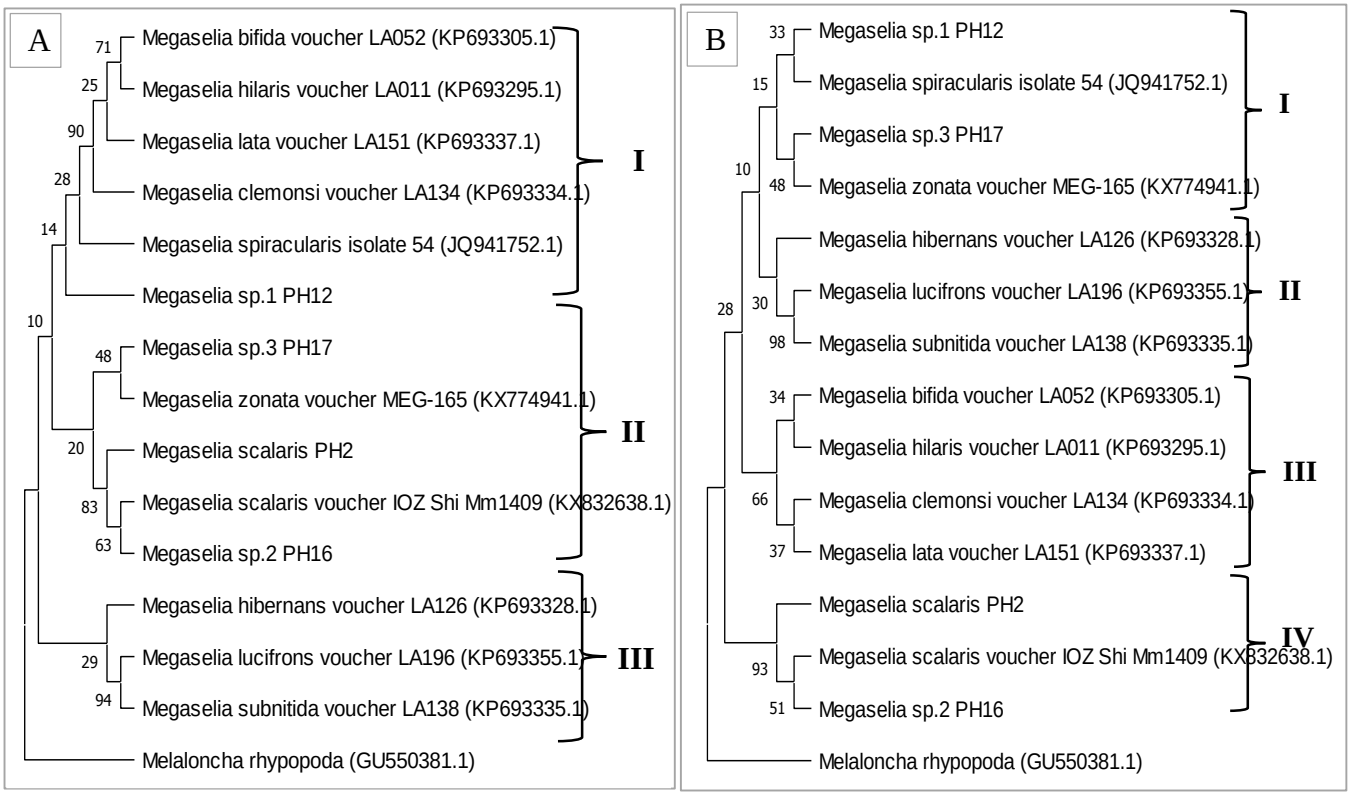


Figure 42. Phylogenetic trees built with sequences of unidentified *Megaselia* sp.1, *Megaselia* sp.2, *Megaselia* sp.3 and *M. scalaris* from our study and some *Megaselia* species from NCBI genebank for evolutionary distance assessment, Bootstrap = 1000. The species *Melaloncha rhypopoda* was used as outgroup to root the trees. **A**= Maximum likelihood's tree built using the General Time Reversible model + Gamma distributed patterns (GTR+G, BIC = 7288.2), **B**= Neighbor-Joining method's tree built using distances from the Jukes-Cantor model. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 758 positions in the final dataset.

III.1.5. Meta-analysis of biological control agents screened on termites and relationships with shade management, termite host groups and seasonality

During this study, we recorded in total 59 species of natural enemies associated with termites in cocoa agroforestry systems of Cameroon (Figure 43). Heavy shaded systems were the most diversified natural enemies' community (32 species), followed by Intermediate

systems (19 species), rustic systems (17 species) and low shaded systems (17 species). Full sun systems recorded 8 species in natural enemies' community. Globally, fungi were the most important group among the community across agroforestry systems except in heavy shaded systems where parasitoid flies were the most important group. In all other systems, parasitoid flies were the poorest represented group. In full sun systems, we did not recorded mites on examined termites.

The correspondence analysis of natural enemies per system revealed significant habitat preference among species (χ^2 = 653.26, P < 0.001), with 3 clusters differentiated (Figure 44). The cluster "Shaded" putting together natural enemies from rustic, heavy shaded and low shaded systems was the most important in species richness with 44 species preferring this habitat. The cluster "Intermediate" that grouped natural enemies *Melaloncha* sp., *Caloglyphus* sp.2, *Aspergillus protuberus*, *Aspergillus carneus*, *Meyerozyma capophila*, Saccharomycetes sp.1, *Candida duobushaemulonis*, *Starmerella etchellsii* and Acaridae gen.1 sp.4 which preferred intermediate systems as habitat. The cluster "Full sun" was the poorest group in species richness with only 2 species (*Candida orthopsilosis* and *Meyerozyma caribbica*) which preferred full sun systems. Regarding termite group, the correspondence analysis revealed significant host preference among natural enemies species (χ^2 = 668.13, P < 0.001) with 5 clusters differentiated for each termite group (Figure 45). *Microcerotermes* spp. clustered the most important community of natural enemies with 27 species preferring this group as host, followed by *Microtermes* spp. (7 species) and *Ancistrotermes* spp. (6 species). *Odontotermes* spp. and *Nasutitermes* sp. clustered 5 species each in their natural enemies' community.

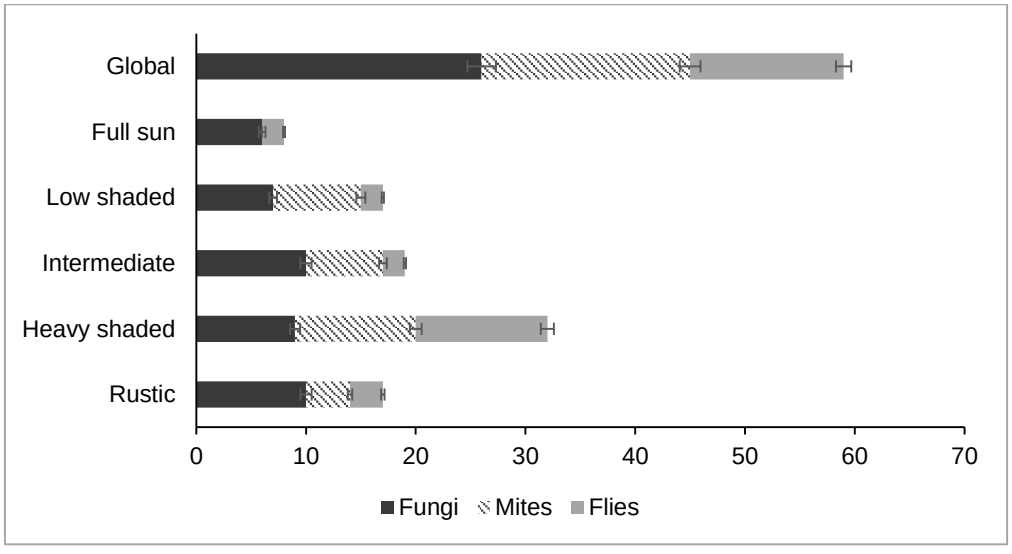


Figure 43. Global species richness of natural enemies isolated from termites per cocoa agroforestry system

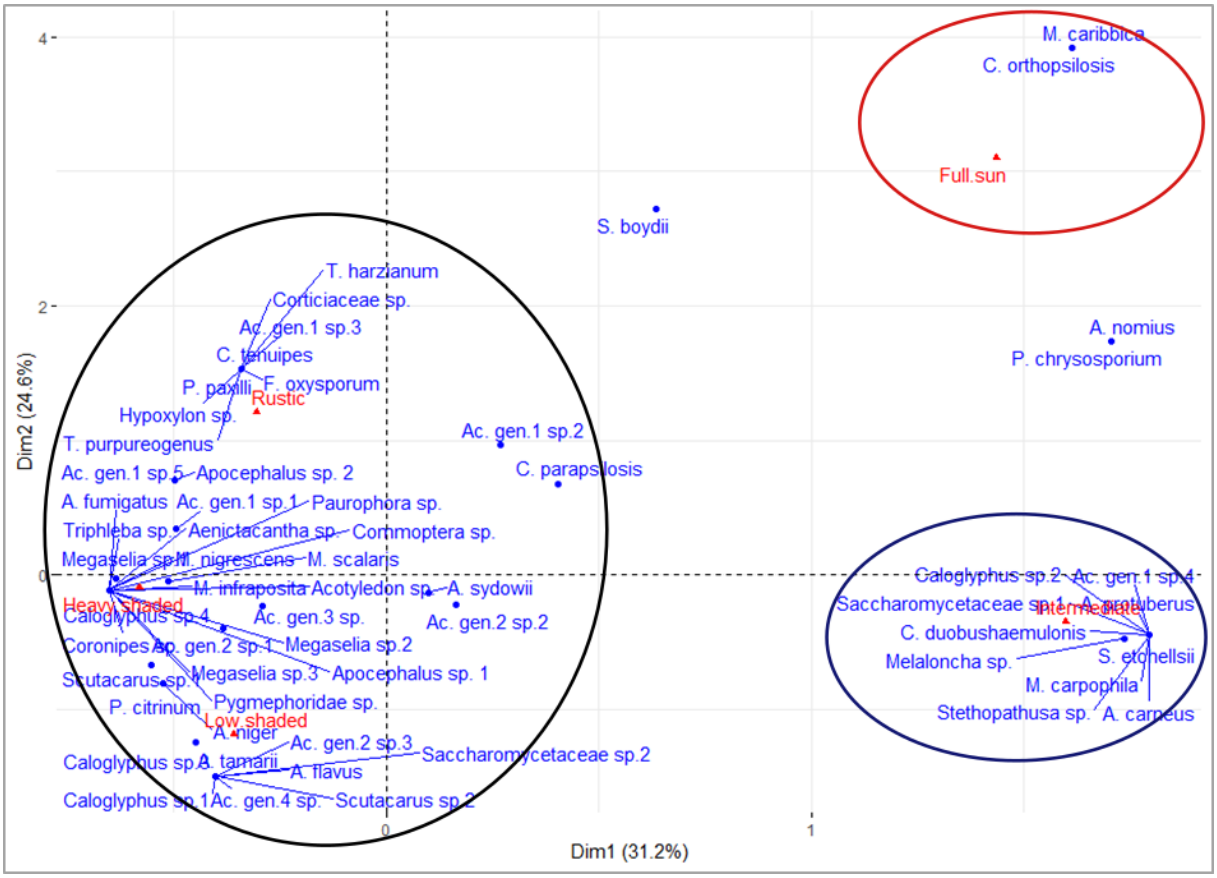


Figure 44. Correspondence analysis of natural enemies species isolated on termites per agroforestry system ($\chi^2 = 653.26$, $P < 0.001$). Black ellipse = cluster "Shaded", red ellipse = cluster "Full sun", blue ellipse = cluster "Intermediate".

Figure 45. Correspondence analysis of natural enemies species isolated per termite group (χ^2 = 668.13, P < 0.001). Black ellipse = cluster "*Nasutitermes*", red ellipse = cluster "*Microcerotermes*", blue ellipse = cluster "*Microtermes*", green ellipse = cluster "*Ancistrotermes*", pink ellipse = cluster "*Odontotermes*".

III.2. Discussion

III.2.1. Taxonomic and functional diversity of termites associated with cocoa trees and effect of shade management and climate variability

III.2.1.1. Taxonomic composition of termites associated with cocoa trees

We showed that cocoa agroforestry systems in Cameroon host a high diversity of termites associated to cocoa trees. For the first time, the family Kalotermitidae (*Neotermes* sp.) was sampled in cocoa farms in Cameroon alongside Rhinotermitidae and Termitidae families. Previous studies in Cameroon identified only one (Termitidae) or two families (Termitidae and Rhinotermitidae) of termites in cocoa agroforestry systems (Norgrove *et al.*, 2009; Ambele *et al.*, 2018b). The present study restricted the termite sampling to crop level, unlike those of Norgrove *et al*. (2009) and Ambele *et al*. (2018b) where termites were sampled at farm level in quadrats. This enabled us to obtain a more precise taxonomic composition of termites that

forage on cocoa. Our result indicates that a more precise sampling at crop level could increase the range of pest species incriminated in yield loss and therefore aid to build up more realistic and effective pest control programs (Southwood & Henderson, 2009). The species *Neotermes* sp. (Kalotermitidae), newly found in cocoa agroforestry systems of Cameroon, is a wood pest species living inside wooden plants and do not build nests as other known pest species (Vos *et al.*, 2003; Rouland-Lefèvre, 2010). It is a major cocoa pest in Nigeria, Equatorial Guinea, Sao Tome and Principe and Samoa islands (Vos *et al.*, 2003). This species is very difficult to control because it lives inside cocoa trunk where it goes often unnoticed and limits any chemical or physical control methods (Verma *et al.*, 2018). Sampling such a destructive and cryptic pest in cocoa farms in Cameroon enlarges the spectrum of bio-attackers in cocoa agroforestry systems and raises the concern about the real impact of termites on cocoa production. Hence, termites could be a major cause of the sudden death of cocoa trees reported by farmers during drought (Djuideu *et al.*, 2020), and a neglected cause of the low yield observed in cocoa farms (Ambele *et al.*, 2018b).

This study also highlighted a change in feeding behavior of species *Anenteotermes humerus* and *Procubitermes undulans* which, previously known as humus feeders and foraging exclusively in the soil, were found foraging on cocoa trees aboveground. Such a discovery can significantly affect cocoa cultivation as behavioral change of some termite species may ruin efforts towards pest control as new bio-attackers can appear at any time and any place. This therefore requires the development of a systemic approach aiming to manage known and emerging pests and avoid the resurgence of new bio-attackers. The causes of such a behavioral change could be attributed to the availability of food sources, their chemistry, and the activities of natural enemies (Bernays, 1998). Abiotic (e.g. land-use and climate changes) and biotic (e.g. soil quality deterioration by chemicals, high predation or competition pressure) factors could play major roles in feeding behavior change (Bernays, 1998; Brodie *et al.*, 2012). Subterranean insects like termites are particularly vulnerable to land disturbance like climate change and habitat fragmentation (Eggleton & Bignell, 1995; Eggleton *et al.*, 2002b) and persistent chemicals (Cowie *et al.*, 1989; Logan *et al.*, 1990). The continuous application of pesticides could render the soil unsuitable to sensitive humus feeders that could displace their ecological niche upward on trees. However, such behavioral changes are more complex than they suggest. Further studies are required to clearly describe the mechanisms or synergistic actions of the intervening factors.

In this study, the rustic systems revealed a higher termite species richness and functional diversity than the low shaded or full sun systems. Analysis from the species accumulation curves corroborated with the results from diversity indexes. In addition, rustic systems recorded the lowest number of trees infested per pest species and a high species richness of beneficial species. These results suggest that the progressive removal of shade trees significantly affects termite diversity associated with cocoa trees as well as increasing termite pest pressure. It also highlights the role of shade trees in cocoa agroforestry systems which ensures a high biodiversity in farm through complex multidimensional processes (Tscharntke *et al.*, 2011; Bisseleua *et al.*, 2013, 2017, 2018; Deheuvels *et al.*, 2014; Ambele *et al.*, 2018b). Shade trees provide a large array of food sources and refugees for several endemic and endangered species (Schroth *et al.*, 2004; Clough *et al.*, 2009, 2010). Cocoa agroforestry systems, therefore, play an important role in biodiversity conservation even at crop level, which enhances positive biotic interactions such as interspecific competition in a pest guild and reduces pest pressure on cocoa (Schroth *et al.*, 2004; Bhagwat *et al.*, 2008; Tscharntke *et al.*, 2011; Bisseleua *et al.*, 2013).

III.2.1.2. Effects of land-use, temperature and relative humidity on pest populations of termites

We showed that shade tree removal and increases in temperature and relative humidity contribute to increase pest pressure on cocoa. This effect was more important on belowground pests than aboveground ones. The effects of environmental disturbances on arthropods have been reviewed and discussed extensively (McCarty, 2001; Yamaguchi *et al.*, 2001; Yamamura & Yokozawa, 2002; Kiritani, 2007). Land-use change by transforming the natural landscape for economic purposes causes direct habitat loss for several species of insects (Oliver & Morecroft, 2014). Moreover, shade tree removal in cocoa induces the fragmentation of remaining habitats in association with the increased of agrochemical inputs which threaten local biodiversity (Root, 1973; Yachi & Loreau, 1999; Donald *et al.*, 2001; Bisseleua *et al.*, 2013). The detrimental impacts of habitat destruction and fragmentation in tropical ecosystems are considerably magnified via synergies between climatic warming and obstructed shifts in the ranges of species (Brodie *et al.*, 2012). Land disturbances may directly affect natural processes of pest control such as predation, parasitism and interspecific competition (Yachi & Loreau, 1999; Cassano *et al.*, 2009; Martin *et al.*, 2013). In addition, the stress induced by drought on cultivated crops may increase in open systems and enhances their susceptibility to termite attacks (Logan *et al.*, 1990).

This study also revealed that termites are more affected by relative humidity than temperature. We noted that the density of termite pest species on trees was higher when the R.H decreased than when the temperature increased, though both significantly affected termite density. In fact, termites prefer highly moistened and warm microhabitats for the establishment of their colony (Grassé, 1986; Logan *et al.,* 1990). Termites are also more affected by desiccation than temperature (Woon *et al.*, 2019). The increase of pest density at crop level when the R.H. decreases is probably due to the high moisture that plants conserve around their roots during drought (Metcalfe *et al.*, 2008). Plants may lose water through the roots during drought to keep a high soil moisture and ensure root growth and nutrient absorption (Metcalfe *et al.*, 2008; Brunner *et al.*, 2015). Termites are then attracted by the high humidity surrounding roots of plants and feed on roots or aboveground parts of the plant. Dibog *et al*. (1998) noticed that seasonality affect termite abundance in soil, with high abundance during dry season. Vos *et al.* (2003) and Asare & David (2010) also reported that termites damage on cocoa is more severe during dry seasons. With the ongoing climate change phenomenon, tropical ecosystems are the most affected and this situation can become worse if agricultural intensification continues (Brodie *et al.*, 2012). Fortunately, this study showed that while termite pest densities are increasing with rising temperatures in open systems, rustic shaded systems keep pest populations at low level. Therefore, multistrata cocoa agroforestry systems can mitigate climate change at the micro-environment surrounding cocoa trees and impair the synergistic effect of climate change on biodiversity and pest pressure (Tscharntke *et al*., 2011; Sonwa *et al.*, 2019). Shade trees also keep a high relative humidity understorey and limit termite accumulation in highly moistened roots during droughts. Such cocoa farming model should therefore be promoted nationwide or at regional scale to ensure a sustainable cocoa production (Bisseleua *et al.*, 2018; Bisseleua, 2019).

III.2.2. Termites services and disservices provided to cocoa trees

III.2.2.1. Termites' ecosystem services and response to land disturbance

This study showed that all feeding groups where represented among termite assemblages per system and the group III (soil feeders) was the most common on cocoa. The group II (mainly pest species) became the main group on cocoa after progressive shade tree removal. Our results corroborate that of Norgrove *et al*. (2009), who reported the group III (soil feeders) as main group in cocoa farms in Mbalmayo. In this zone, cocoa trees are grown under a dense shade cover resembling a secondary forest (Norgrove *et al.*, 2009). Ambele *et al*. (2018b) also noticed that the group II becomes the main group in cocoa farms when shade trees are reduced. Therefore, the reduction of shade trees in cocoa farms results in the increase of

pest guild and decrease of soil feeders which are beneficial to agriculture (Attignon *et al.*, 2005; Ambele *et al.*, 2018b). Tscharntke *et al*. (2011) and Clough *et al*. (2010) documented the role of agroforestry trees in maintaining low pest pressure through predation and interspecific competition enhancement. Bisseleua *et al*. (2017) also suggested that the activity and services of ants, main enemies of termites, are more important in shaded systems than unshaded systems. In addition, this study showed that the number of cocoa trees attacked by termites increases in relation with shade reduction and shade tree removal. We can therefore affirm that the reduction of shade in cocoa agroforestry systems lead to termite outbreaks in low shaded or unshaded systems (Ambele *et al.*, 2018b; Djuideu *et al.*, 2020). With very limited approaches to control termite pests (Ambele *et al.*, 2018a; Verma *et al.*, 2018), such a trend towards monoculture systems (which yield better than agroforestry systems), will result in collapse of farm yield at short-term and further abandonment of the cocoa farm (Saj *et al.*, 2017). The establishment of new cocoa farms after forest clearing, increased deforestation rate (Bitty *et al.*, 2015; Riedel *et al.*, 2019). There is therefore a need to educate cocoa farmers on the importance of agroforestry in supporting lower pest incidence and sustaining cocoa production, as well as additional food and financial resources from shade trees to avoid forest clearing (Bisseleua *et al*., 2019).

We observed a high occurrence of soil feeders (groups III and IV) in heavy shaded systems that decreased with shade tree removal, suggesting that soil feeding termites are associated to complex processes of restoring soil organic matter and nutrient cycling in cocoa agroforestry systems (Attignon *et al.*, 2005; Jouquet *et al.*, 2011). It results in a high humification score which translates into an important activity of termites in soil fertilization process (Davies *et al.*, 2003; Attignon *et al.*, 2005). This could be one of the reasons why complex shaded agroforestry systems ensure a sustainable production, and maintain yield for longer period than other farming systems (Jagoret *et al.*, 2016).

Microtermes osborni was the main damaging termite species. The genus *Microtermes* is among the main subterranean pests of cultivated crops in Africa, attacking crops such as maize, rice, peanut, and cassava (Sands, 1998b; Sekamatte, 2001; Rouland-Lefèvre, 2010). In cocoa, this genus was reported as main cocoa root destroyer alongside *Ancistrotermes* (Vos *et al.*, 2003; Ambele *et al.*, 2018b, 2018a). In this study, the number of trees attacked by *M. osborni* increased when the shade cover decreased. The increase in the number of cocoa trees attacked from rustic to full sun systems is a proof of the role of agroforestry systems in maintaining pest populations under economic injury level (Bos *et al.*, 2007; Tscharntke *et al.*, 2011; Bisseleua *et al.*, 2013, 2018). By conserving biodiversity in farm landscape, agroforestry

systems also enhance predator-prey and host-parasitoid interactions for pest control, and help to reduce pest pressure on cultivated crops (Klein *et al.*, 2002; Perfecto & Vandermeer, 2008; Cassano *et al.*, 2009).

III.2.2.2. Ecosystem disservices provided by termites to cocoa trees, dispersion patterns and sampling plans for pest management

III.2.2.2.1. Termites' infestation in relation to shade management

In this study, we showed that termite infestation in cocoa farms is very high (about 73 % of trees infested). This infestation level was more pronounced belowground (44 % of trees infested) than aboveground (12 % of trees infested). These results corroborate with previous studies (Sekamatte, 2001; Vos *et al.*, 2003). Termites are frequently encountered in agricultural fields where they cause damage to cultivated crops especially belowground (Logan *et al*., 1990; Rouland-Lefèvre, 2010; Sands, 1973), with significantly more damage during dry seasons (Asare & David, 2010). In annual crops, researches have reported high infestation levels with up to 100 % of infested stands in maize, cassava and rice (Umeh & Ivbijaro, 1997; Sekamatte, 2001; Riekert & Van den Berg, 2003; Togola *et al.*, 2012). In perennial crops, Ténon *et al*. (2014) reported infestation between 10 to 93% in mango farms in Benin. Tra-Bi *et al*. (2015) reported termite infesting between 25 to 66 % of cocoa trees in Cote d'Ivoire. The higher infestation rate in Cameroon as compared to Côte d'Ivoire is probably due to the ageing of Cameroon cocoa orchards (Jagoret *et al*., 2016), as ageing may strongly affects the resistance of cocoa trees to pest infestation (Trippi, 1990; Bergman *et al.*, 1991). Other factors may include ongoing land-use change policy of shade removal (Bisseleua, 2019) and pedoclimatic conditions.

The correlations obtained in Table VI highlight the disastrous effects of termite aboveground infestation on cocoa. Nyeko & Olubayo (2005) and Djuideu *et al*. (2020) also suggested that termite infestation may lead to the death of branches or the whole tree. This observation should alert farmers and pest managers on the need to develop management strategies to prevent establishment of shelter tubes in cocoa agroforestry systems. In addition, the arboreal nests associated with shelter tubes could also affect tree vigor and yield by reducing flower production on the trunk of cocoa.

This study also suggests that shade management in cocoa agroforestry systems strongly affect termite infestation, with increased aboveground infestation in heavily shaded systems and increased belowground infestation in poorly shaded systems. By mimicking the forest cover, agroforestry systems create a stable and resilient environment (Schroth *et al.*, 2004;

Tscharntke *et al.*, 2011), where some termite species (i.e. forest species like *Microcerotermes* spp.) thrive better (Cowie *et al.*, 1989; Eggleton *et al.*, 1995). Forest pest species which usually attack forest trees in plantations or in natural habitats (Cowie *et al.*, 1989), would find a conducive environment in heavily shaded systems and increase their range of infestation on cocoa trees. However, belowground infestation, which is the most destructive type of infestation remains low in heavily shaded systems probably because of the diversity of predators (Cassano *et al*., 2009; Clough *et al*., 2009), parasitoids (Sperber *et al.*, 2004) and less niche overlap insured by the presence of shade trees. For instance, the activity and diversity of ants, known as the main natural enemies of termites (Owolabi & Okunlola, 2015), are high in heavily shaded cocoa agroforestry systems and may help to reduce movement of termites and the damages they may cause (Tadu *et al.*, 2014; Bisseleua *et al.*, 2017). In contrast, shifting agricultural practices from heavily shaded to unshaded systems would reduce predation-prey and host-parasitoid interactions (Tscharntke *et al.*, 2011) resulting in more cocoa roots destroyed by termites. Although, higher aboveground infestations were found in heavily shaded systems, the highest densities of termites at both above and belowground were found in poorly shaded systems. This observation was mostly made in poorly shaded cocoa agroforestry systems where farmers are encouraged to reduce shade trees. The ongoing recommendation to remove shade trees is resulting to more termite activity, more roots damages and tree death due to the loss of interaction with beneficial insects such as ants (Sperber *et al.*, 2004; Clough *et al.*, 2009; Tscharntke *et al.*, 2011) and lead to poor yield of cocoa.

III.2.2.2.2. Dispersion models of termite populations in aboveground and belowground infestation

In this study, the three dispersion models used (Taylor, Nachman and Iwao) all showed a strong tendency to aggregated spatial distribution of termite populations on cocoa trees (Ruesink, 1980). The aggregation indices obtained were higher than those reported by Bisseleua *et al*. (2011) for cocoa mirid bug, *Sahlbergella singularis* Haglund (Hemiptera: Miridae) in the same study zone using the same models suggesting that termites are social insects with a highly aggregated spatial pattern (Messier, 1985; Grassé, 1986; Theraulaz *et al.*, 2003). Donovan *et al*. (2007) also showed an aggregated spatial pattern of soil-dwelling termites in Malaysian forests with changing aggregation level from primary site to logged site. In this study, termite dispersion patterns were better described by Taylor's power law ($r^2 = 0.93$). The aggregation indices of Taylor's power law were all significantly >1 ($P < 0.001$), indicating that termite populations were strongly aggregated on cocoa trees. The aggregation indexes were similar for both aboveground (shelter tubes) and belowground (roots) termite populations with the highest

degree of aggregation recorded on roots. Taylor (1984) suggests that the Power Law with respect to mean density ($S^2 = a \bullet m^b$) could be used for sampling of termites and dispersion studies in cocoa agroforestry systems. Because the mean density is included in the Taylor's regression method, it provides practical tools for handling field sampling data from experiments or surveys. Our analysis also showed that the Taylor Power law takes care of the problems of continuity with changing density of termites (Taylor, 1984; Binns & Nyrop, 1992).

III.2.2.2.3. Sampling plans for ecological and pest management purposes

Sampling plans based on Southwood (1978) and Green (1970) were very similar but Green's sequential sampling required less trees samples compared to Southwood's sampling program for a given mean density of 15.1 termites per tree whatever the precision level considered. The Southwood's sampling program is difficult to implement because the mean density is not known in advance, and a preliminary estimation of this mean density would be required at each sampling date (Smith & Hepworth, 1992). Sequential samplings (i.e. Green's), in contrast, are used when the mean density is not known and a decision is made after each unit is drawn (Ives, 1954). We also found that sample sizes increased dramatically when the precision required increased. Sample sizes were small at the 0.25 and higher at the 0.10 level. In practice, estimates of termite population densities with 0.25 levels of precision can be accomplished with relatively little sampling effort. The level of precision needed is a choice made according to the purpose of a sampling plan. Estimates of population densities with precision levels of 0.10 are recommended for ecological or applied research studies, and those of 0.25 are recommended for pest management decision-making (Southwood, 1978). Monte Carlo simulation (Naranjo & Flint, 1994) and resampling (Naranjo & Hutchison, 1997) studies showed that the precision level specified in a sequential sampling plan can differ from that actually achieved when the plan is executed. Consequently, sampling plans should be taken as provisional guidance until they are validated through simulation or against independent field data. The fact that we collected data in spatially distant cocoa plantations give more importance to this study. Indeed, definitive monitoring plans should be based on data gathered at different localities if they are to be adopted by farmers or agronomists (Garat *et al.*, 1999). Considering the complete lack of information for monitoring the pest termites of cocoa in West and Central Africa, this work provides a research tool for termite monitoring in cocoa plantations. Furthermore, these sampling plans could also be used to estimate termite densities as part of a decision making program, in which the relationship between termite density and plant damage is used to estimate economic thresholds, or in conjunction with possible natural enemies to develop management decisions (Nyrop & Van der Werf, 1994).

We know termites to cause severe damage to a wide diversity of trees crops (Rouland-Lefèvre, 2010). However, our sampling plan recommendation should not be generalized to other tree crops for the reason that cocoa is a cauliflory plant producing about 90% of its pods on the trunk (Mossu, 1990). This special architecture is different from other tree crops. Thus, the severity of termite damage on cocoa trunk will be assessed differently from termite attacks on trunk/stems of other tree crops, although belowground attacks are relatively comparable. We therefore suggest that our sampling recommendations from this study should be implemented in cocoa plantations only, and further studies are needed to assess the suitability of these sampling recommendations to other tree crops.

III.2.3. Termites disservices provided to cocoa trees and consequences on plant health and yield

III.2.3.1. Disservices of termites on cocoa trees and plant health

The description of termite damage during this study revealed that although they use different methods to attack cocoa trees (belowground vs aboveground species), they all often show superficial signs of ongoing attacks. The most frequent superficial sign was the gallery along the cocoa trunk, whatever the species concerned. This result is in accordance with the claims of Han & Ndiaye (1996) who argued that a termite species is considered as a pest if it builds galleries on an attacked plant. Also known as earthruns, tunnels or mud tubes, termite galleries are made from soil and wood combined with termite saliva and help to connect their colonies in the soil underground (or upper on branches for aboveground termites species) to their above-ground food sources (Miller, 2010). These galleries protect termites from desiccation and predation from ants while foraging aboveground. Termites build different types of galleries following their use (Miller, 2010): (i) exploratory galleries, thin, fragile and near the soil or the nest they are potential signs of early termite infestation on crop; (ii) utility galleries, larger highways running from the underground termite galleries directly to the food source, they are signs of ongoing attack; (iii) drop or suspended galleries, non-attached tubes similar to utility galleries to make food source more accessible and facilitate foraging, they are signs of long-lasting infestation; (iv) swarming galleries, built seasonally to provide the exit port for winged swarmers leaving the colony, they are signs of ancient infestation (more visible for aboveground termites). Thus, recognizing the different types of galleries on a cocoa tree could be a good tool for preliminary assessment of termite infestation and to take a decision toward pest management.

This study also revealed the categorization of different types of damage between aboveground and belowground termite damaging species. The former attacking wooden part of cocoa tree and the latter concentrating damage on belowground roots. Given the differences in damage and pest settlement on crops, a general control approach may not be appropriate to control termites on cocoa and should instead focus on species-specific control of termite group (belowground vs aboveground). In addition, target-specificity problems may be more important in complex faunal communities in which a large number of potential non-target species occur, or in which the pest animal is sympatric with other functionally similar species (Bengsen *et al.*, 2008). The damage reported during this study are similar to those reported by Ackonor (1997) in Ghana, Tra-Bi (2013) and Tra-Bi *et al.* (2015) in Côte d'Ivoire, and Cowie *et al*. (1989). Regarding the damage per termite species, our results are similar to descriptions of Tra-Bi (2013), revealing that termite pest species display the same damaging behavior on cocoa trees with very similar attack patterns. This finding implies that a species-specific management of termite pest species could be applied at a regional scale in West African cocoa producing countries and may induce a coordinated actions between countries and strategies.

This study also revealed that shade reduction in cocoa agroforestry systems induces higher levels of damage by termites. Indeed, although rustic shaded systems were the most infested, the highest level of damage was observed in low shaded systems. This result could be explained by the resource concentration hypothesis (Root, 1973) which predicts that specialist herbivorous insects should be more abundant in large patches of host plants, because the insects are more likely to find and stay longer in those patches than in less concentrated host plant patches. By reducing shade trees, farmers create larger patches of cocoa trees where pest species of termites will thrive better and therefore induce important damage to crops. Moreover, Ambele *et al*. (2018b) and Djuideu *et al*. (2020) demonstrated that pest species of termites are significantly more abundant in poorly shaded cocoa agroforestry systems. Thus, promoting shaded agroforestry systems could help to reduce damage induced by termites on cocoa.

III.2.3.2. Relationship between termites' ecosystem disservices and cocoa yield

III.2.3.2.1. Shade management and termites disservices affect cocoa tree vigor and development

The results of this study demonstrated the enormous effects of termite infestation at different cocoa yield levels from tree growth to final beans. We demonstrated that shade regime and termite infestation affected the height and the basal area of cocoa trees. Globally, shaded systems were more vigorous than full sun systems. The effect of companion trees on cocoa tree

vigor has been documented by several studies (Bisseleua & Vidal, 2008; Ngala, 2015; Jagoret *et al.*, 2017; Blaser-Hart *et al.*, 2021). Shades trees commonly affect negatively cocoa growth due to the interspecific competition for soil nutrients. Cocoa trees in full sun systems are generally taller and larger (in basal area) than those of complex shaded systems (Jagoret *et al.*, 2017; Blaser-Hart *et al.*, 2021). However, we found in this study that cocoa trees in rustic systems displayed higher tree development characteristics than those in full sun systems. This finding was also highlighted by Bisseleua & Vidal (2008) in the same area. The age of cocoa trees could explain this unexpected finding as cocoa trees in rustic systems are very old (more than 60 years old) while trees in full sun systems are relatively young (15-20 years old) (Jagoret *et al.*, 2017). This difference in age could lead to significant differences in tree development characteristics. We can also note the differences in farms management practices between production systems. Indeed, some farmers especially in rustic systems favor tall cocoa trees (height > 6 m) with the assumption that tall trees produce more pods, although difficult to harvest. Meanwhile farmers in low shaded and full sun systems prune frequently their trees to ease the pod harvest.

Our results also showed that termite attacks on cocoa affect their height and basal area, with infested trees significantly shorter and thinner than healthy ones. Indeed, insect attacks usually affect negatively the growth of crops. Pest assessment studies frequently show that crops vary greatly between sites and between years in their response to attacks by similar numbers of insects (Bardner & Fletcher, 1974). Pest infestation affects crop physiology, especially in terms of effective photosynthetic area and the production of dry matter and its distribution between various organs of the plant (Bardner & Fletcher, 1974). By gnawing plant tissues termites in a back-and-forth movement, termites then affect the sap transport and redistribution system in cocoa tree, as well as inducing local tissue inflammations that stress the plant. The plants are then less vigorous and may easily die in case of severe droughts (Asare & David, 2010; Djuideu *et al.*, 2020).

III.2.3.2.1. Termite disservices and shade management affect cocoa flowering and pod formation

This study demonstrated that cocoa trees in intermediate systems produced more pods than those in rustic and poorly shaded systems. Several studies have documented the trade-off relationship between agroforestry system complexity and yield return in farm (Bisseleua *et al.*, 2013, 2017; Ambele *et al.*, 2018b; Djuideu *et al.*, 2020). Indeed, intermediate shade cover on cocoa should provide benefits from natural biological control ensured by companion trees and

higher yield return from low resource competition between cocoa and companion trees. Ambele *et al*. (2018b) suggested that shade levels between 45% and 65% may be optimal to balance between richness of termite pest species and marketable yield and may help to increase the role of beneficial termites. Because productivity of cocoa was predicted to decrease under dense shade regimes (Zuidema *et al.*, 2005), agricultural intensifications led to large scale landscape homogenization, turning heterogeneous, shaded agroforestry systems into poorly shaded monocultures at local and regional scales (Siebert, 2002; Bos *et al.*, 2007; Bisseleua *et al.*, 2013). However, recent studies showed that it is more profitable to keep shade trees in farm because of the tremendous environmental and economic functions they provide to the landscape and the smallholders (Bos *et al.*, 2007; Tscharntke *et al.*, 2011; Djuideu *et al.*, 2020).

Our results also highlighted the negative effects of termites on cocoa flowering and pod formation. Infested trees produced significantly lesser flowers and pods than healthy trees. By building galleries running off the tree or forming large feeding patches on the trunk covered with soil mixture, termites may mechanically cover sites of flowering on trunk and reduce pod formation. Also, when feeding on cocoa bark, they may destroy budding sites and affect tree production at long term. Furthermore, unlike other major pests whose attacks mainly occur when cocoa trees are in their productive stage (e.g. mirids, pod borers, aphids), termite attacks occur at any development stage of cocoa (from seedlings to old trees). Termite attacks are mainly carried out by workers and start on the taproot and sometimes on the trunks through sites of previous injuries (Ackonor, 2001; Tra Bi, 2013). Ackonor (1997) even noticed some attacks of termites on cocoa pods. By affecting the production of raw sap and availability of elaborated sap to be used for flowering and pod formation, termites directly affect cocoa production upstream of the chain (Trippi, 1990). It is also possible that termite belowground infestation plays a role in the "cherelle wilt" phenomenon as it is linked to a deficiency of nutriments in the plant (Wood & Lass, 2008), but it is hard to conclude without further studies. In the same way, infested trees will show less vigor because of the reduced access to elaborated sap that their tissue feed on. As reported by Akilan *et al.* (1995), vigorous plants use more water and produce more flowers than less vigorous plants. Therefore, unlike other pests, termites may require continuous management plans throughout the year and not only during pre-productive stage of cocoa as it is the case of mirids (Mahob *et al.*, 2014). Control programs may take note of the damaging patterns for effective control.

III.2.3.2.3. Termites' disservices and shade management affect pod quality and marketable yield

This study reveals that, in contrary to common assumption, shaded systems were more productive than unshaded systems. The most productive systems were intermediate systems followed by rustic systems. The tradeoff relationship between biodiversity conservation and marketable yield is well known (Bos *et al*., 2007; Bisseleua *et al*., 2013; Ambele *et al*., 2018b; Djuideu *et al*., 2020). Indeed, producing cocoa on unshaded plantations affect local biodiversity, therefore reducing important ecosystem services provided such as pollination, soil fertilization and natural pest biocontrol (Yachi & Loreau, 1999; Bos *et al.*, 2007; Clough *et al.*, 2010; Tscharntke *et al.*, 2011; Bisseleua *et al.*, 2013). This intensive cocoa production may effectively increase cocoa yield in farm but at short term as soils get rapidly depleted because of lack of soil turnover and may require important inputs to keep the yielding standards (Jagoret *et al*., 2017). This is probably the case in this study where full sun systems showed lower marketable yield than shaded systems, suggesting that soil may have been emptied of its nutriment reserves and the farm may need important amount of inputs to regain its full production in an unsustainable way. As a consequence, development of African cocoa production has been largely based on the shift of growing areas at the expanse of forests (Vaast & Somarriba, 2014; Jagoret *et al.*, 2017) strongly affecting landscape biodiversity (Riedel *et al*., 2019). In the other end, an overabundance of companion shade trees, although ensuring important ecosystem services (Tscharnkte *et al*., 2011), may contribute to reduce farm yield as result of interspecific competition for available resources. As reported by Blaser-Hart *et al.* (2021), shade trees compete with cocoa for light and may reduce farm yield if the cover range is too high. In fact, cocoa needs to receive a certain incidence of light to produce flowers and then pods (Mossu, 1990; Koko *et al.*, 2013). Optimal shade management is necessary to combine both environmental advantages from shade trees and cocoa production which is the case in intermediate systems with shade range comprised between 45 and 65% (Bos *et al*., 2007; Ambele *et al*., 2018b). Therefore, such a system should be promoted for an eco-friendly cocoa production with good yield.

Our results also demonstrated the negative effects of termites on cocoa quality and marketable yield. Cocoa trees infested by termites produced smaller pods, fewer beans per pod and lighter beans with lower water content than healthy trees. All these successive drawbacks at different production scales resulted in a high yield gap of 55% in severe termite infestation. We did not note a significant difference in weight between infested and healthy trees, but infested trees produced significantly fewer beans. In comparison, a study carried out by Babin

et al. (2012) revealed that mirid attacks significantly slightly reduced pod weight but did not significantly reduce the number of beans per ripe pod. This contrast in yield component impact between these two major pests could be explained by their infestation sites and damaging patterns. Indeed, mirids by feeding on pod induce lesions on pod tissue affecting bean formation inside it and may lead to pod abortion in very young pods (Babin *et al.*, 2012; Babin, 2018); meanwhile termites by disturbing the production and availability of elaborated sap, termites may directly affect the quality and amount of pods/beans produced by the tree. The fact that termite damage affect pod formation upstream production chain make it even worse, because when flowers and pods appear during the productive stage, termite damage have been already done and the cryptic behavior hinders their presence in farms (Ambele *et al*., 2018a). Unexperienced farmers will only noticed yield slowdown without really know what is going on in their farms (Djuideu *et al*., 2020). Our result also highlighted the negative effect of termites in cocoa quality as we noted a reduced amount of water in cocoa beans from infested trees. According to Schwan & Wheals (2004) during fermentation, the water content of cocoa beans is utilized for enzymatic reactions in the beans cotyledon and also for microbial growth in cocoa beans pulp. Water will bring the enzyme to the substrate so that the hydrolysis and oxidation of the precursor of flavor, color, and aroma of the cocoa beans occurred (Apriyanto, 2016). Thus, termites may affect the quality of cocoa during fermentation process.

Our results showed that termites reduce total marketable yield from 29 to 55% in the case of severe infestation. Yield loss was more severe in poorly shaded systems than heavy shaded systems. This latter observation supports the importance of agroforestry systems in containing pest damage at low levels thanks to natural enemies ensuring natural regulation of pests (Tscharnkte *et al*., 2011; Bisseleua *et al*., 2013; Ambele *et al*., 2018b). In addition, this yield gap is relatively high compared to other major pest of cocoa. For instance, Babin (2018) reported that mirids (*Sahlbergella singularis* Hagl. and *Distantiella theobroma* Distant) are responsible of economic losses of about 25–30% of the cocoa production in four of the five most important producing countries of the world. The cocoa pod borer, *Conopomorpha cramerella*, can reduced yield by 60–84% in the case of severe infestation, and dry bean quality is also affected (Babin, 2018). Hence, based on the results of this study termites should be counted among the major pest of cocoa not only because of their damage to seedlings but also in regards to the economic losses they induce in farm during productive stage. Therefore, policy makers, cocoa pest managers, researchers, extension officers and cocoa farmers should be aware of the danger of termites in farm landscape and must develop effective control strategies to address the yield gap induced in cocoa farms.

III.2.4. Diversity of biological control agents screened on termites and potentiality for termite control

III.2.4.1. Diversity of fungi screened on termites and potentiality of entomopathogenic strains in termite control

In this study, we have isolated and identified 26 fungi species belonging to Ascomycota and Basidiomycota Divisions associated with termites. This fungi species richness was higher than that of Moharram *et al.* (1992) with 12 fungi species isolated on sand termite *Psammotermes hypostoma* in Egypt, but lower than that of Zoberi & Grace (1990) with 40 fungi species isolated on *Reticulitermes flavipes* in Canada. Many studies have documented the relationship between ectoparasitic fungi (Wilson *et al.*, 2021), pathogenic fungi (Rath, 2000; Hassan *et al.*, 2021), symbiotic fungi (Blackwell, 2017) and termites. Termites and fungi have been at odds for millions of years, termites inevitably encountering them during nesting and foraging in soil or in wood (Hassan *et al.*, 2021; Wilson *et al.*, 2021). The difference between the fungi species richness that we recorded during this study and other studies could be explained by the differences in geographic regions, in termite hosts and in isolation techniques. Indeed, tropical and temperate rainforests are considered as the most biologically diversified regions in the globe (Agoramoorthy, 2002). However, our culture medium (Molisch's agar) is specific to entomopathogenic fungi and may restrict the diversity of fungi that may grow in petri dishes. Furthermore, the cleaning of termite exoskeleton during the fungi cultivation process may also reduce the diversity of fungi, especially ectoparasitic fungi. The richest Orders during this study were Eurotiales, Saccharomycetales and Hypocreales. These orders are commonly associated to insects including termites (Zoberi & Grace, 1990; Oliveira *et al.*, 2012; Hassan *et al.*, 2021). The genera such as *Aspergillus*, *Penicillium* and *Fusarium* are commonly isolated on termites (Zoberi & Grace, 1990; Moharram *et al.*, 1992). *Aspergillus* spp. and *Penicillium* spp. are primarily saprophytic fungi occurring commonly in soils and on other organic and inorganic susbstrates (Bhabhra & Askew, 2005). However, a number of species of *Aspergillus* and *Penicillium* are facultative parasites of insects (Moharram *et al.*, 1992; Foley *et al.*, 2014). A study carried out by Seye *et al.* (2014) demonstrated that *Aspergillus* isolates (*A. flavus* and *A. clavatus*) induced higher mortalities pea aphid (*Acyrthosiphon pisum*) than *Metarhizium anisoplae*, which is well-known entomopathogen in literature. Lin *et al.* (2021) also found *A. nomiae* highly efficient to control ant pest *Dolichoderus thoracicus*. These authors conclude on the potentiality of *Aspergillus* isolates as pest control agents despite their saprophytic lifestyle. This affirmation should nevertheless be taken with great caution given the nuisance of these fungi species in cultivated plants. For instance, many species of *Penicillium*

(i.e. *P. citrinum*), *Aspergillus* (i.e. *A. flavus*) and *Fusarium* (i.e. *F. oxysporum*) have been reported to produce mycotoxins (secondary metabolites produced in foods during fungal growth and causing a toxic response, termed a mycotoxicosis, when ingested by higher vertebrates like humans) (Sweeney & Dobson, 1998). In addition, these fungi species are also responsible of crop loss in agricultural fields (Klich, 2007; Khan *et al.*, 2008; Al-Hatmi *et al.*, 2019) be also However, it is important to note that all strains of a same fungi species do not exhibit the same biochemical characteristics and can even be beneficial for plants (Khan *et al.*, 2008; Waqas *et al.*, 2015). Further studies are then needed to conclude on the real status of our fungi isolates.

During this study, *Candida parapsilosis* was the only fungi species found in all production systems and all termite groups. The association of termites with yeasts species like Candida is well known in literature (Prillinger *et al.*, 1996; Větrovskỳ *et al.*, 2020). The core gut mycobiome of wood feeding insects like termites covers a relatively narrow set of ubiquitous yeasts fungi such as *Candida* spp. (Větrovskỳ *et al.*, 2020). Saccharomycetes associated with termites can act as nutritional symbionts assisting with digestion, detoxification and essential nutrients synthesis, or as protective symbionts (Gurung *et al.*, 2019; Ali *et al.*, 2022). It is a bit surprising to obtain yeasts during this study given that we used an entomopathogenic fungi-specific culture medium (Molisch's agar) for fungi isolation on termites but this can be explained by two reasons: (1) the crushing of termites allows fast-growing gut mycoflora to colonize the culture medium in the opposite to use uncrushed termites, (2) the use of PDA (potato dextrose agar) for fungi culture during the molecular identification procedure may have stimulated yeast growth that was more or less blocked in the specific culture medium. Our results also showed that heavily shaded systems harbored a higher fungi richness than poorly shaded systems, and that aboveground termites (i.e. *Microcerotermes* spp.) were hosts of more fungi species than belowground termites (i.e. *Microtermes* spp.). Suitable EPF (non-pathogen to plants) were more frequently isolated on aboveground termites than on belowground termites. Cocoa agroforestry systems are known to preserve the natural soil biota including fungi (Koohafkan & Altieri, 2011). Fungi in soil are involved in the decomposition of structurally complex materials and play a significant role in nutrient availability to plants (Gilbert & Sousa, 2002), probably playing a role in the long-term cocoa production of agroforestry systems. By moving, nesting and foraging in soil, termites are exposed to the plethora of fungi inhabiting in it and get in contact with them despite their avoidance behavior. In studies on soil quality comparing coffee management systems, Bolaños *et al.* (2012) found that the abundance of soil fungal population was greater in organic, sustainable, conventional and forestry systems than in systems with full sun. Moreover, the

finding that aboveground termites are more associated with fungi than belowground termites totally makes sense. Indeed, fungi are mainly encountered in soil but when they grow on a substrate, their mycelia are observed at its surface. Therefore, termites moving aboveground in galleries are more susceptible to get in contact of fungi sporulating structures appearing at the surface of decaying substrate such as leaves and woods or insect cadavers. Meanwhile belowground, due to important interspecific competition, saprophytic fungi produce extracellular substances that inhibit the growth other fungi (Boddy, 2000), thus reducing their contagiousness towards soil-dwelling insects.

During this study, we did not recorded commonly studied entomopathogenic fungi (*Metarhizium anisoplae* and *Beauveria bassiana*) from termite isolates. This result corroborated the finding of Hussain *et al*. (2010) and Mburu *et al*. (2009) who reported that termites can detect volatiles of most virulent EPF species and easily avoid contamination in field trials. Indeed, although *M. anisopliae* is extremely lethal to insects (Gillespie *et al.*, 2000; Rath, 2000), it has non-significant effect on termites in the field (Chouvenc *et al.*, 2013) due to behavioral and physiological adaptations by termites to defend against entomopathogenic fungi (Liu *et al.*, 2019). Termites can also drastically reduce *M. anisoplae* infestation on termtes through self- and allogrooming (Yanagawa and Shimizu, 2008). Therefore, although these EPF are common in soil in all regions of the world, soil-dwelling termites can avoid contamination during their foraging activities and thus avoid epizooty in termite colonies. However, our study revealed the presence of other important EPF species like *Cordyceps tenuipes*. *C. tenuipes* is a forest entomopathogenic fungus that infest mostly pupae of several lepidopteran families (Castillo *et al.*, 2018). It has shown high efficiency in pest control against the potato tuber moth *Phthorimaea operculella* with up to 100% of mortality recorded in laboratory experiments (Zheng *et al.*, 2019). In the same way, *P. paxilli* and *P. citrinum* have been reported as good agents for pest control based on their ability to kills insects meanwhile producing small quantities of ochratoxin, reflecting a negligible contribution to the presence of mycotoxins in fruits and grains (Nicoletti *et al.*, 2014). *P. citrinum* was also used for the control of the mosquito *Culex quinquefasciatus* in Thailand (Maketon *et al.*, 2014). *Fusarium oxysporum* was also reported among *Fusarium* species exhibiting natural entomopathogenicity (Humber, 2012; Sharma & Marques, 2018). In addition, *F. oxysporum* produces the beauvericin, a cyclic hexadepsipeptide belonging to the enniatin antibiotic family and one of the active constituents of the EPF *Beauveria bassiana* and *C. tenuipes* (Hypocreales: Cordycipitaceae) (Sharma & Marques, 2018), suggesting its good potential for pest control. Given that these fungi species have been recorded naturally infesting termites during this study, this suggests that they were

able to bypass the immune and behavioral defenses of termites, and may be good agents for field control trials. Further studies are however needed to assess the potentiality of *C. tenuipes*, *P. citrinum*, *P. paxilli* and *F. oxysporum* for effectively control termite pest populations.

III.2.4.2. Analysis of the phylogeny of fungi species screened on termites

The topologies of the trees resulting from the two different analyses (Cladogram vs Phenogram) were generally congruent although some differences were noted in the number of differentiated lineages and in the placement of some taxa. The cladogram (maximum livelihood) showcased 8 different lineages meanwhile the phenogram displayed up to 12 different lineages. The tree result from the cladogram seems more coherent as fungi species are grouped according to their class taxonomic level and showed evolutionary relationships between fungi classes. This difference between the two types of dendrograms is clearly explained by the features underlying each dendrogram: a phenogram using phenotypic information for tree constructions including homoplasies between taxa, while a cladogram conveys information about genealogical relationship between taxa (Mayr, 1965). Our study revealed that Eurotiomycetes are closely related to Sordariomycetes as sister group. The group of *Aspergillus* was monophyletic and close to *Penicillium* group. This finding is in accordance with the study of Samson *et al.* (2014) who affirmed that *Aspergillus* species form a monophyletic clade closely related to *Penicillium* group. Our findings are also close to those of Schoch *et al.* (2009) who, when studying the phylogeny the phylum Ascomycota, showed that Eurotiomycetes are a distant sister group of Sordariomycetes with members of Leotiomycetes at mid-term between the two Classes. This results help to shed some light on the evolutionary distances and phylogeny between fungi species associated with termites but they are too limited to a few samples to make strong conclusions about the phylogeny of termites-associated fungi. Further studies are needed to deepen knowledge about the evolutionary history of fungi associated with termites, including ectoparasitic fungi Laboulbeniomycetes not found during this study (Blackwell & Rossi, 1986).

III.2.5.2. Diversity of parasitic mites screened on termites and potentiality for termite control

Our results showed a very low parasitism rate of mites (2.02%) on termites in all production systems. This result is below our expectations as nests of social insects are known to harbor a wide diversity of arthropods among which the most numerous are mites (Eickwort, 1990). In termite nests in part, termitophilous mites have been reported as obviously abundant (Grassé, 1986). Other studies that evaluated mite parasitism rate in termite colonies resulted in

significantly higher values in comparison with our study, 20.4% in *Cryptotermes secundus* (Fuchs & Korb, 2006), 28.6% in *Reticulitermes flavipes* and *R. virginicus* (Wang *et al.*, 2002). This low parasitism rate could be explained by the grooming behavior practiced in termite colonies to prevent diseases and epizooties in their nest (Zhukovskaya *et al.*, 2013). Allogrooming (grooming another individual) is common practice in eusocial insects and is important to eliminate pathogens, parasites and parsitoids (Peng *et al.*, 1987; Yanagawa *et al.*, 2008). Our study also suggest that termite colonies in Cameroon may display different patterns of grooming behavior resulting in very low level of infestation. Although very weak, our results showed that termites from heavily shaded systems were more parasitized by mites than those from poorly shaded systems. In addition, no mites was found on termites harvested in full sun systems. Shaded cocoa systems are known to support much higher ecological and functional biodiversity than unshaded systems (Tscharntke *et al.*, 2011; Bisseleua *et al.*, 2013; Ambele *et al.*, 2018b). Studies from Sperber *et al*. (2004) suggested that agroforestry systems harbor a high diversity of parasites and parasitoids increasing parasitism rates in insect communities. By reducing shade in cocoa agroforestry systems, it induces changes in the composition and structure of the landscape causing a reduction in the number of species, size of populations, and changes in the composition of biotic communities such as soil fauna (Altieri *et al.*, 2015).

During this study, we found that aboveground termites were more parasitized by mites than belowground termites and that termites were more parasitized during the rainy season than the dry season. Some studies have also documented the difference in mite infestation as far as the life cycle of termite species is concerned (Phillipsen & Coppel, 1977; Wang *et al.*, 2002; Fuchs & Korb, 2006). From these studies, drywood termites (*C. secundus*) were reported less parasitized (20.4%) than aboveground termites (*R. flavipes*, *R. virginicus* and *C. formosanus*) (28.6%). The reduction of mite infestation in drywood or belowground termites could be attributed to the size of their occupied niche. Indeed, in contrary to aboveground termites which build galleries on tree/building to move far and forth during foraging, drywood and belowground termites are confined to their limited environment but not only (wood or soil) in which they find all their necessary nutrients (Grassé, 1986; Vos *et al.*, 2003). Therefore, aboveground termites are more exposed to mite contact than belowground termites. Regarding the effect of seasonality on mite infestation, documented studies are reporting contradictory results. For instance, a study of parasitic mites of bats in coffee agroforestry systems of Mexico showed that bats were more parasitized during dry season than rainy season (Colín-Martínez *et al.*, 2018). Naveen *et al.* (2022) also reported that the maximum infestation of *Varroa* spp., in *Apis mellifera* colonies occurs during April (summer), meanwhile Sharma *et al.* (2011)

observed maximum incidence during November (Pre-winter period). Naveen *et al*. (2022) suggested that mite populations increases when food availability of their hosts decrease and during brood rearing periods. In the case of pest termite species, they are more active during period of drought (Asare & David, 2010) and rainfalls often affect their mobility in farm (Dibog *et al*., 1998). Hence their food availability is reduced during rainy season and their reduce mobility may lead to increased mite infestation.

From this study, we recorded 19 mite species belonging to Orders Sarcoptiformes (Astigmata) and Trombidiformes (Prostimata). Prostigmata and Astigmata acarid species are commonly found associated to termites and are numerically the most abundant ectoparasites in termite colonies (Eickwort, 1990). From all mite families sampled during this study, Acaridae was the richest and the most abundant on termites. In accordance with this finding, Wang *et al*. (2002) also found the family Acaridae as the most diversified and common mite family on subterranean termites. Astigmatid mites are nonpredators that specialize in the exploitation of nutrient-rich temporary habitats, especially the nests of insects and vertebrates. The facultative development of a specialized nonfeeding deutonymph enables them to locate and attach to phoretic hosts (Eickwort, 1990). Among astigmatid mites, Acaridae are typically the most abundant mites in nests of social insects (Eickwort, 1990). Unfortunately, our study did not confirm identification at the species level (and even at the genus level) of our specimens. This study is the first of this kind in Cameroon and probably a pioneer in sub-Saharan Africa. From available identification keys, no one is documenting the fauna of Acariforme mites associated with insects in sub-Saharan Africa, what represents a huge knowledge gap in this important group. Among the organisms associated with termites, the most numerous and least studied are the mites (Acari) (Eickwort 1990). According to Wang *et al*. (2002), information on taxonomy and biology of the mites associated with termites is still very limited and needs to be further clarified. However, in this study, the genera *Acotyledon*, *Caloglyohus*, *Coronipes* and *Scutacarus* were clearly identified. Some authors have reported these genera associated with termites in literature (Phillipsen & Coppel, 1977; Eickwort, 1990; Wang *et al.*, 2002; Fakeer *et al.*, 2014; Khaustov *et al.*, 2016). Before concluding on the suitability of these mites in pest control, we would need to clarify the type of association with their host. According to Eickwort (1990) and Wang *et al*. (2002), most mites associated with termites were considered saprophagous or phoretic and do not have any significant effect on the health of their termite hosts in nature (especially the deutonymph stage). Few mites however feed on termites when they become adults (Phillipsen & Coppel, 1997). The species *Acotyledon formosani* Phillipsen & Coppel (Acarina: Acaridae), in association with the genus *Australhypopus* (Acaridae) can

cause death in weak colonies of termite (Phillipsen & Coppel, 1997). In contrary, Wang *et al*. (2002) evaluating the suitability of *Australhypopus* sp. to control on R. flavipes colonies found that they had little effect on termites even at high densities. Therefore, a thorough assessment of the potential of the mite samples during this study on the control of pest populations of termites is needed as well as their taxonomy.

III.2.5.3. Diversity of parasitoid flies screened on termites and potentiality for termite control

During this study, we have recorded a very poor fly parasitism rate on termites (0.24%). Although termites are associated with large diversity of termitophilous and parasitoid flies (Sze *et al.*, 2008; Dupont & Pape, 2009), their potentiality to regulate termite populations remains poor. Culliney & Grace (2000) suggested that parasitoids have a negligible contribution in termite control because of their protected, underground location in fields. However, the fly parasitism rate on termites was very weak in comparison to the parasitism rate recorded by Noknoy *et al.* (2020) who reported 0.89 to 47.43% of major soldiers of *Macrotermes gilvus* parasitized by *Megaselia scalaris*. Based on their findings, these authors suggest that parasitoid flies could be possible agents in the biological control of termites. Indeed, with a very short life cycle (Wineriter & Walker, 1990) parasitoid flies are among the best elements to control pest populations given they would reproduce rapidly on their hosts during a short time. But in the case of termites, their cryptic behavior (Logan *et al*., 1990) is the main barrier to adopting parasitoid flies in control program. However, it is not a bad idea to take time to develop a methodological approach to increase host-parasitoid encounters in natural environments given that available control methods are not efficient enough (Ambele *et al*., 2018a; Djuideu *et al*., 2020). We think that it will be interesting to explore how these parasitoid flies can become a solution to termite control through innovative approaches. During this study, we also found that termites from heavily shaded systems were more parasitized than termites from poorly shaded systems, and that aboveground termites were more parasitized than belowground ones. As we said above, shaded cocoa systems are known to support much higher ecological and functional biodiversity than unshaded systems and therefore will support much more parasitoid infestations than unshaded systems (Sperber *et al*., 2004; Tscharntke *et al*., 2011). Encouraging agroforestry systems in cocoa farming is a good step towards enhancing natural pest control in farms (Bos *et al*., 2007; Bisseleua *et al*., 2013). The fact that aboveground termites were more parasitized than belowground ones could be explained by the size of their occupied niche, the same we observed for mites. Aboveground termites are more susceptible to encounter parasitoid flies which may lay eggs in their bodies than belowground termites. We also found no

significant differences in fly parasitism rate between rainy and dry seasons during this study. According to (Folgarait *et al.*, 2003), parasitoid phorids do not display the same seasonal patterns across species, even in the same genus. The sometimes show opposite patterns, the one preferring cooler periods meanwhile others are more active during warm months. This difference in seasonal patterns between parasitoid flies may be explained as an adaptive response to interspecific competition for the available resource, therefore separating their feeding niches in different period of time. During this study, all the parasitoid flies belonged to family Phoridae and *Megaselia* was the most represented genus. Phorid flies are the dipterans the most commonly associated with termites either for their termitophilous or their parasitoid-host relationship (Dupont and Pape, 2009). For some phorid species, at least one life phase or stage must be completed in direct association with termites whether it is egg, larvae or adult phase (Dupond & Pape, 2009). Among Phoridae, the genus *Megaselia* is numerously the most diversified genus with more than 1700 species described worldwide (Disney, 1994). Associations of *Megaselia* species and termites are documented by several authors, especially for *M. scalaris* (Sekamatte *et al.*, 2000; Noknoy *et al.*, 2020). In Cameroon, *M. scalaris* have been reported as opportunist parasitoid of honey bees by Cham *et al.* (2018), it acts as a facultative parasitoid on insects mainly parasitizing moribund individuals in laboratory cultures (Disney, 2008). Its facultative parasitoidism towards insects however makes of *M. scalaris* a bad agent for pest control because in the overcrowded conditions of laboratory cultures, the infestation rates reported will bear no relation to the likely rates of infestation in the field (Disney, 2008). Obligatory parasitoid flies are the only ones to be consider for pest control program. The genus *Melaloncha*, also find during this study, is a fast, agile parasitoid of bees and can be seriously considered for pest control program due to their obligatory parasitoid life style (Brown, 2016). In addition, unidentified *Megaselia* species of this study may also be obligatory parasitoids and further studies are needed to confirm their life style.

The topologies of the trees resulting from the two different analyses (Cladogram vs Phenogram) were largely congruent and differed in the number of differentiated lineages and in the placement of some taxa. Following the BLAST results, both trees showcased the same relationships between unidentified *Megaselia* species and their corresponding sister species in NCBI database, suggestion strong relatedness between these sister species. This study confirms the monophyly of *Megaselia* species as suggested by Hartop *et al.* (2021) in the 'Core *Megaselia*' clade. However, the taxon sampling is neither geographically diverse nor dense enough to draw any definite conclusions regarding higher-level relationships within the genus.

CONCLUSION, RECOMMENDATIONS AND PERSPECTIVES

Conclusion

(1) This study revealed that termites associated with cocoa trees are functionally diversified and shade tree removal in cocoa agroforestry systems encourages termite pest outbreaks and the damages of more cocoa trees. Roots of cocoa trees were the most susceptible plant parts to damages caused by subterranean termites such as *Microtermes osborni* and *Ancistrotermes cavithorax*. The study also reported changes in the feeding behavior of some soil-feeding termite species (*Procubitermes undulans* and *Anenteotermes humerus*) and could be attributed to the extinction of their primary food sources due to shade trees removal in the cocoa agroforestry systems.

(2) The study also highlighted ecosystem services and disservices provided by termites to the cocoa agroforestry systems and showed that poor shaded systems reduce the beneficial role of termites to enhancing soil aeration and their fertility. The study also showed that belowground infestation of termites was significantly lower in shaded cocoa agroforestry systems as compared to unshaded ones, suggesting the importance of shade trees and habitat complexity in managing complex pest species such as termites.

(3) The study revealed that the Taylor's power law was the best fitted model to describe the distribution pattern of termites in cocoa agroforestry systems and on cocoa trees. Green's sequential sampling was the most precise sampling model because it requires less number of cocoa trees. The sampling plans estimated from these models are designed to monitor termite infestations in cocoa agroforestry systems and could be used by farmers and agroecologists for decision-making and termite management in agricultural lands.

(4) The study identified a diversity of biological control agents to be further tested against termites. They included entomopathogenic fungi (26 species), *Cordyceps tenuipes*, *Penicillium paxilli*, *P. citrinum* and *Fusarium oxysporum*; mites (19 species), most of them classified as phoretic on termites with *Acotyledon* sp. suspected to be good candidates for further assessment on termite species; the parasitic fly group (14 species), *Megaselia* spp., (However, could be a victim of facultative parasitoidism in natural environment) and *Melaloncha* sp. (could be explored as potential biocontrol agent against termites). Shade trees in farms contributed to increase the diversity and the parasitism rate of these biological agents on termites.

(5) The molecular tools and techniques used in this study contributed to improved accuracy during identification and isolation of fungi and parasitoid flies collected on termites. For further studies on the relationship between termites and biological control agents, it is important to combine morphological and molecular approaches. These molecular tools also

contributed to provide more taxonomic insight on the co-evolutive history and mechanism between isolated fungi and *Megasela* species.

Recommendations and future research routes

(1) more research is needed on climate change, anthropogenic disturbance and the feeding behavior of termites in agricultural landscapes with the aim to document adaptation behavior of termite to disturbance and climate change and develop innovative management strategies

(2) more screening work is needed to document the diversity of beneficial insects that could play significant role in biological control programs of termites in agricultural landscapes either inondative, augmentative or conservative including the socio-economic importance of termites in agricultural landscape and other ecosystems where termites pose a threat

(3) Further research should also be performed to improve the molecular techniques used in this study with the aim of refining these techniques and make them ready available for universities and research laboratories in sub-Saharan Africa.

(4) Explore the role of ecological disturbance and environmental factor on the eco-evolutionary history of entomopathogenic fungi and parasitoid flies living on termites in agricultural landscapes and other environments where termites pose a threat

(5) And last, it will be important to look at how best new formulations could be developed using the biological control agents identified during this study including protocols for their successful delivery and use by pest managers in an IPM program.

Overall, this study is the first study to screen for biological control agents of termites in cocoa agroforestry systems. I have developed and make available new methodological approaches and tools to collect and identify beneficial organisms in agricultural landscape. I am also making available sampling plans and dispersion patterns to study the population dynamics of complex species such as termites that can provide ecosystem services and disservices within the same ecosystem and landscape. These tools and methodological approaches could help further research on the bio-ecology and the economic importance of termites in agricultural landscape and other ecosystems where termite is a threat. This study highlighted the need to develop target-specific biological control strategies against termites in tree crops (belowground vs aboveground damages). I have been able to screen new biological control agents that could be explored in further studies and to develop new formulation against termites in agricultural

landscape and other ecosystems where termite is a threat. This study also provide a first step in the phylogenetic study of fungi and *Megaselia* species isolated on termites and may impulse new research interest to explore evolutionary history of these taxa with their hosts. I can strongly affirm that our objectives have been met.

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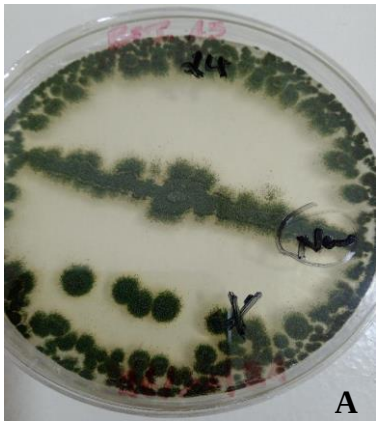
APPENDIXES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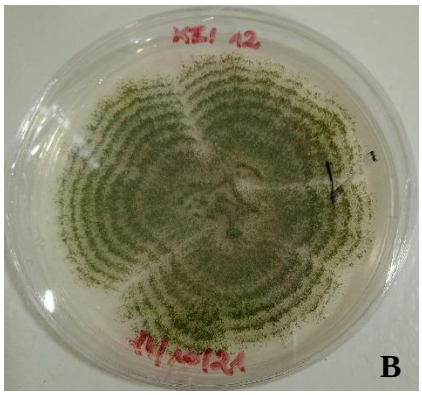
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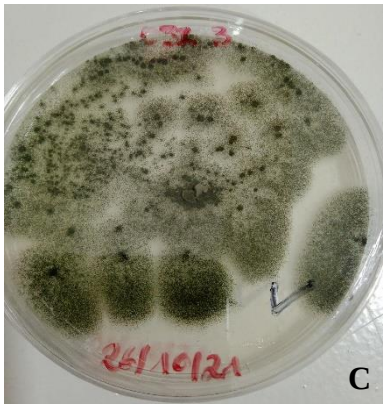
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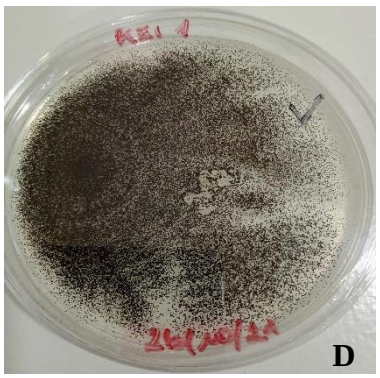
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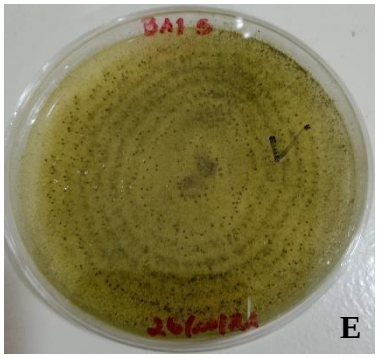
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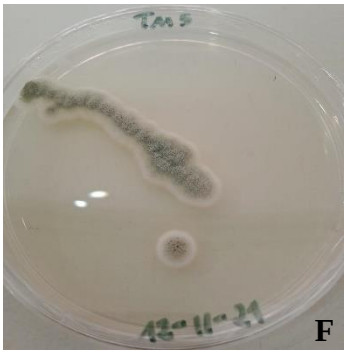
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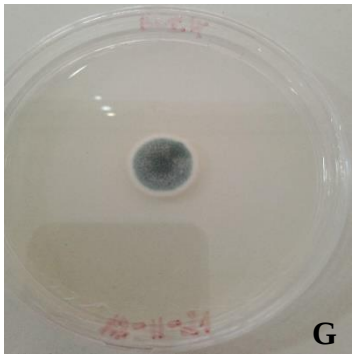
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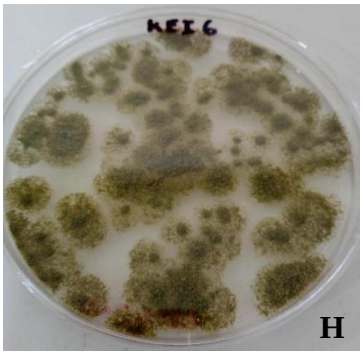
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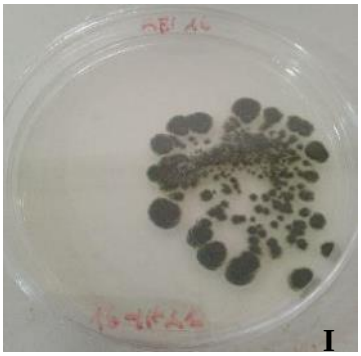
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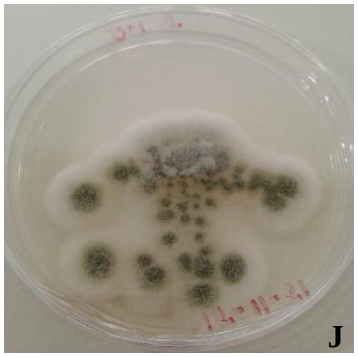
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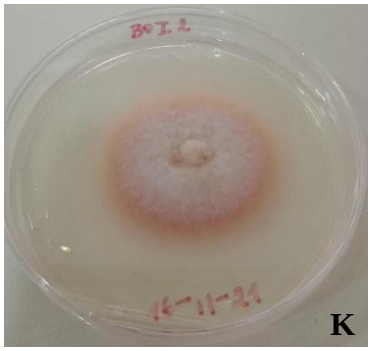
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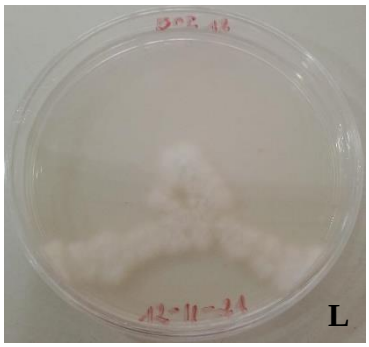
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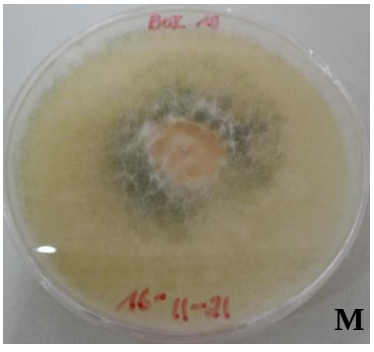
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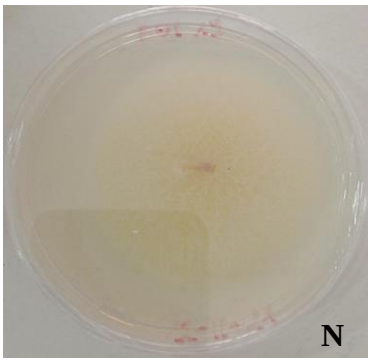
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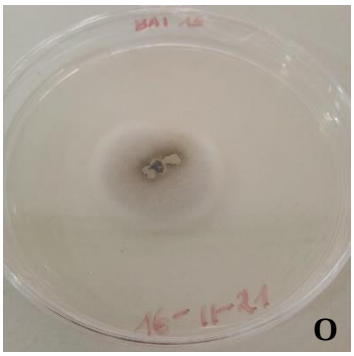
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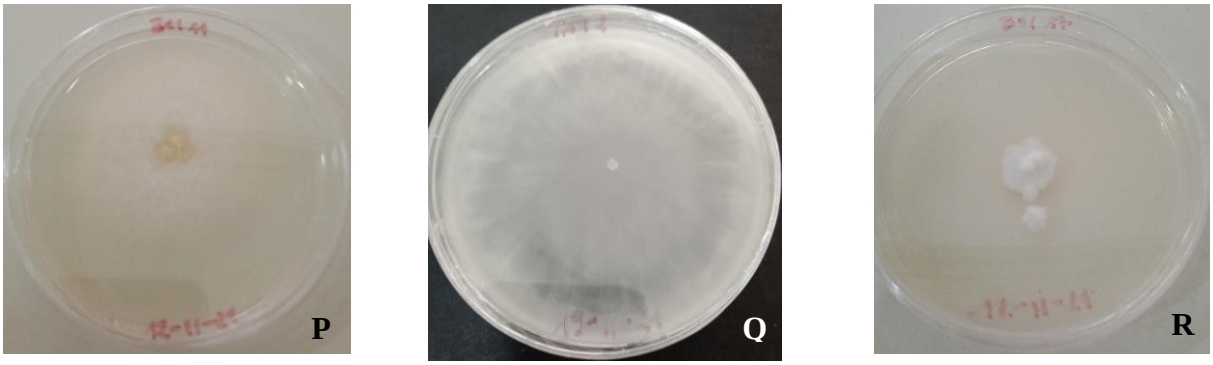
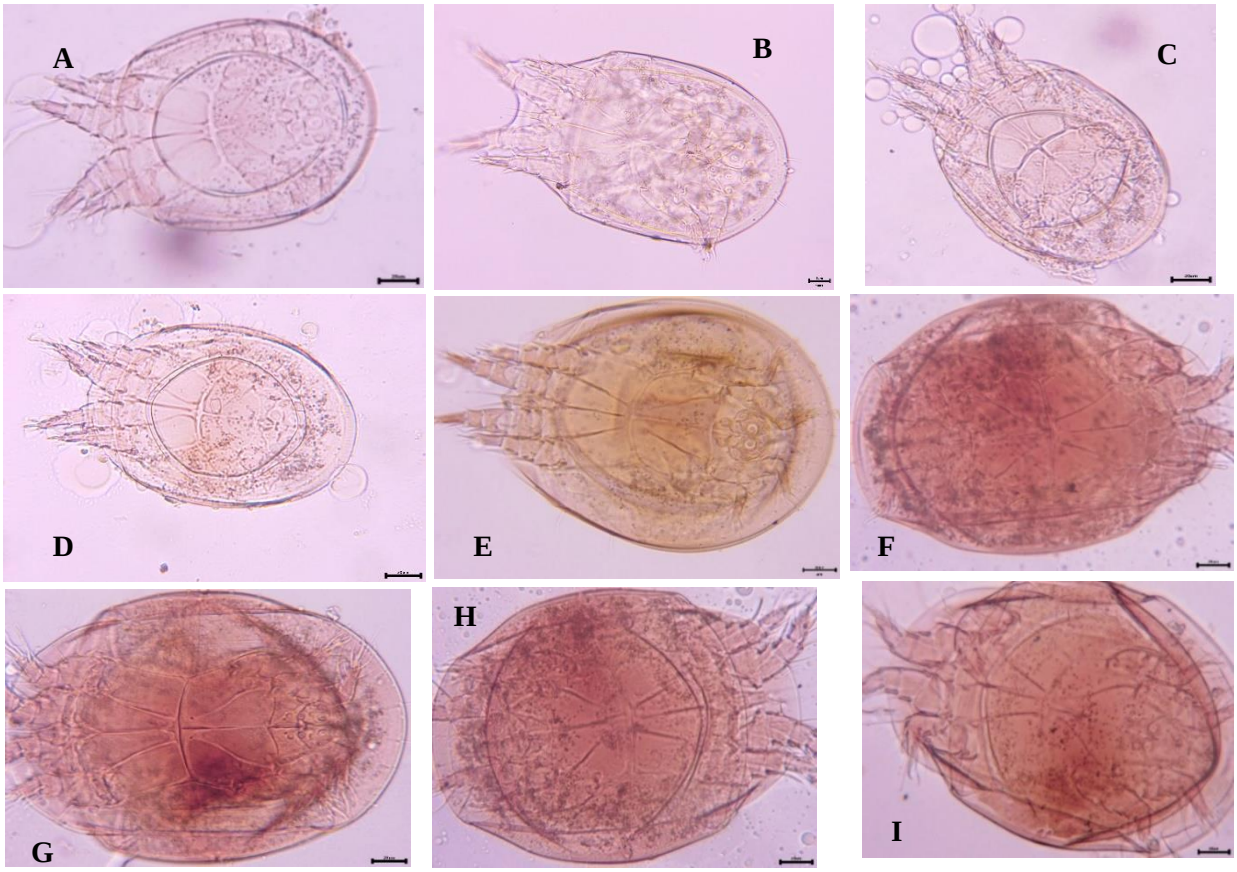
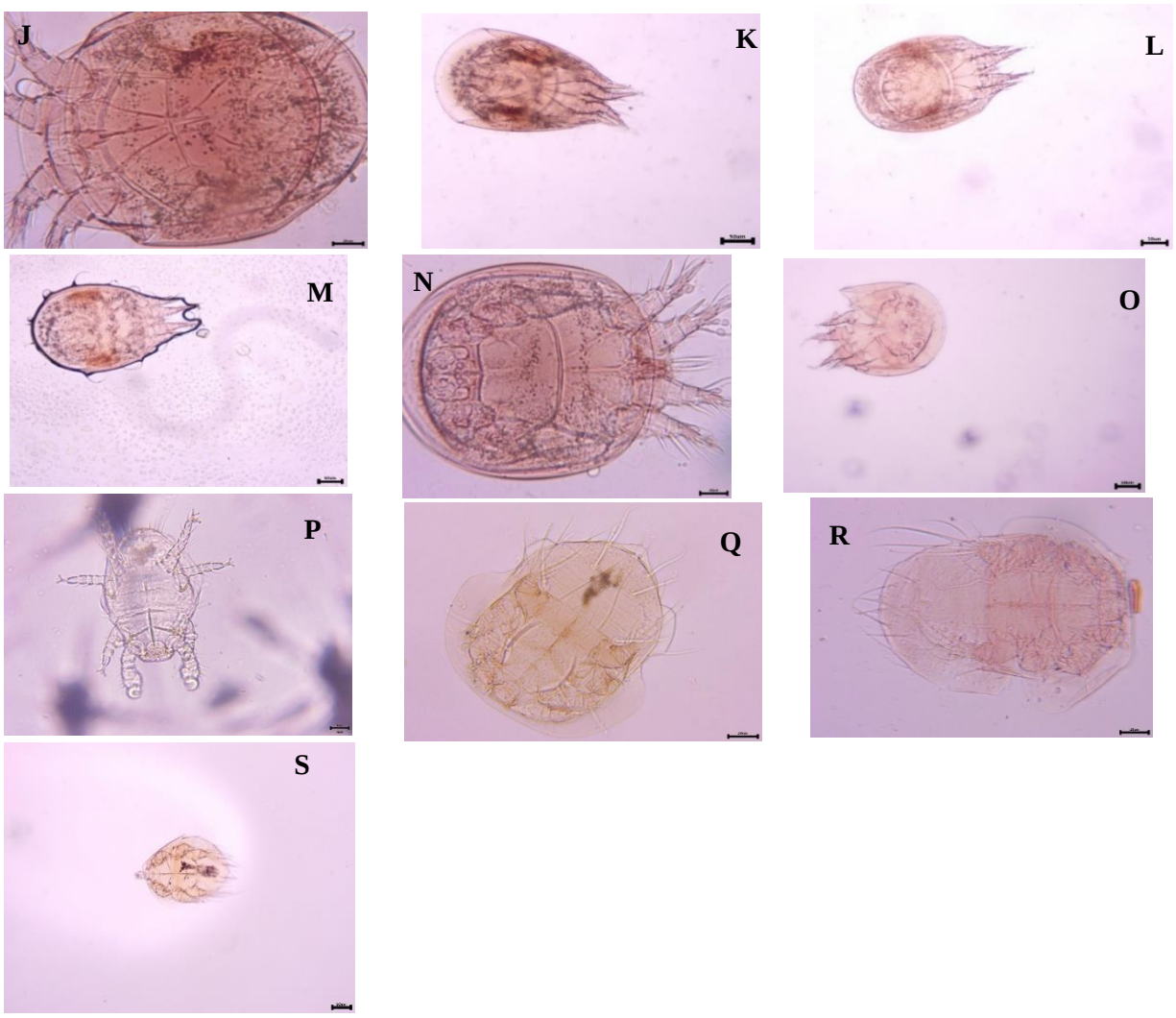
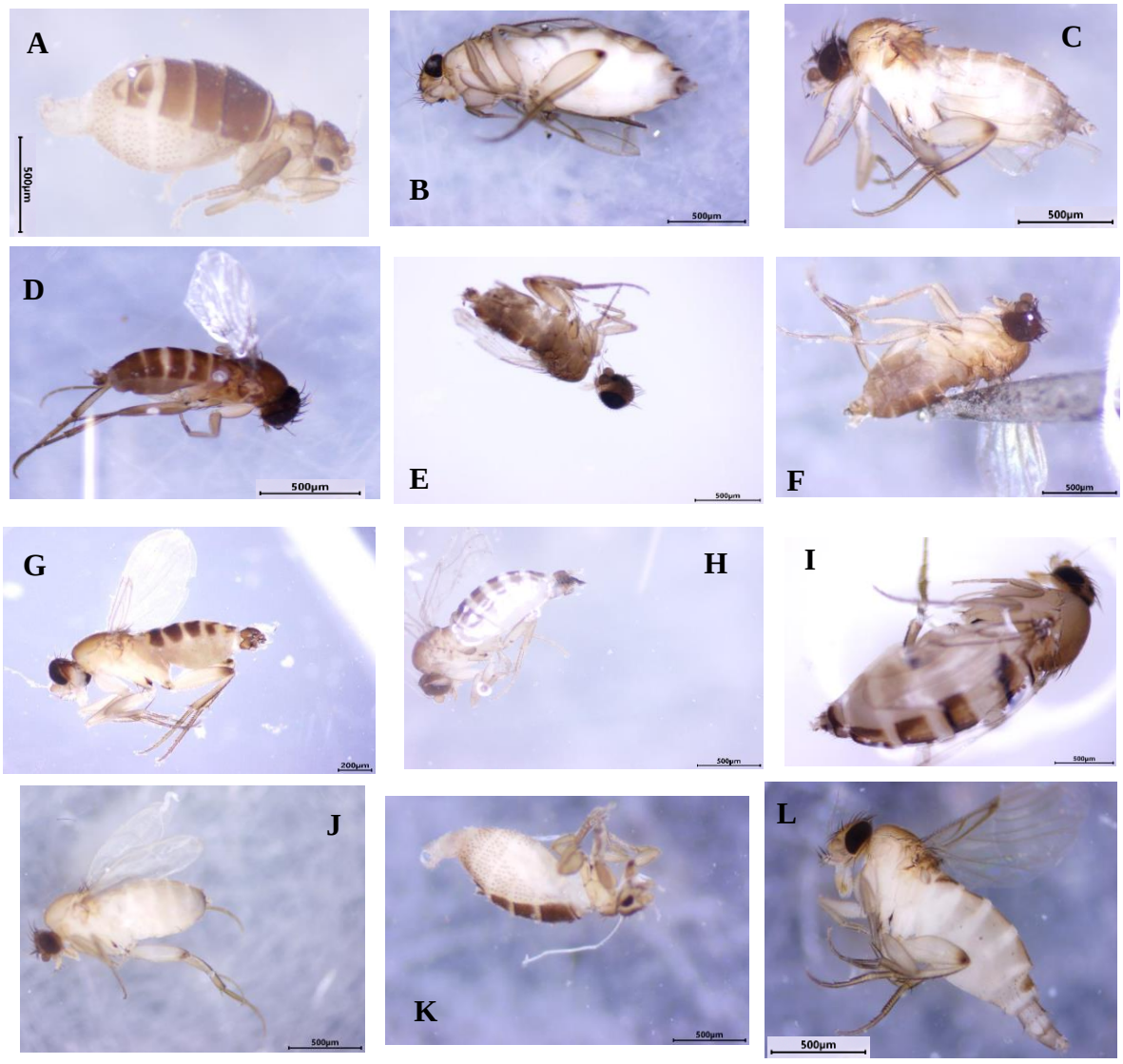


Appendix 1. Image catalog of fungi isolated from termites and inoculated on PDA culture medium in petri dishes (2-3 weeks after inoculation). **A**- *Aspergillus carneus*, **B**- *A. flavus*, **C**- *A. fumigatus*, **D**- *A. niger*, **E**- *A. nomiae*, **F**- *A. protuberus*, **G**- *A. sydowii*, **H**- *A. tamarii*, **I**- *Penicilium citrinum*, J- *A. paxilli*, **K**- *Talaromyces purpureogenus*, **L**- *Cordyceps tenuipes*, **M**- *Trichoderma harzianum*, **N**- *Fusarium oxysporum*, **O**- *Scedosporium boydii*, **P**- *Hypoxylon* sp., **Q**- *Phanerochaete chrysosporium*, **R**- Corticiaceae sp.





Appendix 2. Image catalog of mites isolated from termites in dorsal view under light microscope. **A**- *Caloglyphus* sp.1, **B**- *Caloglyphus* sp.2, **C**- *Caloglyphus* sp.3, **D**- *Caloglyphus* sp.4, **E**- *Acotyledon* sp., **F**- Acaridae gen.1 sp.1, **G**- Acaridae gen.1 sp.2, **H**- Acaridae gen.1 sp.3, **I**- Acaridae gen.1 sp.4, **J**- Acaridae gen.1 sp.5, **K**- Acaridae gen.2 sp.1, **L**- Acaridae gen.2 sp.2, **M**- Acaridae gen.2 sp.3, **N**- Acaridae gen.3 sp., **O**- Acaridae gen.4 sp., **P**- Pygmephoridae sp., **Q**- *Scutacarus* sp.1, **R**- *Scutacarus* sp.2, **S**- *Coronipes* sp.



Appendix 3. Image catalog of parasitoid flies emerging from termites. **A**- *Aenictacantha* sp. ♂, **B**- *Apocephalus* sp.1 ♂, **C**- *Apocephalus* sp.2 ♀, **D**- Commoptera sp. ♂, **E**- *Megaselia nigrescens* ♂, **F**- *M. infraposita*, **G**- *M. scalaris* ♂, **H**- *Megaselia* sp.1 ♂, **I**- *Megaselia* sp.2 ♂, **J**- *Megaselia* sp.3 ♂, **K**- *Stethopathusa* sp. ♂, L- *Triphleba* sp. ♂.