



**IMPACT OF COVER CROP SPECIES, TERMINATION STAGE AND TERMINATION
METHOD ON SOIL NITROGEN, ORGANIC CARBON AND ENZYME ACTIVITIES**

by

ADEWOLE TOMIWA ADETUNJI

Thesis submitted in fulfilment of the requirements for the degree

Doctor of Technology: Environmental Health

In the Faculty of Applied Science

At the

Cape Peninsula University of Technology

Supervisor: Prof. F.B. Lewu

Co-supervisor: Dr. A.R. Mulidzi

Dr. B. Ncube

**Cape Town
July 2019**

CPUT copyright information

The dissertation/thesis may not be published either in part (in scholarly, scientific or technical journals), or as a whole (as a monograph), unless permission has been obtained from the University

DECLARATION

I, **ADEWOLE TOMIWA ADETUNJI**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.



Signed

July 1st, 2019

Date

ABSTRACT

Soil is an essential resource and it is vital to manage its quality in order to improve agricultural yield and environmental quality. A number of methods have been used for decades to improve soil quality including cover cropping. Cover cropping can serve as a sustainable approach to combat soil degradation, provide organic carbon and improve microbial processes and fertility in agricultural soils. Thus, it is crucial to adopt the best cover crop management practices in order to obtain their full benefits. Although, there is a considerable body of literature on cover crop use and management, research assessing how specific cover crop species, termination stage and termination method affect soil organic carbon, nitrogen and enzyme activities remains limited. Currently, no combined study of such processes has been reported in Africa, including the South African cropping systems. Two separate studies were conducted in the greenhouse and field in the Western Cape Province to examine the impact of two termination stages (vegetative and flowering) on the biomass, total C, N and C:N ratios of four cover crops. The study assessed the immediate effect of living cover crops and residues, two termination stages and two termination methods (slash and spray) on soil β -glucosidase, phosphatase and urease activities as well as soil pH, soil organic carbon (SOC) and total soil N. Species tested as cover crops were, grazing vetch (*Vicia dasycarpa* Ten.), field pea (*Pisum sativum*), oats (*Avena sativa* L.), rye (*Secale cereal* L.) and a control (no cover crop). The experimental design was a randomized block design with 3 replications. Soils were sampled at the beginning of the experiment, at kill and at one year after cover crop kill. Cover crops were sampled at kill. The first phase of the study (greenhouse experiment) was carried out from 2016 to 2017, with soils obtained from the Agricultural Research Council (ARC) Nietvoorbij Research Farm. The second phase of the study (field experiment) was carried out at two sites, ARC Nietvoorbij and Bien Donne Research Farms, from 2017 to 2018.

It was observed in the greenhouse study that delay in termination of cover crops till flowering stage significantly increased total C and C:N ratio content of all the cover crops. However, delaying termination from vegetative till flowering stage decreased N in the tissue of pea, oats, vetch and rye by 59%, 65%, 44% and 56%, respectively. Cover crop presence in the soil had no effect on soil pH. At kill, living cover crop species did not influence SOC and total soil N but stimulated β -glucosidase, urease and phosphatase activities compared to the control. At one year, rye, oats and pea residues increased SOC by 8%, 7% and 4%, respectively. However, only pea and vetch residues increased soil N level in the short-term. At one year, β -glucosidase activity was greater under rye (77.58 $\mu\text{g PNP g}^{-1}$ soil h^{-1}), oats (76.53 $\mu\text{g PNP g}^{-1}$ soil h^{-1}) and pea (74.64 $\mu\text{g PNP g}^{-1}$

soil h^{-1}) residues than vetch ($67.99 \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) and control ($61.06 \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$). Unlike urease, all cover crop residues had a positive impact on phosphatase activity at one year. Termination of cover crops at flowering stage resulted in greater β -glucosidase activity and SOC levels than the vegetative stage at one year. Compared to flowering, termination at vegetative stage improved soil N levels and phosphatase activity at both sampling times. Termination by slash had a positive impact on SOC and β -glucosidase activity compared to spraying. However, termination method had no effect on soil N, urease and phosphatase activity at one year. The significant interaction ($P < 0.05$) of sampling time, cover crop and termination stage effects on SOC, total soil N, β -glucosidase and phosphatase activities observed in this study indicates that these management approaches can maximize cover crop benefits and improve soil fertility in the short-term.

Field experiment results indicate that delaying termination from vegetative till flowering stage increased the biomass yield, total C and C:N ratios of vetch, pea, oats and rye, respectively, while their tissue N decreased, at both sites. Cover crop biomass yields at the Nietvoorbij site varied from $207 - 4480 \text{ kg ha}^{-1}$ while those at the Bien Donne site varied from $835 - 9891 \text{ kg ha}^{-1}$ with indices being higher under rye and oats than pea and vetch. Cover crop presence reduced soil pH at both sites. At kill, living rye and pea considerably increased SOC at the Bien Donne site, while at one year, the residues of oats raised SOC by 7% and 9% at Nietvoorbij and Bien Donne sites, respectively. Generally, cover crops were slow to impact SOC and the activities of β -glucosidase, urease and phosphatase, at both sites. Total soil N varied and was elevated by all the tested living cover crops and residues, at both sites. Results at both sampling times showed that cover crop termination at the flowering stage promoted the activities of the three enzymes than the vegetative stage, at both sites. At kill, higher SOC and total soil N was also observed at flowering stage compared to vegetative stage. However, at one year, total soil N was greater at vegetative stage than the flowering stage at both sites. Although, termination methods had no effect on SOC and total soil N at the Nietvoorbij site, termination by slashing favoured SOC compared to spraying while total soil N was more promoted by spraying than slashing at the Bien done site. Soil enzymes did not respond to termination methods at the Bien Donne site, whereas slash stimulated β -glucosidase, urease and phosphatase activities than spraying at the Nietvoorbij site. The significant interaction ($P < 0.05$) of sampling time, cover crop, termination stage and termination method effects on soil N level observed at both sites of this study (field and the greenhouse studies), indicates that these management factors can optimize cover crop benefits and improve soil fertility. However, results from this field study indicate that longer time spans may

have been needed to see the impact of cover crops on soil enzyme activities and SOC levels in the Cape Mediterranean environment.

Keywords: Cover crop, nitrogen, organic carbon, phosphatase, soil management, termination, urease and β -glucosidase.

ACKNOWLEDGEMENTS

I wish to thank:

- God, for divine strength, courage and grace that sustained me throughout my research work.
- My supervisors, Prof. F.B. Lewu, Dr. R. Mulidzi and Dr. B. Ncube for their persistent support, guidance and technical input in the course of the study.
- My wife and son, for their love, encouragement and holding forth in my absence.
- My parents and the entire Adetunji family, for their support and prayers throughout my PhD career.
- The Bioresource Engineering Research Group especially the principal investigator, Prof. S.K.O. Ntwampe for their support and motivation in the course of the study.
- The staff of the department of chemistry especially Dr. O.S. Olatunji, Mrs. Z. Mthembu and Prof. A. Mohammed for their support during the experimental set-up for soil chemical test.
- The technical staff of the Agrifood Technology Station especially Ndumiso Mshicileli for their support during the experimental set-up for total soil nitrogen analysis.
- The Soil Science Department, ARC - Infruitec/Nietvoorbij, for allowing me to use their resources. I appreciate Dr. N.M. Lewu for her support during the course of the project. I also thank Mr. Ncedo Ndololwana and Mrs. Isabella Van Huyssteen for their technical support on the field and in the lab.
- The Department of Agriculture CPUT staff and colleagues, especially Mr. M. Tanga, Ms. N. Mfeka and Ms. A. Bolilitye for their support and encouragement throughout the course of my PhD program.
- Cape Peninsula University of Technology, University Research Fund (URF) for their financial support.

DEDICATION

TO MY FAMILY

PREFACE OF THE THESIS

The research work presented in this thesis was conducted under the Department of Agriculture, Cape Peninsula University of Technology, Cape Town, South Africa. The experimental work was conducted at the Agricultural Research Council (ARC) Infruitec-Nietvoorbij, Western Cape Province, South Africa.

The thesis is made-up of six chapters.

Chapter 1 is a brief general introduction, hypothesis, research objectives, delineation and the significance of the study.

Chapter 2 presents a detailed review on the impacts and benefits of cover crops on soil quality in the cropping systems in terms of species selection, termination stage and termination methods. It reviews the roles of soil enzymes such as β -glucosidase, phosphatase and urease in the cropping systems, as well as the use of their activities as soil quality indicator.

Chapter 3 focuses on examining the impact of termination stages on the chemical composition (C and C:N) of four cover crops in a greenhouse experiment. It presents the immediate effect of living cover crops and their residues, two termination stages and two termination methods on soil organic carbon level as well as soil β -glucosidase activity.

Chapter 4 centres on exploring the impact of termination stages on the chemical composition (N and C:N) of four cover crops in a greenhouse experiment. It presents the immediate effect of living cover crops and their residues, two termination stages and two termination methods on soil nitrogen as well as soil urease and phosphatase activities.

Chapter 5 explores the effect of termination stages on the chemical composition (C, N and C:N) and biomass of four cover crops grown under field conditions at two sites at the Agricultural Research Council. It focuses on the short-term impacts of living cover crops and residues on soil pH, total organic carbon, total soil nitrogen, β -glucosidase, urease and phosphatase activities at the two termination stages, and under two termination methods.

Chapter 6 contains the general conclusions and recommendations.

RESEARCH OUTPUTS

The following research outputs represent the contributions by the candidate to scientific knowledge and development during his doctoral candidacy (2015 - 2019):

A. Journal Papers

Published

Adetunji, A.T., Lewu, F.B., Mulidzi, R. & Ncube, B. **2017**. The biological activities of β -glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of Soil Science and Plant Nutrition* 17: 794-807. <http://dx.doi.org/10.4067/S0718-95162017000300018> (Chapter 2)

Adetunji, A.T., Ncube, B., Meyer, A.H., Mulidzi, R. & Lewu, F.B. **2019**. Soil alteration index three and soil organic matter in response to cover crop species and management practices. 16th South Africa International Conference on Agricultural, Chemical, Biological and Environmental Science proceedings. <https://doi.org/10.17758/EARES8.EAP1119113> (Appendices)

Accepted

Adetunji, A.T., Ncube, B., Meyer, A.H., Mulidzi, R. & Lewu, F.B. β -glucosidase activity, soil organic carbon and nutrient in plant tissue in response to cover crop species and management practices. Submitted to *South African Journal of Plant and Soil* (Manuscript ID: TJPS-2019-0076) (Chapter 3)

Submitted

Adetunji, A.T., Ncube, B., Mulidzi, R. & Lewu, F.B. Management impact and benefit of cover crop on soil quality: a review. Submitted to *Soil & Tillage Research* (Manuscript ID: STILL_2019_690) (Chapter 2)

Adetunji, A.T., Ncube, B., Meyer, A.H., Olatunji, O.S., Mulidzi, R. & Lewu, F.B. The impact of cover crop species, termination stage and termination method on soil nitrogen and enzyme activities. Submitted to *Soil Science and Plant Nutrition* (Manuscript ID: SSPN-19-254-F) (Chapter 4)

B. Book chapters

Published

Adetunji, A.T., Ncube, B., Mulidzi, R. & Lewu, F.B. **2020**. Potential use of soil enzymes as soil quality indicators in agriculture. In: Mishra, B.B & Nayak, SK (eds.) *Frontiers in Soil and Environmental Microbiology*. 1st Edition, Florida, United States: CRC press (Chapter 2)

C. Conferences Attended

Adetunji, A.T., Ncube, B., Meyer, A.H., Mulidzi, R. & Lewu, F.B. 2019. Soil alteration index three and soil organic matter in response to cover crop species and management practices. 16th South Africa International Conference on Agricultural, Chemical, Biological and Environmental Science (ACBES – 19), held at Johannesburg, South Africa, 18th – 19th November 2019 (Oral presentation)

Adetunji, A.T., Ncube, B., Meyer, A.H., Mulidzi, R. & Lewu, F.B. 2019. Soil nitrogen and enzyme activities in response to cover crop species and management practices, Proceedings of 7th international U6 consortium conference, held at University of Calabar, Nigeria, 2nd – 6th September 2019 (Oral presentation)

TABLE OF CONTENTS

DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENTS	vi
DEDICATION.....	vii
PREFACE OF THE THESIS	viii
RESEARCH OUTPUTS.....	x
TABLE OF CONTENTS.....	xii
LIST OF FIGURES	xv
LIST OF TABLES	xvi
LIST OF APPENDICES	xviii
GLOSSARY	xix
CHAPTER 1	1
GENERAL INTRODUCTION.....	1
1.1 Introduction	1
1.2 Hypotheses	3
1.3 Objectives	3
1.4 Delineation of the study	3
1.5 Significance of the study.....	3
References.....	4
CHAPTER 2	6
LITERATURE REVIEW	6
2.1 Management impact and benefit of cover crop on soil quality.....	6
2.1.1 Introduction	6
2.1.2 Cover crop use and benefits for soil quality improvement	7
2.1.3 Selection of cover crop species.....	14
2.1.4 Termination stage	15
2.1.5 Termination method.....	18
2.1.6 Herbicides.....	20
2.2 The biological activities of β -glucosidase, phosphatase and urease as soil quality indicators.....	22
2.2.1 Introduction.....	22
2.2.2 General characteristics and use of soil enzymes.....	23
2.2.3 β -glucosidases.....	25
2.2.4 Phosphatases	27
2.2.5 Urease	30
2.3 Conclusion	32
References.....	32
CHAPTER 3	45

β -GLUCOSIDASE ACTIVITY, SOIL ORGANIC CARBON AND NUTRIENT IN PLANT TISSUE IN RESPONSE TO COVER CROP SPECIES AND MANAGEMENT PRACTICES	45
3.1 Introduction	45
3.2 Materials and methods	46
3.2.1 Experimental description	46
3.2.2 Plant/soil sampling and analysis	47
3.2.3 Soil enzyme activity assay	48
3.2.4 Statistical analysis.....	48
3.3 Results	49
3.3.1 Effect of termination stage on total C concentration and C:N ratio in the plant tissue of cover crops	49
3.3.2 Soil organic carbon	49
3.3.3 β -glucosidase activity.....	52
3.4 Discussion.....	54
3.4.1 Cover crop tissue.....	54
3.4.2 Soil organic carbon	55
3.4.3 β -glucosidase activity.....	57
3.5 Conclusion	58
References.....	59
CHAPTER 4	62
THE IMPACT OF COVER CROP SPECIES, TERMINATION STAGE AND TERMINATION METHOD ON SOIL NITROGEN AND ENZYME ACTIVITIES	62
4.1 Introduction	62
4.2 Materials and methods	63
4.2.1 Soil enzyme activity assay	63
4.2.2 Statistical analysis.....	64
4.3 Results	64
4.3.1 Combined statistical analysis	64
4.3.2 Effect of termination stage on total N concentration and C:N ratio in the plant tissue of cover crops	65
4.3.3 Soil pH as affected by cover crop species and sampling time	65
4.3.4 Total soil nitrogen.....	66
4.3.5 Soil enzyme activity	68
4.4 Discussion.....	71
4.4.1 Cover crop tissue.....	71
4.4.2 Soil pH.....	71
4.4.3 Total soil nitrogen.....	72
4.4.4 Soil enzymes	74
4.5 Conclusion	76

References.....	76
CHAPTER 5	81
COVER CROP SPECIES AND MANAGEMENT EFFECT ON SOIL NITROGEN, ORGANIC CARBON AND ENZYME ACTIVITIES	81
5.1 Introduction	81
5.2 Materials and methods	82
5.2.1 Study sites	82
5.2.2 Experimental design	83
5.2.3 Cover crop sampling and analysis	84
5.2.4 Soil sampling and chemical analysis	84
5.2.5 Soil enzyme activity assay	84
5.2.6 Statistical analysis.....	85
5.3 Results	85
5.3.1 Initial soil characteristics	85
5.3.2 Effect of termination stage on cover crop biomass, total C, total N and C:N ratio	85
5.3.3 Effect of cover crop species and sampling time on soil pH.....	86
5.3.4 Soil organic carbon and total soil nitrogen.....	87
5.3.5 Soil enzyme activity	90
5.4 Discussion.....	93
5.4.1 Cover crop	93
5.4.2 Soil pH.....	93
5.4.3 Soil organic carbon and total nitrogen.....	94
5.4.4 Soil enzymes	96
5.5 Conclusion	98
References.....	98
CHAPTER 6	103
GENERAL CONCLUSIONS AND RECOMMENDATIONS	103
6.1 Summary and conclusions	103
6.2 Recommendations	105
APPENDICES.....	106

LIST OF FIGURES

Figure 3.1: Cover crop species and sampling time effects on soil organic carbon. Bars (means - n = 3) with the same letter are not significantly different based on Fisher's LSD ($P < 0.05$)	50
Figure 5.1: Cover crop species and sampling time effects on soil organic carbon. Bars (means - n = 3) with the same letter are not significantly different based on Fisher's LSD ($P < 0.05$)	88
Figure 5.2: Cover crop species and sampling time effects on soil organic carbon. Bars (means - n = 3) with the same letter are not significantly different based on Fisher's LSD ($P < 0.05$)	89

LIST OF TABLES

Table 2.1: Common cover crop species used in cropping system	6
Table 2.2: Some cover crop benefits in agricultural cropping systems	13
Table 2.3: Soil enzymes as indicators of soil quality	24
Table 3.1: Interaction effects of cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) on total C and C:N ratio in the plant tissue	49
Table 3.2: Effect of cover crop species and termination stage on soil organic carbon at different sampling times (kill and one year).....	51
Table 3.3: Overall impact of termination stage (vegetative and flowering) on soil organic carbon (%) at different sampling times (kill and one year).....	51
Table 3.4: Overall impact of termination method (vegetative and flowering) on soil organic carbon at one year.....	52
Table 3.5: Effect of cover crop species and sampling time on β -glucosidase activity ($\mu\text{g PNP g}^{-1}$ soil h^{-1}).....	52
Table 3.6: Overall impact of termination stage (vegetative and flowering) on β -glucosidase activity ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) at different sampling times (kill and one year).....	53
Table 3.7: Effect of cover crop species and termination stage on β -glucosidase activity ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) at different sampling times (kill and one year).....	53
Table 3.8: Overall impact of termination method (slash and spray) on β -glucosidase activity ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) at one year	54
Table 4.1: Analysis of variance ($\text{Pr} > \text{F}$) for total soil nitrogen, urease activity and phosphatase activity data in combined sampling times as affected by cover crop species, termination stage, and termination method	64
Table 4.2: Interaction effects of cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) at kill on tissue N concentration and C:N ratio.....	65
Table 4.3: Cover crop species and sampling time effects on soil pH and nitrogen	66
Table 4.4: Cover crop species and termination stage effects on soil nitrogen at different sampling times (kill and one year).....	67
Table 4.5: The overall effect of termination stage (vegetative and flowering) on total soil nitrogen at different sampling times (kill and one year)	67
Table 4.6: The overall effect of termination method (slash and spray) on total soil nitrogen at one year	68
Table 4.7: The activities of urease ($\mu\text{g NH}_4^+ \text{g}^{-1}$ soil 2 h^{-1}) and phosphatase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) as affected by cover crop species and sampling time	69

Table 4.8: Cover crop species and termination stage effects on urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) activities at different sampling times (kill and one year) .	70
Table 4.9: The overall effect of termination stage (vegetative and flowering) on the activities of soil urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) at different sampling times (kill and one year)	70
Table 4.10: The overall effect of termination method (slash and spray) on the activities of urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) at one year	71
Table 5.1: Cover crop growth termination date and sampling times for four cover crops at Nietvoorbij and Bien Donne study sites.....	84
Table 5.2: Initial physiochemical characteristics of Nietvoorbij and Bien Donne soils.....	85
Table 5.3: Interaction effects of cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) on cover crop biomass, total C, total N and C:N ratio at Nietvoorbij and Bien Donne study sites.....	86
Table 5.4: Cover crop species and sampling time effects on soil pH at Nietvoorbij and Bien Donne study sites	87
Table 5.5: Average effect of cover crop termination stage (vegetative and flowering) on soil organic carbon and total soil nitrogen at different sampling times (kill and one year) at Nietvoorbij and Bien Donne study sites	89
Table 5.7: Average effect of cover crop termination method (slash and spray) on soil organic carbon and total soil nitrogen at one year at Nietvoorbij and Bien Donne study sites.....	90
Table 5.8: Cover crop species and sampling time effects on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$), urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) at Nietvoorbij and Bien Donne study sites.....	91
Table 5.9: Average effect of cover crop termination stage (vegetative and flowering) on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$), urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) at different sampling times (kill and one year) at Nietvoorbij and Bien Donne study sites	92
Table 5.10: Average effect of cover crop termination method (slash and spray) on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$), urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) at one year at Nietvoorbij and Bien Donne study sites.....	92

LIST OF APPENDICES

Appendix A: Chapter 3 analysis of variance ($Pr > F$) for soil organic carbon and β -glucosidase activity in combined sampling times as influenced by cover crop species, termination stage, and termination method	106
Appendix B: Chapter 3 Effect of cover crop species and termination method (slash and spray) on soil organic carbon (%) and β -glucosidase activity ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) at one year	107
Appendix C: Chapter 4 Cover crop species and termination method (slash and spray) effects on soil nitrogen and the activities of urease and phosphatase at one year.....	108
Appendix D: Chapter 5 analysis of variance ($Pr > F$) for SOC and total soil nitrogen data in combined sampling times as affected by cover crop species, termination stage, and termination method at Nietvoorbij and Bien Donne sites	109
Appendix E: Chapter 5 analysis of variance ($Pr > F$) for soil β -glucosidase, urease and phosphatase activities data in combined sampling times as affected by cover crop species, termination stage, and termination method at Nietvoorbij and Bien Donne sites	110
Appendix F: Chapter 5 cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) effects on SOC and total soil nitrogen at different sampling times (kill and one year) at Nietvoorbij and Bien Donne sites	111
Appendix G: Chapter 5 effects of cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$), urease ($\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ soil 2 h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) over two sampling times at Nietvoorbij and Bien Donne study sites.....	112
Appendix H: Chapter 5 effects of cover crop species (oats, pea, rye and vetch) and termination method (slash and spray) on soil organic C and total N at one year in Nietvoorbij and Bien Donne study sites	113
Appendix I: Chapter 5 effects of cover crop species (oats, pea, rye and vetch) and termination method (slash and spray) on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$), urease ($\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ soil 2 h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) at one year in Nietvoorbij and Bien Donne study sites	114

GLOSSARY

°C	Degree Celsius
µg	Microgram
AI3	Soil alteration index three
ANOVA	Analysis of variance
ARC	Agricultural Research Council
C	Carbon
C:N	Carbon to nitrogen ratio
CC	Cover crop
cm	Centimetre
CPUT	Cape Peninsula University of Technology
DF	Degree of freedom
EC	Enzyme Commission
g	Gram
ha	Hectare
K	Potassium
kg	Kilogram
LSD	Least significant difference
m	Meter
mg	Milligram
min	Minute
mm	Millimetre
N	Nitrogen
NH ₄ ⁺	Ammonium
P	Phosphorus
S	Sulphur
SOC	Soil organic carbon
SOM	Soil organic matter
ST	Sampling time
UV	Ultraviolet

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

There is a growing concern about the degradation of agricultural soils due to conventional farming practices and misuse of soil resources. Intensive tillage, short or no fallow, monoculture practices, inadequate biomass input and excessive inorganic fertilizer use, among other practices, result in the decline of soil organic matter and fertility in many countries, including South Africa (Fourie et al., 2001, Mandiringana et al., 2005, Dabney et al., 2010). This has led to significant decline in agricultural production, biodiversity, and other ecosystem services (Delgado and Gantzer, 2015). Soil degradation can be averted by managing and improving soil fertility in a more sustainable way. In recent years organic farming methods that preserve both nutrients and soil, mitigate climate change, and reduce environmental pollution have become very important; hence, conservation agriculture involving cover cropping is suddenly becoming popular all over the world (Giller et al., 2009, Muzangwa et al., 2013).

The cultivation and integration of cover crops provide a reliable means of improving crop yield, soil and environmental quality. Cover crops are multifunctional and contribute to soil quality by improving soil physical, chemical and biological properties (Blanco-Canqui et al., 2015). The crops also enhance organic matter, carbon (C) sequestration, water retention capacity and increase nutrient release, suppress weeds, and control pests (Dabney et al., 2001, O'Reilly et al., 2011, Blanco-Canqui et al., 2015). Cover crops can be any type of plant, but are generally legumes or non-legumes/grasses. Legume cover crops reduce N fertilization requirement by contributing significant amounts of N to the subsequent cash crops through biological fixation of atmospheric N in the soil (Ketterings et al., 2015, Thilakarathna et al., 2015). Non-legume/grasses, on the other hand, supply a considerable amount of carbon (Poeplau and Don, 2015) and have the potential to sequester and recycle inorganic N that may leach below the rooting zone (Cline and Silvernail, 2002, O'Reilly et al., 2012). However, the best management options for cover cropping are largely unknown, including the growth patterns of cover crop species, optimum termination stages and termination methods. There is a need to continually explore and appropriately manage cover crop utilization to obtain full benefits in different soil types and ecosystems.

Recent findings in the studies of soil microbial diversity have led to a better understanding of the structure and function of soil microbial communities. It is now understood that changes in the whole or particular soil microbial communities can be used as reliable signals of changes in soil quality (Karaca et al., 2010). Soil biological quality measurements are therefore becoming

increasingly important in evaluating the impacts of soil management practices and the sustainability of land-use in agricultural soils (Shukla et al., 2006, Mganga et al., 2016). There is a growing scientific interest in the use of enzymatic activity as an agricultural soil quality indicator because enzymatic relationships with soil biology are integrative, very sensitive, operationally practical, easy to quantify and relate to soil tillage and structure (Hussain et al., 2009, Balota and Chaves, 2010). The highly sensitive nature of soil enzymes makes them react to agricultural soil management changes long before changes in other soil quality indicators are noticeable (Balota and Chaves, 2010, Zhang et al., 2015). Soil enzymes catalyse several biochemical reactions which result in the transformation of organic matter, and the release of inorganic nutrients for plant growth and nutrient cycling (Baležentienė, 2012). Thus the analysis of soil enzyme activity can provide an early and dynamic indication of soil quality changes in agriculture (Mganga et al., 2016). There are several enzymes in the soil. However, those involved in hydrolases and the degradation of main litter components are used most often for evaluating soil quality (Karaca et al., 2010). Soil enzymes such as β -glucosidase, urease and phosphatase are widely distributed in the soil and are involved in carbon, nitrogen and phosphorus cycling, respectively. A number of studies have indicated higher β -glucosidase, urease and phosphatase activities in cover crop and organic residue amended soils compared to fallow soils (Meyer et al., 2015, Mukumbareza et al., 2015, Muzangwa et al., 2019). Nonetheless, there is little or no research on the effect of cover crop species, termination stage and termination method on the activities of these soil enzymes in South African cropping systems, although cover crops are an important component, especially in fruit production systems.

Recent studies in the US have evaluated the effect of cover crop termination stage and termination method on biomass production, weed control, soil moisture and soil N mineralization (Lawson et al., 2015, Wayman et al., 2015, Keene et al., 2017). Earlier studies explored specific growth termination stages and how termination methods effectively killed cover crops and prevented regrowth (Mirsky et al., 2009). Yet, the effect of these management methods on soil organic carbon, microbial processes and enzyme activities is still poorly understood. A better knowledge of the impact of these management options on soil organic carbon build-up, nitrogen level and enzyme activities will aid proper selection and development of management approaches that will result in the restoration of healthy microbial communities, fertility, productivity and sustainability of agricultural soils.

1.2 Hypotheses

The hypotheses were:

1. Biomass production as well as the C and N content and C:N ratio will differ between cover crop species and their termination stages.
2. Living cover crop species and residues will increase soil organic C, total N and soil enzyme activities in the short-term; though the extent of the effect may vary with species.
3. Living cover crops and residues will affect soil pH in the short-term.
4. Termination of cover crops at vegetative stage will increase total soil N level than termination at flowering, and soil organic carbon and soil enzyme activities will be higher at flowering stage relative to vegetative.
5. Soil organic C, total soil N and enzyme activities will respond more to termination stage than termination methods in the short-term.

1.3 Objectives

The overall aim of the study was to determine the short-term effect of cover crop species growth and management on soil chemical and enzyme activity under greenhouse and field conditions.

The specific objectives were to evaluate the effect of:

1. Termination stage on biomass yield, total C, N and C:N ratio of different cover crop species.
2. Living cover crops and residues on soil pH, organic C, total N, β -glucosidase, phosphatase and urease activities.
3. Termination stage on soil organic C, total N, β -glucosidase, phosphatase and urease activities.
4. Termination method on soil organic C, total N, β -glucosidase, phosphatase and urease activities.

1.4 Delineation of the study

The following were not investigated in this study:

- The uptake of soil available N by subsequent cash crop
- The impact of cover crop management on the yield of succeeding crop

1.5 Significance of the study

This research improves the understanding of cover crop benefits and guides soil fertility management in South Africa. This study contributes to the following:

1. Appropriate cover crop species selection

2. Selection of soil ecosystem friendly cover crop termination methods
3. Assist in the selection of suitable cover crop termination stages
4. Understanding of soil conservation, soil biology and ultimately soil quality
5. Minimise fertilizer wastage
6. Minimise environmental pollution
7. Contribute to the available information on soil management practices in grape-based farming systems of the Western Cape Province through the utilization of cover crop technique

References

- Baležentienė, L. 2012. Hydrolases related to C and N cycles and soil fertility amendment: Responses to different management styles of agro-ecosystems. *Polish Journal of Environmental Studies*, 21, 1153-1159.
- Balota, E.L. & Chaves, J.C.D. 2010. Enzymatic activity and mineralization of carbon and nitrogen in soil cultivated with coffee and green manures. *Revista Brasileira de Ciência do Solo*, 34, 1573-1583.
- Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A. & Hergert, G.W. 2015. Cover crops and ecosystem services: Insights from studies in temperate soils. *Agronomy Journal*, 107, 2449-2474.
- Cline, G.R. & Silvernail, A.F. 2002. Effects of cover crops, nitrogen, and tillage on sweet corn. *HortTechnology*, 12, 118-125.
- Dabney, S., Delgado, J. & Reeves, D. 2001. Using winter cover crops to improve soil and water quality. *Communications in Soil Science and Plant Analysis*, 32, 1221-1250.
- Dabney, S.M., Delgado, J.A., Meisinger, J.J., Schomberg, H.H., Liebig, M.A., Kaspar, T., Mitchell, J. & Reeves, W. 2010. Using cover crops and cropping systems for nitrogen management. In: Delgado, J.A. & Follett, R. (eds.) *Advances in nitrogen management for water quality*. Ankeny, Iowa: Soil water conservation society
- Delgado, J.A. & Gantzer, C.J. 2015. The 4Rs for cover crops and other advances in cover crop management for environmental quality. *Journal of Soil and Water Conservation*, 70, 142A-145A.
- Fourie, J., Louw, P. & Agenbag, G. 2001. Effect of seeding date on the performance of grasses and broadleaf species evaluated for cover crop management in two wine grape regions of South Africa. *South African Journal of Plant and Soil*, 18, 118-127.
- Giller, K.E., Witter, E., Corbeels, M. & Tittonell, P. 2009. Conservation agriculture and smallholder farming in Africa: the heretics' view. *Field Crops Research*, 114, 23-34.
- Hussain, S., Siddique, T., Saleem, M., Arshad, M. & Khalid, A. 2009. Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. *Advances in Agronomy*, 102, 159-200.
- Karaca, A., Cetin, S.C., Turgay, O.C. & Kizilkaya, R. 2010. Soil enzymes as indication of soil quality. *Soil enzymology*. Berlin, Heidelberg: Springer.
- Keene, C., Curran, W., Wallace, J., Ryan, M., Mirsky, S., VanGessel, M. & Barbercheck, M. 2017. Cover crop termination timing is critical in organic rotational no-till systems. *Agronomy Journal*, 109, 272-282.
- Ketterings, Q.M., Swink, S.N., Duiker, S.W., Czymmek, K.J., Beegle, D.B. & Cox, W.J. 2015. Integrating cover crops for nitrogen management in corn systems on northeastern US dairies. *Agronomy Journal*, 107, 1365-1376.

- Lawson, A., Cogger, C., Bary, A. & Fortuna, A.-M. 2015. Influence of seeding ratio, planting date, and termination date on rye-hairy vetch cover crop mixture performance under organic management. *PLoS one*, 10, e0129597.
- Mandiringana, O., Mnkeni, P., Mkile, Z., Van Averbeke, W., Van Ranst, E. & Verplancke, H. 2005. Mineralogy and fertility status of selected soils of the Eastern Cape Province, South Africa. *Communications in Soil Science and Plant Analysis*, 36, 2431-2446.
- Meyer, A.H., Wooldridge, J. & Dames, J.F. 2015. Variation in urease and β -glucosidase activities with soil depth and root density in a 'Cripp's Pink'/M7 apple orchard under conventional and organic management. *South African Journal of Plant and Soil*, 32, 227-234.
- Mganga, K.Z., Razavi, B.S. & Kuzyakov, Y. 2016. Land use affects soil biochemical properties in Mt. Kilimanjaro region. *Catena*, 141, 22-29.
- Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R. & Shumway, D.L. 2009. Control of cereal rye with a roller/crimper as influenced by cover crop phenology. *Agronomy Journal*, 101, 1589-1596.
- Mukumbareza, C., Muchaonyerwa, P. & Chiduzza, C. 2015. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. *South African Journal of Plant and Soil*, 32, 189-197.
- Muzangwa, L., Chiduzza, C. & Muchaonyerwa, P. 2013. Feasibility of winter cover crop production under rainfed conditions in the eastern Cape Province of South Africa. *African Crop Science Journal*, 21, 173-184.
- Muzangwa, L., Mnkeni, P.N.S. & Chiduzza, C. 2019. The use of residue retention and inclusion of legumes to improve soil biological activity in maize-based no-till systems of the Eastern Cape Province, South Africa. *Agricultural Research*, 1-11.
- O'Reilly, K.A., Lauzon, J.D., Vyn, R.J. & Van Eerd, L.L. 2012. Nitrogen cycling, profit margins and sweet corn yield under fall cover crop systems. *Canadian Journal of Soil Science*, 92, 353-365.
- O'Reilly, K.A., Robinson, D.E., Vyn, R.J. & Van Eerd, L.L. 2011. Weed populations, sweet corn yield, and economics following fall cover crops. *Weed Technology*, 25, 374-384.
- Poeplau, C. & Don, A. 2015. Carbon sequestration in agricultural soils via cultivation of cover crops—A meta-analysis. *Agriculture, Ecosystems & Environment*, 200, 33-41.
- Shukla, M., Lal, R. & Ebinger, M. 2006. Determining soil quality indicators by factor analysis. *Soil and Tillage Research*, 87, 194-204.
- Thilakarathna, M.S., Serran, S., Lauzon, J., Janovicek, K. & Deen, B. 2015. Management of manure nitrogen using cover crops. *Agronomy Journal*, 107, 1595-10607.
- Wayman, S., Cogger, C., Benedict, C., Burke, I., Collins, D. & Bary, A. 2015. The influence of cover crop variety, termination timing and termination method on mulch, weed cover and soil nitrate in reduced-tillage organic systems. *Renewable Agriculture and Food Systems*, 30, 450-460.
- Zhang, L., Chen, W., Burger, M., Yang, L., Gong, P. & Wu, Z. 2015. Changes in soil carbon and enzyme activity as a result of different long-term fertilization regimes in a greenhouse field. *PLoS one*, 10, e0118371.

CHAPTER 2 LITERATURE REVIEW

2.1 Management impact and benefit of cover crop on soil quality

2.1.1 Introduction

Cover crops are plants grown to cover and improve the soil (Benedict et al., 2014). They may be used as a living or dead mulch on the soil surface, or they can be ploughed into the soil as green manure (Benedict et al., 2014, Finney et al., 2017). The Soil Science Society of America (SSSA), defines cover crops as “close-growing crops that provide soil protection, and soil improvement between periods of normal crop production, or between trees in orchards and vines in vineyards” (Fageria et al., 2005). Cover crops can be any type of plant but are generally legumes or non-legumes/grasses. Table 2.1 is a list of the most commonly used cover crop species.

Table 2.1: Common cover crop species used in cropping system

Type	Common name	Scientific name
Legume	Vetch	<i>Vicia villosa</i>
	Pea	<i>Pisum sativum</i>
	Cowpea	<i>Vigna unguiculata</i>
	Faba bean	<i>Vicia faba</i>
	Soybean	<i>Glycine max</i>
	Crimson clover	<i>Trifolium incarnatum</i>
	Sunn hemp	<i>Crotalaria juncea</i>
	Alfalfa	<i>Medicago sativa</i>
Non-legume	Rye	<i>Secale cereal</i>
	Oats	<i>Avena sativa</i>
	Wheat	<i>Triticum aestivum</i>
	Barley	<i>Hordeum vulgare</i>
	Rapeseed	<i>Brassica napus</i>
	Ryegrass	<i>Lolium perenne</i>
	Oilseed radish	<i>Raphanus sativus</i>
	Triticale	<i>Triticosecale</i>
	Buckwheat	<i>Fagopyrum esculentum</i>
	Serradella	<i>Ornithopus sativus</i>

Several studies have reported the effect of cover crops in agricultural systems (Fourie et al., 2001, Dube et al., 2014, Mukumbareza et al., 2015b, Coombs et al., 2017). The benefits can be on soil physical properties, improved soil chemical properties, biological benefits, and other benefits such as reduced production costs. The beneficial impacts include enhancing soil organic matter, carbon (C) sequestration, improving microbial biodiversity, soil aggregate stability, water retention capacity, weed suppression and pest management (Dabney et al., 2001, O'Reilly et al., 2011). In

addition, cover crops reduce particulate emissions, reduce soil erosion from wind and water and improve nutrient cycling (Blanco-Canqui et al., 2015). Cover crops also increase N availability from biological nitrogen fixation and retain excess soil N for the following growing season (O'Reilly et al., 2012). When used in combination with conservation tillage, cover crops contribute to a system that improves soil quality and crop production (Schomberg et al., 2009).

Improperly managed cover crops may have detrimental effects on the cash crop either by competing for water and nutrients, building up diseases or retarding seed germination (Thorup-Kristensen et al., 2003, Stipešević and Kladičko, 2005). The direct and indirect costs of cultivation and management, as well as possible economic losses, may also constitute possible restraints to the utilization of cover crop as soil improvement and conservation practice (Snapp et al., 2005). However, several studies have shown that cover crop benefits surpass the disadvantages (Miguez and Bollero, 2005, Sarrantonio and Gallandt, 2008).

Proper management is required to realize many of the benefits associated with cover crops (Ashford and Reeves, 2003). Successful cover cropping requires advanced planning and selecting appropriate varieties, seeding rates, planting period, termination stage and termination methods (Wortman et al., 2013, Alonso-Ayuso et al., 2014). An increased understanding of cover crop species selection, termination time and termination method is crucial to aid better management decisions that most improve soil quality and crop yield. This chapter is a review of the importance of cover crops for soil quality improvement, including species selection, termination stage and termination methods.

2.1.2 Cover crop use and benefits for soil quality improvement

2.1.2.1 Benefits on soil physical properties

Soil organic matter is an important soil quality indicator and its increase leads to improved aggregate stability and water retention capacity and nutrient cycling (Lehman et al., 2015). Cover crops may offer a cheap on-site management choice to improve soil organic matter and soil quality (Dabney et al., 2010). Though chemical fertilizers are easy to store, apply, and manage, they do not contribute to soil organic matter which is often derived from adding plant residues over a period of time (Newman et al., 2007). Indeed, the presence or addition of organic matter enhances water percolation in heavy clay soils and increases water retention capacity in sandy soils (Newman et al., 2007). Regardless of management method, cover crop residues from rye, oats, grazing vetch, faba bean and clover increased organic matter content of vineyard topsoil in Western Cape, South Africa (Fourie et al., 2007).

Cover crops reduce water erosion involving sediment and dissolved nutrient loss but the impact varies with cover crop species and biomass production (Blanco-Canqui et al., 2011). Soils amended with rye and oats showed a decrease in rill erosion after 3 years (Kaspar et al., 2001). The effect of water erosion was lessened under winter triticale, lentil and pea residues compared with control fields (Blanco-Canqui et al., 2011). A study in New York indicated a marked reduction in P loss to runoff under cover crop plots relative to plots without cover crops (Kleinman et al., 2005). Thus, cover crop enhances water quality, soil fertility, crop productivity and decrease water pollution by reducing water erosion (Kaspar et al., 2001, Blanco-Canqui et al., 2015). Blanco-Canqui et al. (2011) concluded that cover crops decrease nutrient and sediments losses through runoff by “providing protective cover to the soil, absorbing raindrop energy, reducing soil aggregate detachment, increasing soil surface roughness, delaying runoff initiation, intercepting runoff, reducing runoff velocity, increasing the opportunity time for water infiltration, and promoting the formation of water-stable aggregates”. A number of studies have reported that cover crops reduced wind erosion as a result of increased soil cover and soil organic carbon as well as improved soil structural properties (Bilbro and Hargrove, 1991, Colazo and Buschiazzo, 2010).

Soils managed with cover crops have been shown to improve soil aggregation under conventional tillage (Liu et al., 2005) in less than 3 years (Blanco-Canqui et al., 2013b) compared to no cover crop plots. An increase in soil aggregate stability through cover cropping can improve water quality, nutrient availability, carbon build-up, soil macroporosity and root development, nonetheless reduce soil erodibility (Blanco-Canqui et al., 2013b). There is a positive correlation between soil organic carbon level and soil aggregate stability (Blanco-Canqui et al., 2013b). Silt loam and loam soils managed for 17 years with crimson clover, winter rye and hairy vetch resulted in an increase in soil absorbency, water retention capacity and saturated hydraulic conductivity (Keisling et al., 1994). In agricultural soils, cover crops enhance hydraulic properties such as water retention capacity, water infiltration and saturated hydraulic conductivity by protecting the soil surface from raindrop impact, increasing macroporosity and pore connectivity, and increasing organic matter and microbial activity (Blanco-Canqui et al., 2015).

Cover crops reduce soil compaction due to their ability to enhance soil aggregation and increase soil organic carbon level. Soil compaction was reduced by 5% in a silt loam treated with sunn hemp and soybean over a long-term (Blanco-Canqui et al., 2012). In addition, cultivation of brassicas (radish) reduced soil compaction by probing dense layers with their taproots and functioning like tillage tools (Chen and Weil, 2010). Soil management studies involving 13 years (Steele et al., 2012) and 15 years (Blanco-Canqui et al., 2011) cover cropping have also been reported to reduce soil bulk density.

Cover crop canopy cover and residue aid soil temperature control in agricultural cropping systems (Dabney et al., 2001). Cover crops can decrease soil temperature during the summer and increase soil temperature in winter (Dabney et al., 2001). The reduced soil temperature during summer may decrease evaporation and increase soil water storage while it can be detrimental in cool regions where crop growth is temperature limited (Drury et al., 1999, Dabney et al., 2001).

2.1.2.2 Improvement on soil chemical properties

Most studies have found that cover crops such as hairy vetch, crimson clover, pea, turnip Brassica, oats and rye significantly increased soil organic C concentrations compared to plots without cover crops (Dabney et al., 2001, Sainju et al., 2002, Olson et al., 2014). Cover crops add to above and belowground biomass in the soil which results in organic carbon increase (Poeplau and Don, 2015). Additionally, cover crop practice may reduce soil organic carbon loss by reducing soil erosion which may serve as a pathway of such loss (Blanco-Canqui et al., 2015). Thus, soil organic carbon is one of the most important properties used to examine the effect of cover crops on soil fertility. Blanco-Canqui et al. (2015) indicated that the magnitude of soil carbon build-up is site-specific and varies with cover crop biomass quantity, duration of life materials prior to termination, initial amount of soil C, soil type, cover crop type, tillage management, and climate. For instance, soil clay content significantly influences how a soil accumulates and preserves soil organic carbon input from cover crops (Hassink and Whitmore, 1997). Owing to the high background of soil carbon level, the effect of cover crop on soil organic carbon is not usually detected early rather than in the long-term of establishment (Acuña and Villamil, 2014). Total soil organic carbon and water-soluble C was increased when maize was rotated with oats and grazing vetch within a period of 5 years (Dube et al., 2012). Cover crops managed under different tillage systems indicated highest soil organic carbon level in no-till plots followed by chisel plough and mouldboard plough (Olson et al., 2014); showing that cover crop benefits are more quickly identified under no-till management due to reduced residue decomposition rates in contrast to conventional tillage (Blanco-Canqui et al., 2015). Non-legumes improve soil organic matter by supplying high levels of organic carbon (Lithourgidis et al., 2006). The practice of cultivating cover crop species mixtures has also been reported to produce more above and belowground biomass, consequently increasing soil organic carbon compared to monocultures (Stavi et al., 2012). Nevertheless, single species practice showed higher biomass compared to cover crop species mixtures in a study by Nielsen et al. (2015). Cover crops not only increase nutrient availability as well as storage of C and N in the soil, but also increase sequestration of atmospheric CO₂ and N, thus reducing the effect of global warming (Dabney et al., 2010). Therefore, cover crop management can be a reliable approach to enhance agricultural production since the increase in the amount and quality of organic matter as well as carbon will improve soil quality and crop productivity.

Grasses and other non-legumes do not fix N, but they can quickly assimilate soil N as they grow (Thorup-Kristensen, 2006). Grass cover crops can assimilate and recycle residual soil NO_3^- left behind at the end of the growing season, which may leach below the rooting zone when it rains (Cline and Silvernail, 2002). Rye, canola, and forage radish exhibited better potential to mine residual soil NO_3^- than legume cover crops (Wagger et al., 1998, Nielsen et al., 2015). A metal analysis in an irrigated cropping system showed that non-legume cover crops reduced NO_3^- leaching by 50% relative to fallow soil (Quemada et al., 2013). The nutrient scavenging ability of non-legumes is a vital approach to managing excess nutrient supplements to the soil from inorganic fertilizers or animal dung; lessening the chances of water pollution (Blanco-Canqui et al., 2015). However, it has also been shown that grasses with high C:N ratio slowly decompose and immobilize soil N, thus increasing the quantity of N fertilizer needed for maximum economic yield of subsequent crops like cotton, corn and sorghum (Dabney et al., 2001, Dabney et al., 2010).

Cover crop cultivation can build up N and enhance N cycling to reduce potential field and economic losses (O'Reilly et al., 2012). Assessments of N input have been based only on the breakdown and N contribution of legume shoot residues while N inputs of roots have mostly been overlooked (Jani et al., 2016). Roots of leguminous crops account for about 30% of total cover crop biomass although the decomposition and N release of the roots is slow compared to the shoots (Sainju et al., 2005). Jani et al. (2016) reported a rapid legume root decomposition and N release of 47 – 62 and 19 – 33 kg ha^{-1} at Goldsboro and Kinston cultivated fields in South-eastern USA, respectively. Legumes have lower C:N ratios (less than 20) (Dabney et al., 2001) and are therefore considered high quality cover crops that rapidly decompose and mineralize N, compared to grass cover crops, which have greater C:N ratios (Kaleem Abbasi et al., 2015). Yet, this process rapidly takes place and limits the persistence of these residues on the soil surface, which reduces benefits linked to surface residues (Reeves, 1994). Cover cropping systems involving a mixture of legumes and non-legumes are crucial in obtaining combined benefits such as acting as C and N sources (Sainju et al., 2000), optimizing the C:N balance, weed suppression, increasing biomass input, improving organic matter production and enhancing diversity (Treadwell et al., 2010, Nielsen et al., 2015). A mixture of legume and grass may also be beneficial when the crops complement each other with regard to growth and nutrient capture (Tiemann et al., 2015). In legume-grass blends, grasses develop faster than legumes consequently shielding the soil from erosion while legumes sequester N that can be used by the companion grass or the following cash crop (Blanco-Canqui et al., 2015). As a result of this, plant residues of the mix decompose faster than sole grass thereby reducing nitrogen immobilization (Newman et al., 2007).

In previous studies, nearly all the N required for optimum crop growth and a considerable amount of soil P and sulphur are found in soil organic matter (Newman et al., 2007, Dube et al., 2014). A study by Dube et al. (2014) showed that the addition of winter oats and grazing vetch residues considerably increased P concentration in topsoil and young maize plants in Eastern Cape, South Africa. Also, oats, rye and ryegrass increased inorganic P under no-tillage field conditions (Varela et al., 2017). This is an indication that in the long-term, P fertilizer application could be reduced in a conservation agriculture system. Soil organic matter increases the soil cation exchange capacity, consequently improving the ability of soil to store other macronutrients, including calcium, potassium and magnesium (Newman et al., 2007). Rye and white mustard residues increased soil P and magnesium level while oilseed rape mulch increased soil K level (Harasim et al., 2016).

Micronutrients form complexes with soil organic matter, which increases their accessibility for plant uptake (Newman et al., 2007). It was suggested that the influence of winter cover crops on soil micronutrient pools namely zinc and copper be examined (Dube et al., 2014). Cover crops add organic matter to the soil thereby supplying additional carbon which serves as energy to sustain the activities of soil microorganisms and enzymes (Du et al., 2014). This, in turn, contributes to efficient nutrient cycling and soil structure improvement (Snapp et al., 2005).

2.1.2.3 Biological benefits

Legume cover crops fix atmospheric N through a symbiotic relationship with soil bacteria (*Rhizobia* spp.), which infects crop roots (Vaughan et al., 2000). Soils treated with hairy vetch and crimson clover showed a significant increase in N concentration compared to fallow soils (Sainju et al., 2002). Hairy vetch provided an equivalent of 50 – 155 kg ha⁻¹ (Seo et al., 2000) and 150 - 250 kg ha⁻¹ (Crandall et al., 2005) of fertilizer N, respectively to the following corn crops. In wheat rotation, soil N levels also increased by 258 kg ha⁻¹ and 279 kg ha⁻¹ under soybean and sunn hemp respectively, compared with no cover crop treatments (Blanco-Canqui et al., 2011). Therefore, legume cover crops supplied significant quantities of plant-held nitrogen to subsequent crops (Thilakarathna et al., 2015), thereby, increasing soil fertility and reducing N fertilizer requirements (Fortuna et al., 2008). Some studies, however, reported that winter legume cover crops did not increase soil N, especially in less than 5 years (Sainju et al., 2003, Villamil et al., 2006).

Cover crop mixtures of varying functional groups can stimulate a broader diversity of organisms (Kramberger et al., 2014) leading to increased organic matter, nutrient release and soil quality. Thus, it is important to improve cover crop mixtures and management for better sequestering, scavenging, cycling and supplying of obtainable nutrient requirements to the cash crop.

Living cover crops and their residues provide shade to the soil and suppress weeds through direct resource competition, niche disruption, and phytotoxic effects (Mirsky et al., 2011). Living cover crops have the ability to suppress weeds than cover crop residues in vineyards and orchards since living cover crop compete with weeds for light, water and nutrients (Kandel et al., 1997, Blanco-Canqui et al., 2015). Straw mulch, vetch, triticale, and rye and faba bean mixture significantly reduced weed population in a grapevine soil of Western Cape, South Africa (Fourie, 2010). Rye residue suppressed weed growth for up to 6 weeks after rye desiccation (Williams et al., 2000). Various small-seeded weeds under different summer cash crops grown across the southern US have been effectively controlled by cover crop residues (Reeves et al., 2005, Price et al., 2007). Thus, cover crops reduce herbicide use and costs as well as labour charges of weed control. Cover crop species mixtures improve allelopathic effects to control weeds and attract beneficial insects or prevent insect pests infestation (Treadwell et al., 2010). Crop rotation involving cover crops enhance diversity which leads to interruption of pest and disease cycle in agricultural cropping system.

Since cover crops add organic matter to agricultural soils, they provide energy and nutrients for microbial growth and activity thereby acting as the driving force for nutrient mineralization/immobilization processes (Murungu et al., 2011, Kaleeem Abbasi et al., 2015). Microorganisms release enzymes which catalyse and increase several biochemical reactions (Kujur and Kumar Patel, 2014) that bring about decomposition of crop residues, nutrient cycling, and release of inorganic nutrients for plant growth (Baležentienė, 2012). Thus, microbial and enzymatic activity is the main link between soil organic matter and nutrient release to plants. Cultivation of cover crops such as crimson clover, pea, wheat and vetch increased microbial biomass C, *arbuscular mycorrhizal* fungi population, bacteria population, and soil enzyme activities compared to control, under different environment and management practices (Kabir and Koide, 2000, Blanco-Canqui et al., 2015, Mukumbareza et al., 2015b). In a 2-year study, soil microbial biomass increased in topsoil treated with sole rye and a mixture of rye and oats rotated with corn silage in Ohio, USA (Faé et al., 2009). Addition of above and belowground cover crop biomass increases soil organic carbon, microbial and enzymatic activities, consequently enhancing soil aggregation, aeration, water infiltration, porosity, and other soil physical properties (Blanco-Canqui et al., 2011). Finney et al. (2017), however, stated that the influence of cover crops on soil microbial community, structure and activity is species specific. Literature has shown that the C:N ratio of cover crop residues incorporated into the soil plays a primary role in controlling microbial activity and the dynamics of mineralization/ immobilization (Brennan et al., 2013, Kaleeem Abbasi et al., 2015). Therefore, there is need to develop soil management strategies that

can manipulate C:N ratios and improve enzyme activity, thereby reducing N immobilization and increasing soil fertility for sustainable agricultural productivity (Haney et al., 2012).

2.1.2.4 Other benefits

Cover crops do not only improve soil fertility, crop productivity and environmental quality, but they can also be grazed (Franzluebbers and Stuedemann, 2014) or prepared as hay (Holman et al., 2012) for livestock or biofuel production to generate some economic return (Baker and Griffis, 2009, Feyereisen et al., 2013). Cover crop management systems involving grass-legume mixtures may reduce the possibility of failure and costs of labour and replanting (Blanco-Canqui et al., 2015). Table 2.2 shows some of the cover crop benefits in agricultural cropping system.

Table 2.2: Some cover crop benefits in agricultural cropping systems

Physical Benefits	Chemical Benefits	Biological Benefits	Other benefits
Increase residue cover	Increase soil organic carbon concentration and pool	Promote N fixation	Reduced chemical costs
Increase water infiltration and retention capacity	Increase N concentration	Increase populations of beneficial insects	Improve landscape aesthetics
Reduce soil and wind erosion	Enhance nutrient cycling	Improve microbial activity and biomass	Potential forage harvest
Improve field trafficability	Reduce nonpoint source pollution	Decrease soil-borne pest and pathogenic microorganisms	Increase or sustain crop yields
Reduce sudden fluctuations in soil temperature		Reduce some diseases	Reduce labour costs
Increase soil aggregate stability		Suppress weeds	
Reduce risk of soil compaction			
Improve soil macroporosity			

Adapted from Blanco-Canqui et al. (2015) and Dabney et al. (2001)

2.1.2.5 Disadvantages of Cover Crops

Cover crops may have a negative impact on cash crops by competing for water and nutrients, building up diseases or delaying seed germination if not appropriately managed (Thorup-Kristensen et al., 2003, Stipešević and Kladvko, 2005). For instance, cover crops require water to grow, hence they compete and reduce soil water content at planting; leading to a potential decrease in yield of cash crops in rain-fed semiarid regions (Reeves, 1994, Dabney et al., 2001). Management of species mixture may be challenging since the system warrants increased costs

of seeds, and perhaps the need for different planting equipment, sowing time, and termination requirements, which can influence the economic returns from cover crops (Blanco-Canqui et al., 2015). Thus, additional costs of cultivation and management, as well as possible economic losses, may also be restraints to cover crop practice (Snapp et al., 2005).

Nevertheless, several studies have shown that the advantages of cover cropping exceed the disadvantages (Dabney et al., 2001, Miguez and Bollero, 2005, Sarrantonio and Gallandt, 2008).

2.1.3 Selection of cover crop species

Cover crop species should be selected based on their adaptation to local climates and the intended purpose of managing the soil (Schomberg et al., 2009). Cover crops comprise of several ideal characteristics which inform the choice of a grower. These include the ability to; easily establish, develop quickly to cover the soil, supply enough biomass in a short-term for residue maintenance, resist disease and not act as a host for cash crop diseases, be easy to terminate and be economically viable (Reeves, 1994). Fourie et al. (2001) selected a number of species suitable for sustainable cover crop management in the different grapevine regions of South Africa.

It has been shown that soil organic matter can greatly be increased by high biomass yielding winter cover crops (Dube et al., 2012). The amount of biomass a cover crop produces varies with species (Wayman et al., 2015). Under adequate fertility levels, grasses usually yield more biomass than legumes and the amount of biomass may also vary within the same species (Sainju et al., 2000, Newman et al., 2007). Oats and grazing vetch have been shown to be the most promising cover crop species, based on biomass production in conservation agriculture systems of the Eastern Cape (Murungu, 2010). Therefore, cover crop types should be chosen on the basis of the ability to produce high quantity of biomass and/or root biomass to increase soil organic matter and enhance soil quality in agricultural systems (Balkcom et al., 2015).

Rapid growing and extensive rooted cover crops have the ability to scavenge scarce soil nutrient and increase soil organic matter at greater depths (Dabney et al., 2001). Deep-rooted cover crops likewise aggregate soil particles to form a porous network that allows for improved water drainage (Monahan et al., 2012). Grasses may have more effective early season weed control than legumes since they sprout earlier and develop root systems quicker (Ranells and Waggar, 1996). Cover crops regulate and reduce extreme summer temperatures which decrease soil evaporation and conserve water, resulting in enhanced soil aggregate, enzyme activity, residue breakdown and water storage (Blanco-Canqui et al., 2013a). However, the amount of cover crop canopy, biomass and residue input controls the extent to which cover crops influence soil temperature (Dabney et

al., 2001, Blanco-Canqui et al., 2013a). Cover crop can reduce wind erosion, water erosion and runoff, but the extent of this benefit depends on cover crop species due to differences in biomass cover, height of standing residues and slower decay of residues (Wortman et al., 2013, Balkcom et al., 2015) Therefore, the selection of appropriate cover crop varieties according to management goals will improve soil fertility in different agroecosystems.

The chemical composition of cover crop residues, such as C:N ratio and lignin are important features influencing breakdown process and nitrogen release in the soil (Sullivan and Andrews, 2012, Alonso-Ayuso et al., 2014). Cover crop chemical composition, however, varies with species and growth stage (Reeves, 1994). Grass residues usually have high C:N ratio, are more effectual at increasing soil carbon levels and persist longer on the soil surface than legume cover crops owing to slower putrefaction of grass cover crop residues (Reeves, 1994, Blanco-Canqui et al., 2013a). Legumes, on the other hand, have higher N contents and therefore lower C:N ratios that permit rapid decomposition and N release for the following crop (Murungu et al., 2011). Thus, it is crucial to consider C:N ratio of cover crop residue to preserve soil cover when preferred, but allow optimum decomposition, nutrient recycling and release rates.

2.1.4 Termination stage

The appropriate growth stage of cover crop termination is a crucial management approach that should be adopted and varied based on location and specific purpose of cultivation (Balkcom et al., 2015). Cover crop termination timing is vital because it can be influenced by the growing season, soil temperature, soil moisture, N management, weed suppression, tillage and planting operation (Balkcom et al., 2015). Cover crop growth stage can be deduced using the Mischler (Mischler et al., 2010) and Zadoks (Zadoks et al., 1974) scale. Determining the optimum cover crop termination stage can be challenging since it often involves trade-offs amongst the different benefits provided by cover crops.

Cover crop termination can be delayed as late as possible in order to attain sufficient biomass and organic matter production (Ruis et al., 2017). Cover crop biomass increases up till flowering stage, nonetheless, quality decrease as cover crops grow from vegetative to flowering stages and beyond (Benedict et al., 2014). Hairy vetch-triticale and rye biomass have been indicated to peak at early flowering and late dough stages, respectively (Keene et al., 2017). Late termination of barley led to considerably larger biomass build-up in a soybean plot (Rosario-Lebron et al., 2018), and the average biomass of black lentil doubled from early bud to full-bloom stages (Brandt, 1996). Therefore, delayed termination of legume cover crops to the pod setting stage of growth can be

an effective management approach. Cover crops produced higher leguminous phytomass at this growth stage (Hirpa, 2013).

It has been shown that the quantity, maturity and type of cover crops incorporated into the soil determine the build-up of soil organic matter and soil organic carbon (Kuo and Jellum, 2002, Sainju et al., 2005). Soil organic carbon increased by 17.84% when termination of legume crops was delayed from mid-vegetative to pod-setting growth stage (Hirpa, 2013). Furthermore, cover crops terminated at mid-vegetative stage did not add much to the soil organic matter formation and grain yield in contrast to late termination (Njunie et al., 2004, Hirpa, 2013). This is due to the limited biomass and quick microbial degradation or loss of residue materials by decomposition (Hirpa, 2013). Thus, growth stage influences the amount and quality of cover crop biomass which are vital features that control soil organic carbon build-up and how much N may become available or unavailable for the cash crop (Alonso-Ayuso et al., 2014, Benedict et al., 2014). Nevertheless, there is a need for more understanding of the influence of cover crop growth stage termination on organic carbon availability, under different soils and ecosystems. This will better inform cover crop management methods that increase soil microbial and enzymatic activity for optimum nutrient cycling.

In a crop rotation system, early termination has been shown to reduce the risk of preventive competition by lessening the N assimilation of cover crop and permitting more time for N release from cover crop residues (Alonso-Ayuso et al., 2014). A number of studies have shown that delaying hairy vetch termination for 8 days (Cline and Silvernail, 2001), 2 weeks (Clark et al., 1995) or beyond early or mid-flowering (Drinkwater et al., 2000) increased vetch biomass but did not increase the amount of N input from N fixation. A delay in cover crop termination led to an increase in rye biomass (Mirsky et al., 2011, Nord et al., 2012), decrease in weed biomass (Mirsky et al., 2011) and reduction in net N mineralization and availability to the subsequent crop (Thorup-Kristensen and Dresbøll, 2010). Furthermore, the nitrogen content of winter rye terminated at flowering stage was 50% less in contrast to cover crops terminated at vegetative stage (Dabney et al., 2010). Depletion of soil available N by cover crops occurs due to delays in termination time and consequently slow mineralization of a high C:N residue (Thorup-Kristensen et al., 2003). However, postponing termination time has also been reported to mitigate nitrate losses and increase N use efficiency by capturing inorganic N that is prone to leaching to deeper soil profile to be released on the topsoil through residue decomposition (Alonso-Ayuso et al., 2014).

Cover crop species, growth stage and management have a great influence on the release of nitrogen in agricultural systems (Schomberg et al., 2009, Sullivan and Andrews, 2012). The optimum termination time to maximize PAN depends on the cover crop type (Balkcom et al., 2015).

Plant available nitrogen from a good stand of legumes peaks at budding growth stage and declines slowly as reproductive growth continues (Sullivan and Andrews, 2012). PAN from grass residues is positive from early up to tillering stage and as stem elongation continues (jointing), PAN declines and it nears zero by the time it reaches Feekes growth stage 8 or Zadoks 37 (Sullivan and Andrews, 2012). Oats terminated at boot stage increased N fertilizer requirement while vetch terminated at flowering growth stage reduced N fertilizer need (Benedict et al., 2014). Sole grass cover crops should be terminated earlier or seeded with legumes in order to control N immobilization (Benedict et al., 2014). However, when seeded with less than 25% legume, termination should be done before grass reaches boot stage (Sullivan and Andrews, 2012). To maximize PAN, it is crucial to terminate legume cover crops before bud stage and grasses at boot stage (Sullivan and Andrews, 2012). Nonetheless, Benedict et al. (2014) indicated that the best time to incorporate cover crop for optimum nitrogen contribution is during the late vegetative and early flowering stages. Delaying the cover crop termination date especially for grasses may reduce residue N concentration, increase C:N ratios, and result in higher concentrations of hemicellulose and lignin in the residue (Alonso-Ayuso et al., 2014), which has an important effect on N release. More studies on termination timing are needed in order to manage grass C:N ratios for optimum N mineralization and reduce immobilization in agricultural soils.

Cover crop termination time is a vital management variable for maximizing the cover crop value in agricultural practices (Alonso-Ayuso et al., 2014). Several studies have shown that altering termination time by few weeks may have a great effect on water consumption, soil moisture conservation and soil nitrate availability (Clark et al., 2007, Krueger et al., 2011, Tosti et al., 2012). The effect of termination date on soil water availability is a balance between the water extracted by the living cover crop and the soil water evaporation prevented by the residue mulch (Clark et al., 2007). For example, some studies indicated that though there was depletion in soil water, moisture conservation was enhanced when cover crop termination was delayed (Clark et al., 2007). However, an earlier termination of cover crop has been shown to reduce water pre-emptive competition, conserve soil moisture content, and increase water availability for the following cash crop (Stipešević and Kladvko, 2005, Krueger et al., 2011). Water use by cover crops can adversely affect yields of subsequent dryland crops in semiarid areas (Dabney et al., 2001). Optimization of cover crop termination date can increase the quantity and quality of above ground biomass and enhance the development of soil surface mulch thereby improving water conservation and weed control (Saini et al., 2008, Alonso-Ayuso et al., 2014). This can be achieved by evaluating the termination date effect in combination with more cover crop species since termination time should be considered independently.

In sub-Saharan Africa, several studies evaluating the effect of cover crops on soil fertility and the following cash crops have been focusing on termination at a particular growth stage. There is a need to further investigate residue quantity and quality through proper selection of cover crop species and termination timing to find the optimum growth stage and quantity of cover species in every ecosystem. More understanding of the effect of cover crop termination stage on soil macro and micronutrients, as well as organic C build-up, will likely result in improvements in environmental parameters such as soil fertility, reduction in greenhouse effects and an increase in agricultural sustainability. Furthermore, a better knowledge of the impacts on microorganisms and enzyme activity is crucial for improving soil management and soil quality.

A number of studies evaluating the effect of cover crop termination have been expressing their kill/termination dates as days after sowing. Alonso-Ayuso et al. (2014) however, suggested that termination date or time should be based on degree-days accumulated and growth stage in order to ease comparison between different climatic regions where cover cropping is practiced.

2.1.5 Termination method

The choice of method for cover crop termination depends on the purpose of cultivation and the equipment available. Cover crops can be terminated by frost, mowing, disking, roller crimping, and herbicide in preparation for the next crop (Sullivan and Andrews, 2012, Wayman et al., 2015).

2.1.5.1 Mowing

Cover crops can be terminated by mowing with a rotary or flail mower that disengages aboveground cover crop stands from roots, mixes, chops and shreds residue on the soil surface (Kornecki et al., 2009). This process is effective as long as mowing is not done at a very early stage in which cover crops re-grow (Creamer and Dabney, 2002). Mowing of rye at vegetative growth stage led to re-growth, and ultimately caused nutrient and water competition with the cash crop planted in spring (Wilkins and Bellinder, 1996, Kornecki et al., 2009). Cover crop termination using rotary mowers can be challenging because the size and dispersal of plant remains are usually irregular. A flail mower serves as a more desirable option to a rotary mower since the remains are uniformly spread and are of a more even length (Creamer and Dabney, 2002). Cover crop termination with a flail mower may regulate the soil temperature and moisture at more suitable levels for microbial activity and residue decomposition relative to cover crops incorporated into the soil (Havstad et al., 2010). However, another study stated that mulch uniformity is difficult to achieve with flail-mowing which leads to weed emergence through thinly covered gaps in the mulch layer (Teasdale et al., 2007). Mechanical mowing of rye and hairy vetch mixture resulted in small pieces of residues that decomposed faster, negating some of the benefits of keeping the

soil surface covered (Teasdale and Mohler, 1993, Morse, 2001). On the other hand, N mineralization was improved when a mixture of hairy vetch, rye and oriental mustard was managed by mowing (Snapp et al., 2005).

2.1.5.2 Roller crimping

Rolling with a roller-crimper is a cover crop termination where biomass is flattened to produce a thick uniform mat of mulch (Mirsky et al., 2009). In contrast to mowing, application of roller crimping in cover crop termination leads to residue decomposition over time, consequently providing better soil cover, weed suppression, as well as soil moisture conservation (Mischler et al., 2010, Mirsky et al., 2011). Effective termination of cover crop with a roller-crimper is crucial to prevent cover crops from competing with cash crop (Ashford and Reeves, 2003, Mischler et al., 2010). Quite a few types of roller crimper have been built and adapted from existing designs to fit their specific situation. Rollers can be front- or rear-mounted on tractors and works by rolling over and crimping cover crop stalks when the crop is at flowering stage, leaving plants levelled on the soil surface as covering mulch (Parr et al., 2011). However, total termination of cover crop by roller crimping can be demanding (Mischler et al., 2010), and timing of the operation is crucial if a grower is depending on the roller alone to terminate the crop.

Grass rye can be successfully terminated with a roller-crimper when rolled at anthesis (Mirsky et al., 2009). Also, the roller-crimper has been effectively used to terminate hairy vetch at full bloom (Creamer and Dabney, 2002) and early pod set (Mischler et al., 2010). The use of the roller-crimper can lessen or eliminate herbicide usage in a management system with thick soil surface layer of mulch derived from high cover crop biomass (Mirsky et al., 2009, Mirsky et al., 2011), offering the possibility of no-till organic farming systems (Jani et al., 2016). Soil and cover crop root systems remain intact after roller crimping compared to disking where soil disturbance usually results in repositioning of cover crop biomass and improved decomposition through elevated soil respiration (Yaduvanshi and Sharma, 2008, Soriano et al., 2014). Cover crop residues terminated by roller crimping resulted in increased cash crop production compared to flail mowing (Morse, 2001). It has been shown that legume cover crops controlled by roller crimping produced 10 - 217 kg N ha⁻¹ depending on species and kill date (Parr et al., 2011), and that soil inorganic N reached its peak 4 - 6 weeks after roller crimping (Parr et al., 2014). Jani et al. (2016) however, indicated that the rapid root decay and N release recorded under vetch, clover and pea plots were primarily as a result of favourable root biochemistry rather than termination by disking or roller-crimping in the humid South-eastern USA. Most studies have so far only reported about the influence of termination methods with regards to their efficiency in controlling cover crop. More knowledge is

needed on the effect of roller crimping as a cover crop termination method on soil C build-up and N release as well as soil enzyme activity in agricultural ecosystem.

2.1.5.3 Disking

The use of a tractor-mounted mechanical disk to control and incorporate cover crop shoots into the soil is a traditional termination approach (Jani et al., 2016). Cover crop termination by disking leads to fast shoot decay and nutrient release, in contrast to kill methods that leave surface mulch (Paul, 2007). This is as a result of better shoot-soil contact and increased levels of soil oxygen in disked systems, both of which stimulate microbial decomposition (Paul, 2007). The quicker rates of shoot decomposition and nutrient mineralization in disking led to greater availability of soil inorganic N at an earlier date relative to herbicide application (Jani et al., 2016). Disking cover crops increased the population of total and gram-negative bacteria while it either decreased or stabilized *actinomycetes* and fungi populations (Elfstrand et al., 2007). The reduction in fungi abundance may be as a result of reduced soil moisture, high lignin materials and increased soil disturbance in disked soils (Elfstrand et al., 2007). Further studies should focus on examining the effect of disking cover crops on decomposition, soil moisture retention, soil organic matter, nitrogen release dynamics and subsequent cash crops.

2.1.6 Herbicides

Application of non-selective herbicides is usually used for cover crop termination since the herbicides are effective at any growth stage (Kornecki et al., 2009). Herbicide is also widely used because most spray equipment permits cover crop termination in a large area of land within a short period of time (Kornecki et al., 2009). Herbicides applied should not only be effective but also cause little or no harmful effects on crop and soil ecosystem (Hoerlein, 1994). Several studies have however indicated that herbicides have significant residual activity in soil which may influence chemical equilibrium reaction and microbial activity or be deleterious to the soil environment (Pandey et al., 2007, Riaz et al., 2007). Moreover, herbicides applied for weed or cover crop termination could persist on the topsoil or be leached thereby polluting groundwater (Sebiomo et al., 2011). Examples of herbicides are glyphosate, atrazine, oxyfluorfen, imazethapyr and paraquat. Glyphosate (N-phosphonomethylglycine) is a systemic non-selective and post-emergence herbicide and it is one of the most often used herbicides in agriculture due to its low production cost, effective weed control and low mammalian toxicity (Nguyen et al., 2016).

There is a growing interest in evaluating the effect of herbicide application on non-target beneficial microorganisms, nutrient cycling and plant nutrition in different ecosystems (Cherni et al., 2015, Newman et al., 2016). However, the findings from previous studies have been inconsistent, which perhaps reflects a site and management specific response of the microbial community to herbicide

additions and may vary with type and application rates (Weaver et al., 2007, Lane et al., 2012). Several studies have indicated that the presence of glyphosate has no significant impact on the diversity and activity of soil beneficial microorganisms (Weaver et al., 2007, Lane et al., 2012). Nevertheless, bacteria diversity and rhizobacterial community structure were negatively influenced when glyphosate was repeatedly applied in maize (Barriuso and Mellado, 2012) and temperate grass (Druille et al., 2016) cropping systems. Seven days after glyphosate treatment, a significant decrease in the total microbial biomass in soybean rhizosphere soil was observed (Lane et al., 2012). Similarly, in an incubatory study, atrazine, glyphosate, primeextra and paraquat applications decreased bacteria, *Actinomycetes* and fungi populations (Sebiomo et al., 2011). García-Orenes et al. (2010) reported that termination of oats straw with paraquat, glyphosate or oxyfluorfen led to a reduction in microbial biomass C, C mineralization coefficients, soluble C and β -glucosidase, phosphatase and urease activities. This is an indication that these herbicides could be adsorbed in soil and be toxic to soil microorganisms which in turn has important implications on biogeochemical reactions, organic matter and soil quality (Abbas et al., 2014). In other studies, there were significant changes in soil organic matter levels under atrazine (Ayansina and Oso, 2006) as well as glyphosate and paraquat (Oladele and Ayodele, 2017) treated soils. The root biomass of radish and vetch reduced in response to imazethapyr and saflufenacil/dimethenamid-p herbicide application (Rojas et al., 2016).

Some micronutrients such as iron, manganese, zinc, and boron have been shown to be deficient in glyphosate-based cropping systems (Neumann et al., 2006, Huber, 2007). Glyphosate may immobilize nutrients or transform them to plant unavailable forms by stimulating certain microbial community members (Johal and Huber, 2009). Yet, the presence of glyphosate enhanced soil bacterial diversity in soybean plots (Arango et al., 2014), increased proliferation of protists and total bacterial composition in barley plots (Imparato et al., 2016) and stimulated microbial activity, as well as N and C mineralization under different soil types (Haney et al., 2002). Similarly, soil inorganic N availability was enhanced when a mixture of rye, hairy vetch and oriental mustard was terminated with glyphosate (Snapp et al., 2005).

There is limited information on the impact of herbicides use on the residue function and soil quality properties in cover cropping systems. The microbiota community and enzymes are sensitive to agrochemicals (Baćmaga et al., 2015, Borowik et al., 2017), and a number of studies have shown that soil enzyme activity reflects soil health status, thus serves as a reliable soil quality indicator under different management systems (Adetunji et al., 2017). It is, therefore, important to further examine the effect of cover crop termination with herbicides on organic carbon build-up, enzyme

activity and soil fertility, to inform better management choices that improve soil quality and ultimately increase agricultural production.

Cover crop termination is an important management method in agricultural cropping systems. But most research has been focusing on how effective these methods control cover crops, ignoring their impacts on nutrient cycling and soil biology. Studies from some regions in the United States of America indicated that the termination method does not affect the amount of PAN; nonetheless, it may affect the timing of PAN release (Sullivan and Andrews, 2012). Information on the implications of the choice of cover crop kill method on soil fertility is still lacking. There is a need for more research on the effect of cover crop termination methods on plant available nitrogen, soil organic carbon accumulation, and microbial activity in agricultural cropping systems.

2.2 The biological activities of β -glucosidase, phosphatase and urease as soil quality indicators

2.2.1 Introduction

The soil is a living, dynamic and non-renewable resource. Soil conditions influence food production, environmental efficiency and the global ecological balance (Binkley and Fisher, 2013). Soil quality is an important indicator of good crop yield in various land use contexts (Almeida et al., 2015). Doran and Parkin (1994) define soil quality as '*the continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries, to sustain biological productivity, promote the quality of air and water environments and maintain plant, animal and human health*'. To manage soil quality, it is essential to have appropriate tools for predicting and evaluating soil changes caused by various management practices and environmental factors (Piotrowska and Wilczewski, 2012). A number of methods have been used to predict changes in soil quality. The chemical, physical and biological properties of soil can serve as indicators of quality (Abbott and Murphy, 2003). Indicators that are sensitive and make rapid, incisive and efficient responses concerning soil quality are preferred (Matsuoka et al., 2003). Soil quality indicators that are linked to microbial activity may respond to disturbances over a shorter period of time than those linked to physical or chemical properties (Garcia-Gil et al., 2000). Thus, soil ecosystem sustainability can be suitably evaluated through the use of biologically-based indicators (Piotrowska-Dlugosz and Charzynski, 2015).

In recent years, soil biology has turned to assessing the degrading capacity of microorganisms by evaluating their enzyme activity (Fioretto et al., 2000). Microbial species release enzymes into the environment in order to degrade complex organic molecules into absorbable simple molecules

(Almeida et al., 2015). Thus, soil enzymes catalyse and increase several biochemical reactions (Dick et al., 1996) that bring about the decay of organic residues, transformation of native soil organic matter, mineralization of nutrients for plant growth, and soil aggregation (Baležentienė, 2012). Decomposition rates are therefore linked to the enzymes that act directly on the main structural parts of plant material and can supply useful information on definite features of the microbial community and succession (Fioretto et al., 2000). This chapter reviews the properties and roles of three enzymes – β -glucosidase, phosphatase and urease – as indicators of soil quality. It also reviews the factors affecting their activity in the soil as well as their application in agriculture.

2.2.2 General characteristics and use of soil enzymes

All soils contain a group of intracellular and extracellular enzymes with different origins that may be synthesized by plants, animals, and microorganisms (Gianfreda et al., 1996, Verdoucq et al., 2003). Intracellular enzymes can be found in various parts of proliferating living cells (Nannipieri and Gianfreda, 1998). Living cells, however, produce and secrete extracellular enzymes which function outside the parent cells as free enzymes in a soil solution or as enzymes that are still associated with the external surface of the root epidermal or microbial cell wall (Nannipieri and Gianfreda, 1998). These enzymes are not only available in dead cells, but may also be taken up on clays or integrated into humic substances. Enzymes play a vital role in agriculture and in nutrient cycling, in particular, since they are constantly being synthesized, accumulated, inactivated and decomposed in the soil (Balota and Chaves, 2010).

The choice to use enzymes to assess soil quality is based on their sensitivity to soil management, organic matter decomposition, and relative ease of analysis (Balota and Chaves, 2010). The determination of soil fertility and plant yield using a single enzyme activity has been proven to be inaccurate (Nannipieri et al., 2012). This is because soil enzyme activities catalyse a particular reaction and cannot therefore be linked to the general soil microbiological activity, which comprises a wide range of different enzymatic reactions (Nannipieri et al., 2012). Furthermore, a given enzyme cannot reflect the whole nutrient status of the soil because it is substrate specific (Nannipieri et al., 2012). Thus the enzymes most widely used for evaluating the factors controlling plant litter decomposition and soil quality are those involved in the degradation of main litter components and hydrolases, which are associated with the carbon (C) (β -glucosidase and β -galactosidase), nitrogen (N) (urease), phosphorus (P) (phosphatase) and sulphur (S) (arylsulphatase) cycle (Karaca et al., 2010). Other soil enzymes may include amylase, amidase, phenol oxidase, cellulase, chitinase, dehydrogenase and protease (Tabatabai, 1994, Karaca et

al., 2010). Table 2.3 shows some of the common soil enzymes that can be used as biological soil quality indicators.

Table 2.3: Soil enzymes as indicators of soil quality

Soil enzyme	Enzyme reaction	Reaction catalysed	Indicator of microbial activity
Dehydrogenase	Electron transport system	$XH_2 + A \rightarrow X + AH_2$	C-cycling
β -glucosidase	Cellobiose hydrolysis	$Glucoside + H_2O \rightarrow ROH + glucose$	C-cycling
Cellulase	Cellulose hydrolysis	Hydrolysis of β -1, 4 - glucan bonds	C-cycling
Phenol oxidase	Lignin hydrolysis	$A + H_2O_2 \rightarrow oxidized\ A + H_2O$	C-cycling
Urease	Urea hydrolysis	$Urea \rightarrow 2NH_3 + CO_2$	N-cycling
Amidase	N-mineralization	$Carboxylic\ acid\ amide + H_2O \rightarrow carboxylic\ acid + NH_3$	N-cycling
Protease	N-mineralization	Proteins \rightarrow peptides and amino acids	N-cycling
Phosphatase	Release of PO_4^-	$Phosphate\ ester + H_2O \rightarrow ROH\ phosphate$	P-cycling
Arylsulphatase	Release of SO_4^-	$ROSO_3^- + H_2O \rightarrow ROH\ SO_4^{2-}$	S-cycling
Other soil enzymes	Hydrolysis	Hydrolysis	General organic matter degradative enzyme activities

Adapted from Das and Varma (2010)

Soil enzyme activity can be estimated and serve as a valuable pointer to nutrient cycling potential, nitrification, oxidation, and other processes crucial to soil quality (Almeida et al., 2015). This review therefore focuses on the enzymes β -glucosidase, phosphatase and urease, known to play crucial roles in C, P and N cycling respectively, which are important nutrients for plant growth and microbial metabolism. The three enzymes are widely distributed in nature and are very sensitive to the environment and management-induced changes in the soil ecosystem.

2.2.3 β -glucosidases

2.2.3.1 Characteristics and role of β -glucosidases

Glycosidases are a group of enzymes that catalyse the hydrolysis of glycosides (Martinez and Tabatabai, 1997). They are highly diverse enzymes owing to the wide diversity of glycosidic bonds and variations in their substrates (Almeida et al., 2015). Among the glycosidases, α - and β -glucosidase, as well as α - and β -galactosidase are the main members, widely distributed in the soil (Utobo and Tewari, 2015). The α -glucosidase (maltase) catalyses the hydrolysis of α -D-glucopyranosides while β -glucosidase (cellobiase) hydrolyzes maltose and cellobiose (Utobo and Tewari, 2015). β -glucosidase is, however, the most common, important and widely used soil quality indicator (Bandick and Dick, 1999).

β -glucosidase is predominantly found among plants, animals, fungi, bacteria, and yeasts (Veena et al., 2011). Its role in soils is crucial since it is involved in catalysing the hydrolysis and biodegradation of various β -glucosides that are present in plant debris (Martinez and Tabatabai, 1997). β -glucosidase acts in the last phase of the cellulose degradation process by hydrolysing the cellobiose residue (Gil-Sotres et al., 2005). These reactions produce glucose as the final product, an important C energy source for the growth and activity of soil microorganisms (Acosta-Martinez et al., 2011). β -glucosidase's involvement in C cycling has remarkably facilitated its adoption for soil quality testing.

2.2.3.2 Factors affecting β -glucosidase activity

The activity of β -glucosidase decreased as soil pH increased from 4.5 to 8.5 (Eivazi and Tabatabai, 1990), and 4.3 to 7.4 in a paddy soil (Xiao-Chang and Qin, 2006). The sensitivity of β -glucosidase to pH changes can serve as a reliable biochemical indicator for assessing environmental changes caused by soil acidification (Acosta-Martinez and Tabatabai, 2000).

Soil moisture can influence the biochemical processes of soil carbon transformation catalysed by β -glucosidase (Zhang et al., 2011). β -glucosidase activity decreased by 10-80% and 35-83% when soil moisture was reduced by 10% and 21% respectively, depending on the soil depth (Sardans and Peñuelas, 2005). Thus drought influences β -glucosidase activity and its catalytic features, causing a slower nutrient turnover and a reduced nutrient supply to plants.

Increased soil salinity and solidity led to an exponential and linear decline in β -glucosidase activity, respectively (Rietz and Haynes, 2003). It has been reported that plant residues in a soil polluted

with heavy metals neither decompose nor indicate β -glucosidase activities (Geiger et al., 1993), resulting in less glucose for soil microbes. The response of β -glucosidase activity to soil salinity and heavy metal contamination can serve as a good pointer to soil quality status.

Several studies have revealed that β -glucosidase activity decreases with soil depth (Acosta-Martinez et al., 2003a, Xiao-Chang and Qin, 2006). This is because β -glucosidase activity greatly depend on substrate supply and the microorganisms that mainly produce this enzyme are active in the top soil (Xiao-Chang and Qin, 2006). Therefore, β -glucosidase activity can be used to indicate the presence of higher simple sugars for microbial population in the soil surface layer.

2.2.3.3 Application of β -glucosidase activity in agriculture

The activity of β -glucosidase is influenced by crop residue quality and a number of soil management practices. A no-till system and reduction in tillage frequency results in an increase in the activities of β -glucosidase as well as microbial biomass C and N in contrast to a conventional tillage system (Pandey et al., 2014). The reduction in tillage intensity favours and increases β -glucosidase activity due to improvement in microbial biomass, more substrate availability and reduced soil disturbance (Sinsabaugh et al., 2008). Therefore, a conventional tillage system may cause soil organic matter depletion (Miralles et al., 2012), which can result in a reduction in simple sugars for microbial functioning owing to a decrease in β -glucosidase activity.

The activity of β -glucosidase was reported to be lower in arable soils than in woodland and meadow soils (Bandick and Dick, 1999). This may be as a result of varying substrate and organic matter composition associated with each soil. Incorporation of residues of vetch, oat+legume, *Trifolium pratense* L, *Brassica napus* L and *Trifolium pratense* L+*Brassica napus* L, led to an increase in the activity of β -glucosidase compared to oats (Piotrowska-Dlugosz and Wilczewski, 2014a, Mukumbareza et al., 2015a). β -glucosidase activity increased because the soils amended with lower C:N crop residue favours its function, resulting in quick organic matter decomposition and nutrient release. The high capacity to respond to substrate and soil type make β -glucosidase activity an efficient soil quality indicator.

Several studies have shown that β -glucosidase activity was higher in fertilization treatments with compost, vermicompost, municipal solid waste compost and straw mulch, than in those without compost as well as those with synthetic fertilizer and herbicide (Crecchio et al., 2004, Saha et al., 2008, Meyer et al., 2015). Furthermore, β -glucosidase activity increased remarkably in various soils amended with sewage sludge and irrigated with winery wastewater rather than municipal water (Kizilkaya and Bayrakli, 2005, Mulidzi and Wooldridge, 2016). β -glucosidase activity

increased due to the inducement caused by the addition of simple organic substrates contained in those residues, which makes this enzyme a reliable indicator of soil quality.

In general, β -glucosidase activity is closely related to soil organic matter, biological activity and C cycling, and it can provide an advanced sign of alterations in organic carbon long before this can be correctly determined by other routine techniques. These qualities have significantly enabled its adoption for soil quality testing in agriculture. More knowledge is however needed to show the dynamics of β -glucosidase and other factors influencing their activity, to enhance the understanding of biological soil fertility management in agriculture.

2.2.4 Phosphatases

2.2.4.1 Characteristics and role of phosphatases

Phosphatases are a group of enzymes that catalyse the hydrolysis of esters and anhydrides of phosphoric acid (Condrón et al., 2005). Plants and microorganisms are the main sources of phosphatase enzymes in the soil. The amount of phosphatase present in the soil varies with the microbial count and the extent of organic materials, mineral and organic fertilizers, tillage and other agricultural practices (Banerjee et al., 2012).

Since plants make use of only inorganic P and a large amount of soil P is organically bound, the mineralization of this organic portion can be a vital influence in plant nutrition (Nannipieri et al., 2011). When phosphorus is lacking in the soil, plant roots and microorganisms increase the secretion of phosphatase to intensify the solubilisation and remobilization of phosphate, therefore influencing the ability of the plant to cope with phosphorus-stressed conditions (Kai et al., 2002). This shows that the demand for phosphorus by plants and microorganisms can be linked to the production and activity of soil phosphatase (Condrón et al., 2005). Phosphatase activity can therefore be used as an indicator of inorganic phosphorus availability for plants and microorganisms (Piotrowska-Długosz and Charzynski, 2015).

Phosphomonoesterase is the most studied among the phosphatases present in the soil. It hydrolyses phosphate monoester to produce free phosphate for biological uptake (Makoi and Ndakidemi, 2008). Phosphomonoesterase is active under acidic and alkaline conditions, depending on its optimal pH, and acts upon low molecular P compounds with monoester bonds, including nucleotides, sugar phosphates and polyphosphates (Dodor and Tabatabai, 2003). Acid phosphatase activity is therefore found mainly in acid soils, while alkaline phosphatase dominates in alkaline soils (Dodor and Tabatabai, 2003), with ranges of 4-6 and 9-11, respectively.

2.2.4.2 Factors affecting phosphatase activity

Soil pH influences the rate of synthesis, release, and stability of phosphatase (Acosta-Martinez and Tabatabai, 2000). As the soil pH increases, the activity of alkaline phosphatase increases, while acid phosphatase activity decreases (Dick et al., 2000). Alkaline (Pal) and acid (Pac) phosphatase activities can be used to examine the optimum soil pH for crop production and the amount of lime required to achieve it (Dick et al., 2000). Thus, determination of the Pal/Pac ratio may be a better way of evaluating the effective soil pH and liming needs than the chemical method (Acosta-Martinez et al., 2003b).

The activity of phosphatase was influenced in soils affected by forest fire, increasing over the years as the soil recovered (Staddon et al., 1998). The effect of drought has also been reported: when soil moisture was reduced by 21%, there was a 31-40% reduction in acid phosphatase activity (Sardans and Peñuelas, 2005). The presence of lead and other heavy metals in the soil decreased phosphatase activity (Kandeler et al., 1996). Phosphatases are a good soil quality indicator since their activity reflects the situation of the soil.

2.2.4.3 Application of phosphatase activity in agriculture

In agricultural soils, phosphatases play a crucial role in phosphorus cycles, and because their activity is sensitive to management practices they can be used as an index of soil quality (Makoi and Ndakidemi, 2008). To improve soil quality management and agricultural productivity, it is important to evaluate the effect of different management practices on phosphatase activity in the soil. Agricultural management methods responsible for phosphorus stress in the soil may influence the production of these enzymes in the ecosystem (Ndakidemi, 2006).

Previous studies have reported that legumes such as chickpea, cowpea, *Cyclopia* and *Aspalathus* release more phosphatase enzymes than non-legumes (Liu et al., 2004, Makoi et al., 2010, Maseko and Dakora, 2013). This is because legumes require more phosphorus in the symbiotic nitrogen fixation process than cereals do (Makoi and Ndakidemi, 2008). The increase in phosphatase activity in the legume roots and soils leads to a significant increase in plant available P (Makoi et al., 2010). Thus, phosphorus supply and assimilation can be estimated by acid and alkaline phosphatase activity in the low-P soils of legume crops (Maseko and Dakora, 2013).

The activity of phosphatases was higher in a crop rotation system comprising oats or meadow than in a monoculture system of corn or soybean (Dodor and Tabatabai, 2003). Mukumbareza et al. (2015a) revealed that the rotation of maize with vetch and fertilized oat cover crops increased

microbial biomass carbon and the activities of phosphatase in a South African deep alluvial soil. The increased microbial biomass carbon and phosphatase activity in the bicultures as compared to the monocultures showed that the cover crops have synergistic effects in bicultures, and could be valuable for improving P cycling and soil physiochemical properties (Mukumbareza et al., 2016).

Acid and alkaline phosphatase activity increased under various soil management practices where organic fertilizers like plant residues, sewage sludge, manure, compost and vermicompost were applied (Criquet et al., 2007, Nannipieri et al., 2011, Piotrowska-Dlugosz and Wilczewski, 2014b). The increase in phosphatase activity associated with soils amended with organic materials can be attributed to stimulation of microbial growth and soil organic matter enrichment. Phosphatase activity can be considered to be a good index of the quality and quantity of organic matter in soils.

Several studies have reported that the activity of phosphatases increases in soils supplemented with organic matter and inoculated with mycorrhizal species (Joner and Jakobsen, 1995, van Aarle and Plassard, 2010). The association of mycorrhizal with phosphatase activity supports the role this enzyme plays in the degradation of soil-bound phosphorus (van Aarle and Plassard, 2010).

Phosphatase probably requires a considerable amount of N, since nitrogen fertilization has been shown to increase acid phosphatase activity and reduced alkaline phosphatase activity in soils cultivated with corn and wheat (Lemanowicz, 2011, Kalembasa and Symanowicz, 2012). Furthermore, the joint application of vermicompost or municipal solid waste compost and mineral N fertilizer revealed higher phosphate activity than the separate application of the fertilizers to soil (Crecchio et al., 2004, Srivastava et al., 2012). Phosphatase activity increased when P fertilizer was added to soils with low organic matter, yet there were no changes in the activity of this enzyme when P fertilizer was amended with soils containing high organic matter (Piotrowska-Dlugosz and Wilczewski, 2014a). Thus, the synthesis and activity of phosphatase can be influenced by P from mineral fertilization in the soil (Saha et al., 2008). This confirms that phosphatase increases the available reserves of P when this nutrient is limited, and that the addition of P to the soil is an alternative for increasing the availability of this element (Bautista-Cruz and Ortiz-Hernandez, 2015). Phosphatase activity can therefore serve as a good soil quality indicator because of its strong correlation with soil organic matter, organic P, inorganic P, and N availability in the soil.

No-tillage and conventional tillage systems affect soil biological activity and aggregate stability. Greater acid phosphatase activity was observed under a no-till and reduced tillage system compared to a continuous tillage system in Brazilian Cerrado Oxisol and rice grown soil (Green et al., 2007, Pandey et al., 2014). Thus, phosphatase activity is a reliable soil quality indicator since

it promptly detects changes in soil organic matter caused by tillage. Tillage should be reduced to increase the biological activity of surface soils in order to improve P nutrient cycling processes and soil structure.

A larger amount of the inorganic P assimilated by plants is produced from the mineralization of organic P through phosphatase activity. The functions of phosphatase activity and its ability to rapidly detect management changes, as described above, indicate the importance of this enzyme as a biological soil quality indicator. More understanding of the roles of phosphatase and better ways of optimizing its activities in soils managed organically will result in improved soil conservation, P release and increased agricultural sustainability in the ecosystems.

2.2.5 Urease

2.2.5.1 Characteristics and role of Urease

The urease enzyme acts by aiding the hydrolysis of urea into CO_2 and NH_3 , which leads to a rise in soil pH and nitrogen loss to the atmosphere through NH_3 volatilization (Das and Varma, 2010). Urease also catalyses the hydrolysis of hydroxyurea, dihydroxyurea, and semicarbazide, with nickel as a co-factor (Alef and Nannipieri, 1995). The enzyme is widely distributed in nature, and it originates from bacteria, yeasts, fungi, algae, animal waste and plants (Follmer, 2008). Although urease may be constitutively synthesized in some organisms, its expression is usually under N regulation (Mobley et al., 1995). The synthesis of the enzyme is prevented when cells grow in the presence of NH_4^+ as the preferred N source (Geisseler et al., 2010). However, the presence of urea or an alternative N source activates urease production (Mobley et al., 1995). Studies of soil urease activity have been of great interest over the years and have been used as good index of soil quality, because of the role of urease in the regulation of N supply to plants after urea fertilization (Piotrowska-Dlugosz and Charzynski, 2015).

2.2.5.2 Factors affecting urease activity

The stability of urease depends on several factors, including soil moisture and temperature. Urease activity increases with increasing temperature, showing the effect of temperature on urease hydrolysis (Sardans et al., 2008). In addition, a grassland soil incubated over a range of temperatures (-2 to 21°C) showed a positive relationship between urease activity and temperature, with an activation energy (E_a) of 73.4 kJ mol^{-1} and temperature coefficient (Q_{10}) of 2.78 (Fraser et al., 2013). Urea fertilizer should therefore be applied to the soil when the temperature is low and activation energy is low, to minimize the loss of N by the volatilization

process (Makoi and Ndakidemi, 2008). Studies examining the temperature sensitivity of urease activity in soils will enhance knowledge of N cycling.

The reduction of soil moisture by 10% and 21% led to reductions of 10-67% and 42-62% in urease activity, respectively, which explains the link between drought and a slower nutrient turnover (Sardans and Peñuelas, 2005). YANG et al. (2006) investigated the combined effects of cadmium, zinc and lead on urease activities and concluded that urease is very sensitive to toxic concentrations of heavy metals. The highest urease activity was recorded under soil water pH 5.8 and the lowest activity at soil water pH 4.2 (Blonska and Lasota, 2014). The response of urease activity to drought, contamination and pH can be used to assess soil quality status.

2.2.5.3 Application of urease activity in agriculture

Urease activity has been widely used to monitor soil quality because it is influenced by different agricultural management practices (Corstanje et al., 2007, Blonska and Lasota, 2014). Urease activity increased in a soil management system where maize was rotated with vetch and fertilized oat cover crops, which led to an increase in maize yield (Mukumbareza et al., 2015a). The high urease activity recorded in soils treated with vetch and a combination of vetch and oats indicates a greater potential for N cycling through a lower C:N ratio.

The activity of urease increases with organic fertilization such as compost, sewage sludge and straw mulch, and decreases with soil tillage (Crecchio et al., 2004, Kizilkaya and Bayrakli, 2005, Meyer et al., 2015). Furthermore, urease activity increased in four different vineyard soils treated with winery wastewater rather than municipal water (Mulidzi and Wooldridge, 2016). The increase in urease activity recorded under organic fertilization shows the close relationship this enzyme shares with soil organic matter and N cycling. On the other hand, there was a decrease in the activity of urease in soils with long-term nitrogen fertilization, in comparison with unfertilized soils (Mohammadi, 2011). The reduction in urease activity was a result of the absorption of mineral N by soil microorganisms (Meysner et al., 2006), which supports the hypothesis of Konig et al. (1996) that high quantities of ammonia reduce urease activity. Urease activity can be used to effectively evaluate changes in soil quality relating to management, since its activity increases with organic fertilization and decreases with soil tillage.

Urease activities have effectively discriminated between a wide range of soil management practices. Due to its sensitivity and capacity to provide information that integrates environmental factors and N cycling, urease activity can be a useful tool to assess soil fertility. Analysis of urease activity can inform management methods that best improve microbial metabolism and N cycling.

Furthermore, urease activity could be stimulated by legume crops since they have the ability to biologically fix N (Roldan et al., 2003). More studies on urease activity and factors affecting them in soils managed with legume crops will provide better ways to minimize the application of urea fertilizer, reduce NH₃ loss, and optimize soil nitrogen levels.

2.3 Conclusion

The potential for cover crops to supply ecosystem services and improve soil quality has been documented in various cropping systems and environments. Cover crops enhance soil organic matter, organic C sequestration, physical, chemical, and biological properties, nutrient cycling, weed suppression, pest management, wildlife habitat and diversity, regulate soil temperature as well as control soil and wind erosion. Management practices involving effective planning, species selection, seeding rate, planting period, termination timing and termination method, all contribute to successful cover cropping for improved soil quality and agricultural productivity. The impacts of these cover crop management options on soil biology and fertility are still not well understood, particularly cover crop species selection, termination stage and termination methods. There is, therefore, still a lot of research that needs to be done to examine the influence of cover crop termination stage and termination methods on N dynamics, soil organic carbon build-up and soil enzyme activity under different ecosystems.

Soil enzymes have been successfully used as indicators of soil quality in different agricultural systems. A better understanding of the function of soil enzymes and the factors influencing their activity is crucial for improving soil management, soil quality, and food production. Soil enzymes catalyse and facilitate decomposition and nutrient cycling, thereby rendering their activities a biological index of soil quality. The soil enzymes are operationally practical, integrative, easy to measure, and they respond to soil management changes long before other soil quality indicator changes are detectable. Their activities may be influenced by soil depth and type, temperature, moisture, pH, the quality and quantity of available substrate, as well as management regimes. Individual enzyme activity does not reflect soil quality status since single enzyme activities cannot represent the rate of all metabolic processes (unless they catalyse one specific reaction). Thus, several enzyme activities should be assessed in order to measure soil quality effectively.

References

- Abbas, Z., Akmal, M. & Khan, K.S. 2014. Effect of buctril super (Bromoxynil) herbicide on soil microbial biomass and bacterial population. *Brazilian Archives of Biology and Technology*, 57, 9-14.
- Abbott, L.K. & Murphy, D.V. 2003. *Soil biological fertility: A key to sustainable land use in agriculture*, Springer Science & Business Media.

- Acosta-Martinez, V., Klose, S. & Zobeck, T. 2003a. Enzyme activities in semiarid soils under conservation reserve program, native rangeland, and cropland. *Journal of Plant Nutrition and Soil Science*, 166, 699-707.
- Acosta-Martinez, V., Mikha, M.M., Sistani, K.R., Stahlman, P.W., Benjamin, J.G., Vigil, M.F. & Erickson, R. 2011. Multi-location study of soil enzyme activities as affected by types and rates of manure application and tillage practices. *Agriculture*, 1, 4-21.
- Acosta-Martinez, V. & Tabatabai, M. 2000. Enzyme activities in a limed agricultural soil. *Biology and Fertility of Soils*, 31, 85-91.
- Acosta-Martinez, V., Zobeck, T., Gill, T. & Kennedy, A. 2003b. Enzyme activities and microbial community structure in semiarid agricultural soils. *Biology and Fertility of Soils*, 38, 216-227.
- Acuña, J. & Villamil, M.B. 2014. Short-term effects of cover crops and compaction on soil properties and soybean production in Illinois. *Agronomy Journal*, 106, 860-870.
- Adetunji, A.T., Lewu, F.B., Mulidzi, R. & Ncube, B. 2017. The biological activities of β -glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of Soil Science and Plant Nutrition*, 17, 794-807.
- Alef, K. & Nannipieri, P. 1995. *Methods in applied soil microbiology and biochemistry*, Academic press.
- Almeida, R.F.d., Naves, E.R. & Mota, R.P.d. 2015. Soil quality: Enzymatic activity of soil β -glucosidase. *Global Journal of Agricultural Research and Reviews*, 3, 146-150.
- Alonso-Ayuso, M., Gabriel, J.L. & Quemada, M. 2014. The kill date as a management tool for cover cropping success. *PLoS one*, 9, e109587.
- Arango, L., Buddrus-Schiemann, K., Opelt, K., Lueders, T., Haesler, F., Schmid, M., Ernst, D. & Hartmann, A. 2014. Effects of glyphosate on the bacterial community associated with roots of transgenic Roundup Ready® soybean. *European Journal of Soil Biology*, 63, 41-48.
- Ashford, D.L. & Reeves, D.W. 2003. Use of a mechanical roller-crimper as an alternative kill method for cover crops. *American Journal of Alternative Agriculture*, 18, 37-45.
- Ayansina, A. & Oso, B. 2006. Effect of two commonly used herbicides on soil microflora at two different concentrations. *African Journal of Biotechnology*, 5, 129-132.
- Baćmaga, M., Kucharski, J. & Wyszowska, J. 2015. Microbial and enzymatic activity of soil contaminated with azoxystrobin. *Environmental Monitoring and Assessment*, 187, 615.
- Baker, J.M. & Griffis, T.J. 2009. Evaluating the potential use of winter cover crops in corn–soybean systems for sustainable co-production of food and fuel. *Agricultural and Forest Meteorology*, 149, 2120-2132.
- Baležtienė, L. 2012. Hydrolases related to C and N cycles and soil fertility amendment: Responses to different management styles of agro-ecosystems. *Polish Journal of Environmental Studies*, 21, 1153-1159.
- Balkcom, K.S., Duzy, L.M., Kornecki, T.S. & Price, A.J. 2015. Timing of cover crop termination: Management considerations for the Southeast. *Crop, Forage & Turfgrass Management*, 1.
- Balota, E.L. & Chaves, J.C.D. 2010. Enzymatic activity and mineralization of carbon and nitrogen in soil cultivated with coffee and green manures. *Revista Brasileira de Ciência do Solo*, 34, 1573-1583.
- Bandick, A.K. & Dick, R.P. 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochemistry*, 31, 1471-1479.
- Banerjee, A., Sanyal, S. & Sen, S. 2012. Soil phosphatase activity of agricultural land: A possible index of soil fertility. *Agricultural Science Research Journals*, 2, 412-419.
- Barriuso, J. & Mellado, R.P. 2012. Relative effect of glyphosate on glyphosate-tolerant maize rhizobacterial communities is not altered by soil properties. *Journal of Microbiology and Biotechnology*, 22, 159-165.
- Bautista-Cruz, A. & Ortiz-Hernandez, Y.D. 2015. Hydrolytic soil enzymes and their response to fertilization: a short review. *Comunicata Scientiae*, 6, 255.
- Benedict, C., Cogger, C.G. & Andrews, N. 2014. *Methods for successful cover crop management in your home garden*, Washington State University Extension.

- Bilbro, J. & Hargrove, W. Cover crops for wind erosion control in semiarid regions. Cover crops for clean water. Proceedings of an International Conference West Tennessee Experiment Station, Jackson, TN. April, 1991. 9-11.
- Binkley, D. & Fisher, R.F. 2013. *Ecology and management of forest soils*, Wiley Online Library.
- Blanco-Canqui, H., Claassen, M. & Presley, D. 2012. Summer cover crops fix nitrogen, increase crop yield, and improve soil–crop relationships. *Agronomy Journal*, 104, 137-147.
- Blanco-Canqui, H., Holman, J.D., Schlegel, A.J., Tatarko, J. & Shaver, T.M. 2013a. Replacing fallow with cover crops in a semiarid soil: Effects on soil properties. *Soil Science Society of America Journal*, 77, 1026-1034.
- Blanco-Canqui, H., Mikha, M.M., Presley, D.R. & Claassen, M.M. 2011. Addition of cover crops enhances no-till potential for improving soil physical properties. *Soil Science Society of America Journal*, 75, 1471-1482.
- Blanco-Canqui, H., Shapiro, C.A., Wortmann, C.S., Drijber, R.A., Mamo, M., Shaver, T.M. & Ferguson, R.B. 2013b. Soil organic carbon: The value to soil properties. *Journal of Soil and Water Conservation*, 68, 129A-134A.
- Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A. & Hergert, G.W. 2015. Cover crops and ecosystem services: Insights from studies in temperate soils. *Agronomy Journal*, 107, 2449-2474.
- Blonska, E. & Lasota, J. 2014. Biological and biochemical properties in evaluation of forest soil quality. *Folia Forestalia Polonica*, 56, 23-29.
- Borowik, A., Wyszowska, J., Kucharski, J., Baćmaga, M. & Tomkiel, M. 2017. Response of microorganisms and enzymes to soil contamination with a mixture of terbuthylazine, mesotrione, and S-metolachlor. *Environmental Science and Pollution Research*, 24, 1910-1925.
- Brandt, S. 1996. Alternatives to summerfallow and subsequent wheat and barley yield on a Dark Brown soil. *Canadian Journal of Plant Science*, 76, 223-228.
- Brennan, E.B., Boyd, N.S. & Smith, R.F. 2013. Winter cover crop seeding rate and variety effects during eight years of organic vegetables: III. Cover crop residue quality and nitrogen mineralization. *Agronomy Journal*, 105, 171-182.
- Chen, G. & Weil, R.R. 2010. Penetration of cover crop roots through compacted soils. *Plant and Soil*, 331, 31-43.
- Cherni, A.E., Trabelsi, D., Chebil, S., Barhoumi, F., Rodríguez-Llorente, I.D. & Zribi, K. 2015. Effect of glyphosate on enzymatic activities, Rhizobiaceae and total bacterial communities in an agricultural Tunisian soil. *Water, Air, & Soil Pollution*, 226, 145.
- Clark, A., Meisinger, J., Decker, A. & Mulford, F. 2007. Effects of a grass-selective herbicide in a vetch–rye cover crop system on corn grain yield and soil moisture. *Agronomy Journal*, 99, 43-48.
- Clark, A.J., Decker, A.M., Meisinger, J.J., Mulford, F.R. & McIntosh, M.S. 1995. Hairy vetch kill date effects on soil water and corn production. *Agronomy Journal*, 87, 579-585.
- Cline, G.R. & Silvernail, A.F. 2001. Residual nitrogen and kill date effects on winter cover crop growth and nitrogen content in a vegetable production system. *HortTechnology*, 11, 219-225.
- Cline, G.R. & Silvernail, A.F. 2002. Effects of cover crops, nitrogen, and tillage on sweet corn. *HortTechnology*, 12, 118-125.
- Colazo, J.C. & Buschiazzo, D.E. 2010. Soil dry aggregate stability and wind erodible fraction in a semiarid environment of Argentina. *Geoderma*, 159, 228-236.
- Condon, L.M., Turner, B.L., Cade-Menun, B.J., Sims, J. & Sharpley, A. 2005. Chemistry and dynamics of soil organic phosphorus. *Agronomy*, 46, 87.
- Coombs, C., Lauzon, J.D., Deen, B. & Van Eerd, L.L. 2017. Legume cover crop management on nitrogen dynamics and yield in grain corn systems. *Field Crops Research*, 201, 75-85.
- Corstanje, R., Schulin, R. & Lark, R. 2007. Scale-dependent relationships between soil organic carbon and urease activity. *European Journal of Soil Science*, 58, 1087-1095.

- Crandall, S., Ruffo, M. & Bollero, G. 2005. Cropping system and nitrogen dynamics under a cereal winter cover crop preceding corn. *Plant and Soil*, 268, 209-219.
- Creamer, N.G. & Dabney, S.M. 2002. Killing cover crops mechanically: Review of recent literature and assessment of new research results. *American Journal of Alternative Agriculture*, 17, 32-40.
- Crecchio, C., Curci, M., Pizzigallo, M.D., Ricciuti, P. & Ruggiero, P. 2004. Effects of municipal solid waste compost amendments on soil enzyme activities and bacterial genetic diversity. *Soil Biology and Biochemistry*, 36, 1595-1605.
- Criquet, S., Braud, A. & Nèble, S. 2007. Short-term effects of sewage sludge application on phosphatase activities and available P fractions in Mediterranean soils. *Soil Biology and Biochemistry*, 39, 921-929.
- Dabney, S., Delgado, J. & Reeves, D. 2001. Using winter cover crops to improve soil and water quality. *Communications in Soil Science and Plant Analysis*, 32, 1221-1250.
- Dabney, S.M., Delgado, J.A., Meisinger, J.J., Schomberg, H.H., Liebig, M.A., Kaspar, T., Mitchell, J. & Reeves, W. 2010. Using cover crops and cropping systems for nitrogen management. In: Delgado, J.A. & Follett, R. (eds.) *Advances in nitrogen management for water quality*. Ankeny, Iowa: Soil water conservation society
- Das, S.K. & Varma, A. 2010. Role of enzymes in maintaining soil health. *Soil enzymology*. Berlin, Heidelberg: Springer.
- Dick, R.P., Breakwell, D.P. & Turco, R.F. 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Doran, J.W. & Jones, A. (eds.) *Methods for assessing soil quality*. Madison WI, USA: Soil Science Society America.
- Dick, W., Cheng, L. & Wang, P. 2000. Soil acid and alkaline phosphatase activity as pH adjustment indicators. *Soil Biology and Biochemistry*, 32, 1915-1919.
- Dodor, D.E. & Tabatabai, M.A. 2003. Effect of cropping systems on phosphatases in soils. *Journal of Plant Nutrition and Soil Science*, 166, 7-13.
- Doran, J. & Parkin, T. 1994. Defining and assessing soil quality. In 'Defining soil quality for a sustainable environment'. (Eds JW Doran, DC Coleman, DE Bezdicek, BA Stewart) pp. 3–21. *Soil Science Society of America: Madison, WI, USA*.
- Drinkwater, L., Janke, R. & Rossoni-Longnecker, L. 2000. Effects of tillage intensity on nitrogen dynamics and productivity in legume-based grain systems. *Plant and Soil*, 227, 99-113.
- Druille, M., García-Parisi, P.A., Golluscio, R., Cavagnaro, F.P. & Omacini, M. 2016. Repeated annual glyphosate applications may impair beneficial soil microorganisms in temperate grassland. *Agriculture, Ecosystems & Environment*, 230, 184-190.
- Drury, C.F., Tan, C.-S., Welacky, T.W., Oloya, T.O., Hamill, A.S. & Weaver, S.E. 1999. Red clover and tillage influence on soil temperature, water content, and corn emergence. *Agronomy Journal*, 91, 101-108.
- Du, Z., Xie, Y., Hu, L., Hu, L., Xu, S., Li, D., Wang, G. & Fu, J. 2014. Effects of fertilization and clipping on carbon, nitrogen storage, and soil microbial activity in a natural grassland in southern China. *PloS one*, 9, e99385.
- Dube, E., Chiduzza, C. & Muchaonyerwa, P. 2012. Conservation agriculture effects on soil organic matter on a Haplic Cambisol after four years of maize–oat and maize–grazing vetch rotations in South Africa. *Soil and Tillage Research*, 123, 21-28.
- Dube, E., Chiduzza, C. & Muchaonyerwa, P. 2014. High biomass yielding winter cover crops can improve phosphorus availability in soil. *South African Journal of Science*, 110, 01-04.
- Eivazi, F. & Tabatabai, M. 1990. Factors affecting glucosidase and galactosidase in soils. *Soil Biology and Biochemistry*, 22, 891-897.
- Elfstrand, S., Båth, B. & Mårtensson, A. 2007. Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek. *Applied Soil Ecology*, 36, 70-82.
- Faé, G.S., Sulc, R.M., Barker, D.J., Dick, R.P., Eastridge, M.L. & Lorenz, N. 2009. Integrating winter annual forages into a no-till corn silage system. *Agronomy Journal*, 101, 1286-1296.

- Fageria, N., Baligar, V. & Bailey, B. 2005. Role of cover crops in improving soil and row crop productivity. *Communications in Soil Science and Plant Analysis*, 36, 2733-2757.
- Feyereisen, G.W., Camargo, G.G., Baxter, R.E., Baker, J.M. & Richard, T.L. 2013. Cellulosic biofuel potential of a winter rye double crop across the US corn–soybean belt. *Agronomy Journal*, 105, 631-642.
- Finney, D., Buyer, J. & Kaye, J. 2017. Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*, 72, 361-373.
- Fioretto, A., Papa, S., Curcio, E., Sorrentino, G. & Fuggi, A. 2000. Enzyme dynamics on decomposing leaf litter of *Cistus incanus* and *Myrtus communis* in a Mediterranean ecosystem. *Soil Biology and Biochemistry*, 32, 1847-1855.
- Follmer, C. 2008. Insights into the role and structure of plant ureases. *Phytochemistry*, 69, 18-28.
- Fortuna, A., Blevins, R., Frye, W., Grove, J. & Cornelius, P. 2008. Sustaining soil quality with legumes in no-tillage systems. *Communications in Soil Science and Plant Analysis*, 39, 1680-1699.
- Fourie, J. 2010. Soil management in the Breede River Valley wine grape region, South Africa. 1. Cover crop performance and weed control. *South African Journal of Enology and Viticulture*, 31, 14-21.
- Fourie, J., Agenbag, G. & Louw, P. 2007. Cover crop management in a Chardonnay/99 Richter vineyard in the coastal region, South Africa. 3. Effect of different cover crops and cover crop management practices on organic matter and macro-nutrient content of a medium-textured soil. *South African Journal for Enology and Viticulture*, 28, 61.
- Fourie, J., Louw, P. & Agenbag, G. 2001. Effect of seeding date on the performance of grasses and broadleaf species evaluated for cover crop management in two wine grape regions of South Africa. *South African Journal of Plant and Soil*, 18, 118-127.
- Franzluebbers, A.J. & Stuedemann, J.A. 2014. Crop and cattle production responses to tillage and cover crop management in an integrated crop–livestock system in the southeastern USA. *European Journal of Agronomy*, 57, 62-70.
- Fraser, F.C., Hallett, P.D., Wookey, P.A., Hartley, I.P. & Hopkins, D.W. 2013. How do enzymes catalysing soil nitrogen transformations respond to changing temperatures? . *Biology and Fertility of Soils*, 49, 99-103.
- García-Gil, J., Plaza, C., Soler-Rovira, P. & Polo, A. 2000. Long-term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass. *Soil Biology and Biochemistry*, 32, 1907-1913.
- García-Orenes, F., Guerrero, C., Roldán, A., Mataix-Solera, J., Cerdà, A., Campoy, M., Zornoza, R., Bárcenas, G. & Caravaca, F. 2010. Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. *Soil and Tillage Research*, 109, 110-115.
- Geiger, G., Federer, P. & Sticher, H. 1993. Reclamation of heavy metal—contaminated soils: field studies and germination experiments. *Journal of Environmental Quality*, 22, 201-207.
- Geisseler, D., Horwath, W.R., Joergensen, R.G. & Ludwig, B. 2010. Pathways of nitrogen utilization by soil microorganisms—a review. *Soil Biology and Biochemistry*, 42, 2058-2067.
- Gianfreda, L., Bollag, J. & Stotzky, G. 1996. Influence of natural and anthropogenic factors on enzyme activity in soil. *Soil Biochemistry*, 9, 123-193.
- Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M. & Seoane, S. 2005. Different approaches to evaluating soil quality using biochemical properties. *Soil Biology and Biochemistry*, 37, 877-887.
- Green, V., Stott, D., Cruz, J. & Curi, N. 2007. Tillage impacts on soil biology activity and aggregation in a Brazilian Cerrado Oxisol. *Soil and Tillage Research*, 92, 114-121.
- Haney, R., Senseman, S. & Hons, F. 2002. Effect of Roundup Ultra on microbial activity and biomass from selected soils. *Journal of Environmental Quality*, 31, 730-735.
- Haney, R.L., Franzluebbers, A.J., Jin, V.L., Johnson, M.-V., Haney, E.B., White, M.J. & Harmel, R.D. 2012. Soil organic C: N vs. water-extractable organic C: N. *Open Journal of Soil Science*, 2, 269.

- Harasim, E., Gawęda, D., Wesolowski, M., Kwiatkowski, C. & Gocół, M. 2016. Cover cropping influences physico-chemical soil properties under direct drilling soybean. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, 66, 85-94.
- Hassink, J. & Whitmore, A.P. 1997. A model of the physical protection of organic matter in soils. *Soil Science Society of America Journal*, 61, 131-139.
- Havstad, L.T., Aamlid, T.S. & Henriksen, T.M. 2010. Decomposition of straw from herbage seed production: Effects of species, nutrient amendment and straw placement on C and N net mineralization. *Acta Agriculturae Scandinavica Section B—Soil and Plant Science*, 60, 57-68.
- Hirpa, T. 2013. Effect of stage at termination of legume green manures on soil organic carbon, yield and economic performance of subsequent maize crop. *International Journal of Current Research and Academic Review*, 1, 84-101.
- Hoerlein, G. 1994. Glufosinate (phosphinothricin), a natural amino acid with unexpected herbicidal properties. *Reviews of Environmental Contamination and Toxicology*. Springer.
- Holman, J., Dumler, T., Roberts, T. & Maxwell, S. 2012. Fallow replacement crop effects of wheat yield. *Kansas State University Cooperative Extension Services Report of Progress, Manhattan KS*.
- Huber, D. Strategies to ameliorate glyphosate immobilization of Mn and its impact on disease. *Phytopathology*, 2007. Amer Phytopathological Soc 3340 Pilot Knob Road, St Paul, MN 55121 USA, S168-S168.
- Imparato, V., Santos, S.S., Johansen, A., Geisen, S. & Winding, A. 2016. Stimulation of bacteria and protists in rhizosphere of glyphosate-treated barley. *Applied Soil Ecology*, 98, 47-55.
- Jani, A.D., Grossman, J., Smyth, T.J. & Hu, S. 2016. Winter legume cover-crop root decomposition and N release dynamics under disking and roller-crimping termination approaches. *Renewable Agriculture and Food Systems*, 31, 214-229.
- Johal, G. & Huber, D. 2009. Glyphosate effects on diseases of plants. *European Journal of Agronomy*, 31, 144-152.
- Joner, E. & Jakobsen, I. 1995. Growth and extracellular phosphatase activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter. *Soil Biology and Biochemistry*, 27, 1153-1159.
- Kabir, Z. & Koide, R. 2000. The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize. *Agriculture, Ecosystems & Environment*, 78, 167-174.
- Kai, M., Takazumi, K., Adachi, H., Wasaki, J., Shinano, T. & Osaki, M. 2002. Cloning and characterization of four phosphate transporter cDNAs in tobacco. *Plant Science*, 163, 837-846.
- Kaleem Abbasi, M., Mahmood Tahir, M., Sabir, N. & Khurshid, M. 2015. Impact of the addition of different plant residues on nitrogen mineralization-immobilization turnover and carbon content of a soil incubated under laboratory conditions. *Solid Earth*, 6, 197-205.
- Kalembasa, S. & Symanowicz, B. 2012. Enzymatic activity of soil after applying various waste organic materials, ash, and mineral fertilizers. *Polish Journal of Environmental Studies*, 21, 1635-1641.
- Kandel, H., Schneiter, A. & Johnson, B. 1997. Intercropping legumes into sunflower at different growth stages. *Crop Science*, 37, 1532-1537.
- Kandeler, F., Kampichler, C. & Horak, O. 1996. Influence of heavy metals on the functional diversity of soil microbial communities. *Biology and Fertility of Soils*, 23, 299-306.
- Karaca, A., Cetin, S.C., Turgay, O.C. & Kizilkaya, R. 2010. Soil enzymes as indication of soil quality. *Soil enzymology*. Berlin, Heidelberg: Springer.
- Kaspar, T., Radke, J. & Laflen, J. 2001. Small grain cover crops and wheel traffic effects on infiltration, runoff, and erosion. *Journal of Soil and Water Conservation*, 56, 160-164.
- Keene, C., Curran, W., Wallace, J., Ryan, M., Mirsky, S., VanGessel, M. & Barbercheck, M. 2017. Cover crop termination timing is critical in organic rotational no-till systems. *Agronomy Journal*, 109, 272-282.

- Keisling, T., Scott, H., Waddle, B., Williams, W. & Frans, R. 1994. Winter cover crops influence on cotton yield and selected soil properties. *Communications in Soil Science and Plant Analysis*, 25, 3087-3100.
- Kizilkaya, R. & Bayrakli, B. 2005. Effect of N-enriched sewage sludge on soil enzyme activities. *Applied Soil Ecology*, 30, 192-202.
- Kleinman, P.J., Salon, P., Sharpley, A.N. & Saporito, L.S. 2005. Effect of cover crops established at time of corn planting on phosphorus runoff from soils before and after dairy manure application. *Journal of Soil and Water Conservation*, 60, 311-322.
- Konig, C., Kaltvasser, H. & Schiegel, H. 1996. The formation of urease after use of other nitrogen sources in *Hydrogenomonas*. *Archives of Microbiology*, 53, 231-241.
- Kornecki, T., Price, A., Raper, R. & Arriaga, F. 2009. New roller crimper concepts for mechanical termination of cover crops in conservation agriculture. *Renewable Agriculture and Food Systems*, 24, 165-173.
- Kramberger, B., Gselman, A., Kristl, J., Lešnik, M., Šuštar, V., Muršec, M. & Podvršnik, M. 2014. Winter cover crop: the effects of grass-clover mixture proportion and biomass management on maize and the apparent residual N in the soil. *European Journal of Agronomy*, 55, 63-71.
- Krueger, E.S., Ochsner, T.E., Porter, P.M. & Baker, J.M. 2011. Winter rye cover crop management influences on soil water, soil nitrate, and corn development. *Agronomy Journal*, 103, 316-323.
- Kujur, M. & Kumar Patel, A. 2014. Kinetics of soil enzyme activities under different ecosystems: An index of soil quality. *Chilean journal of agricultural research*, 74, 96-104.
- Kuo, S. & Jellum, E.J. 2002. Influence of winter cover crop and residue management on soil nitrogen availability and corn. *Agronomy Journal*, 94, 501-508.
- Lane, M., Lorenz, N., Saxena, J., Ramsier, C. & Dick, R.P. 2012. Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate. *Pedobiologia*, 55, 153-159.
- Lehman, R.M., Cambardella, C.A., Stott, D.E., Acosta-Martinez, V., Manter, D.K., Buyer, J.S., Maul, J.E., Smith, J.L., Collins, H.P. & Halvorson, J.J. 2015. Understanding and enhancing soil biological health: the solution for reversing soil degradation. *Sustainability*, 7, 988-1027.
- Lemanowicz, J. 2011. Phosphatases activity and plant available phosphorus in soil under winter wheat (*Triticum aestivum* L.) fertilized minerally. *Polish Journal of Agronomy*, 4, 12-15.
- Lithourgidis, A., Vasilakoglou, I., Dhima, K., Dordas, C. & Yiakoulaki, M. 2006. Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. *Field Crops Research*, 99, 106-113.
- Liu, A., Ma, B. & Bomke, A. 2005. Effects of cover crops on soil aggregate stability, total organic carbon, and polysaccharides. *Soil Science Society of America Journal*, 69, 2041-2048.
- Liu, Y., Mi, G., Chen, F., Zhang, J. & Zhang, F. 2004. Rhizosphere effect and root growth of two maize (*Zea mays* L.) genotypes with contrasting P efficiency at low P availability. *Plant Science*, 167, 217-223.
- Makoi, J.H., Chimphango, S.B. & Dakora, F.D. 2010. Elevated levels of acid and alkaline phosphatase activity in roots and rhizosphere of cowpea (*Vigna unguiculata* L. Walp.) genotypes grown in mixed culture and at different densities with sorghum (*Sorghum bicolor* L.) *Crop and Pasture Science*, 279-286.
- Makoi, J.H. & Ndakidemi, P.A. 2008. Selected soil enzymes: examples of their potential roles in the ecosystem. *African Journal of Biotechnology*, 7, 181-191.
- Martinez, C. & Tabatabai, M. 1997. Decomposition of biotechnology by-products in soils. *Journal of Environmental Quality*, 26, 625-632.
- Maseko, S. & Dakora, F. 2013. Rhizosphere acid and alkaline phosphatase activity as a marker of P nutrition in nodulated *Cyclopia* and *Aspalathus* species in the Cape fynbos of South Africa. *South African Journal of Botany*, 89, 289-295.

- Matsuoka, M., Mendes, I. & Loureiro, M. 2003. Microbial biomass and enzyme activities in soils under native vegetation and annual and perennial agricultural systems in Primavera do Leste region (MT). *Journal of Soil Science*, 27, 425-433.
- Meyer, A.H., Wooldridge, J. & Dames, J.F. 2015. Variation in urease and β -glucosidase activities with soil depth and root density in a 'Cripp's Pink'/M7 apple orchard under conventional and organic management. *South African Journal of Plant and Soil*, 32, 227-234.
- Meysner, T., Szajdak, L. & Kuś, J. 2006. Impact of the farming systems on the content of biologically active substances and the forms of nitrogen in the soils. *Agronomy Research*, 4, 531-542.
- Miguez, F.E. & Bollero, G.A. 2005. Review of corn yield response under winter cover cropping systems using meta-analytic methods. *Crop Science*, 45, 2318-2329.
- Miralles, I., Ortega, R., Almendros, G., Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M. & Soriano, M. 2012. Modifications of organic matter and enzymatic activities in response to change in soil use in semi-arid mountain ecosystems (southern Spain). *European Journal of Soil Science*, 63, 272-283.
- Mirsky, S., Curran, W., Mortensen, D., Ryany, M. & Shumway, D. 2011. Timing of cover-crop management effects on weed suppression in no-till planted soybean using a roller-crimper. *Weed Science*, 59, 380-389.
- Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R. & Shumway, D.L. 2009. Control of cereal rye with a roller/crimper as influenced by cover crop phenology. *Agronomy Journal*, 101, 1589-1596.
- Mischler, R., Duiker, S.W., Curran, W.S. & Wilson, D. 2010. Hairy vetch management for no-till organic corn production. *Agronomy Journal*, 102, 355-362.
- Mobley, H., Island, M.D. & Hausinger, R.P. 1995. Molecular biology of microbial ureases. *Microbiological Reviews*, 59, 451-480.
- Mohammadi, K. 2011. Soil microbial activity and biomass as influenced by tillage and fertilization in wheat production. *American-Eurasian Journal Agricultural & Environmental Sciences, Deira*, 10, 330-337.
- Monahan, S., Cummings, E., Harwood, H. & Madden, R. 2012. Cover Crop Termination & Reduced Tillage Study. In: Vermont, U.o. (ed.) *Northwest Crops and Soils Program*. Vermont: University of Vermont Libraries.
- Morse, R. No-herbicide, no-till summer broccoli—quantity of rye and hairy vetch mulch on weed suppression and crop yield. Proc. 24th annual southern conservation tillage conference for sustainable agriculture, Oklahoma City, OK, 2001. 85-94.
- Mukumbareza, C., Chiduzza, C. & Muchaonyerwa, P. 2015a. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. *South African Journal of Plant and Soil*, 32, 189-197.
- Mukumbareza, C., Muchaonyerwa, P. & Chiduzza, C. 2015b. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. *South African Journal of Plant and Soil*, 32, 189-197.
- Mukumbareza, C., Muchaonyerwa, P. & Chiduzza, C. 2016. Bicultures of oat (*Avena sativa* L.) and grazing vetch (*Vicia dasycarpa* L.) cover crops increase contents of carbon pools and activities of selected enzymes in a loam soil under warm temperate conditions. *Soil Science and Plant Nutrition*, 62, 447-455.
- Mulidzi, A.R. & Wooldridge, J. 2016. Effect of irrigation with diluted winery wastewater on enzyme activity in four western cape soils. *Sustainability in Environment*, 1, 141.
- Murungu, F.S. 2010. *Evaluation and management of cover crop species and their effects on weed dynamics, soil fertility and maize (Zea mays L.) productivity under irrigation in the Eastern Cape Province, South Africa*. University of Fort Hare.
- Murungu, F.S., Chiduzza, C., Muchaonyerwa, P. & Mkeni, P.N. 2011. Decomposition, Nitrogen, and Phosphorus Mineralization from Residues of Summer-Grown Cover Crops and Suitability for a Smallholder Farming System in South Africa. *Communications in Soil Science and Plant Analysis*, 42, 2461-2472.

- Nannipieri, P., Giagnoni, L., Landi, L. & Renella, G. 2011. Role of phosphatase enzymes in soil. *Phosphorus in action*. Springer.
- Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M.C. & Marinari, S. 2012. Soil enzymology: classical and molecular approaches. *Biology and Fertility of Soils*, 48, 743-762.
- Nannipieri, P. & Gianfreda, L. 1998. Kinetics of enzyme reactions in soil environments. *In: Huang, P., Senesi, N. & Buffle, J. (eds.) Structure and surface reactions of soil particles*. New York: Wiley.
- Ndakidemi, P.A. 2006. Manipulating legume/cereal mixtures to optimize the above and below ground interactions in the traditional African cropping systems. *African Journal of Biotechnology*, 5.
- Neumann, G., Kohls, S., Landsberg, E., Stock-Oliveira Souza, K., Yamada, T. & Romheld, V. 2006. Relevance of glyphosate transfer to non-target plants via the rhizosphere. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz-Sonderheft*, 20, 963.
- Newman, M.M., Hoilett, N., Lorenz, N., Dick, R.P., Liles, M.R., Ramsier, C. & Kloepper, J.W. 2016. Glyphosate effects on soil rhizosphere-associated bacterial communities. *Science of the Total Environment*, 543, 155-160.
- Newman, Y., Wright, D., Mackowiak, C., Scholberg, J. & Cherr, C. 2007. Benefits of cover crops for soil health. *University of Florida, IFAS Extension, SS AGR*, 272.
- Nguyen, D.B., Rose, M.T., Rose, T.J., Morris, S.G. & Van Zwieten, L. 2016. Impact of glyphosate on soil microbial biomass and respiration: a meta-analysis. *Soil Biology and Biochemistry*, 92, 50-57.
- Nielsen, D.C., Lyon, D.J., Hergert, G.W., Higgins, R.K. & Holman, J.D. 2015. Cover crop biomass production and water use in the Central Great Plains. *Agronomy Journal*, 107, 2047-2058.
- Njunie, M., Waggar, M. & Luna-Orea, P. 2004. Residue decomposition and nutrient release dynamics from two tropical forage legumes in a Kenyan environment. *Agronomy Journal*, 96, 1073-1081.
- Nord, E.A., Ryan, M.R., Curran, W.S., Mortensen, D.A. & Mirsky, S.B. 2012. Effects of management type and timing on weed suppression in soybean no-till planted into rolled-crimped cereal rye. *Weed Science*, 60, 624-633.
- O'Reilly, K.A., Lauzon, J.D., Vyn, R.J. & Van Eerd, L.L. 2012. Nitrogen cycling, profit margins and sweet corn yield under fall cover crop systems. *Canadian Journal of Soil Science*, 92, 353-365.
- O'Reilly, K.A., Robinson, D.E., Vyn, R.J. & Van Eerd, L.L. 2011. Weed populations, sweet corn yield, and economics following fall cover crops. *Weed Technology*, 25, 374-384.
- Oladele, S. & Ayodele, O. 2017. Glyphosate, 1, 1'-dimethyl-4, 4'-bipyridinium dichloride and Atrazine induces changes in Soil organic carbon, bacterial and fungal communities in a tropical alfisol. *Eurasian Journal of Soil Science*, 6, 238-248.
- Olson, K., Ebelhar, S.A. & Lang, J.M. 2014. Long-term effects of cover crops on crop yields, soil organic carbon stocks and sequestration. *Open Journal of Soil Science*, 4, 284.
- Pandey, D., Agrawal, M. & Bohra, J.S. 2014. Effects of conventional tillage and no tillage permutations on extracellular soil enzyme activities and microbial biomass under rice cultivation. *Soil and Tillage Research*, 136, 51-60.
- Pandey, R., Sharma, G., Tripathi, S. & Singh, A. 2007. Litterfall, litter decomposition and nutrient dynamics in a subtropical natural oak forest and managed plantation in northeastern India. *Forest Ecology and Management*, 240, 96-104.
- Parr, M., Grossman, J., Reberg-Horton, S., Brinton, C. & Crozier, C. 2011. Nitrogen delivery from legume cover crops in no-till organic corn production. *Agronomy Journal*, 103, 1578-1590.
- Parr, M., Grossman, J.M., Reberg-Horton, S.C., Brinton, C. & Crozier, C. 2014. Roller-crimper termination for legume cover crops in North Carolina: Impacts on nutrient availability to a succeeding corn crop. *Communications in Soil Science and Plant Analysis*, 45, 1106-1119.
- Paul, E.A. 2007. Soil microbiology, ecology, and biochemistry in perspective. *In: Paul, E.A. (ed.) Soil Microbiology, Ecology and Biochemistry*. Third ed. San Diego, CA: Elsevier.

- Piotrowska-Dlugosz, A. & Charzynski, P. 2015. The impact of the soil sealing degree on microbial biomass, enzymatic activity, and physicochemical properties in the Ekranic Technosols of Toruń (Poland). *Journal of Soils and Sediments*, 15, 47-59.
- Piotrowska-Dlugosz, A. & Wilczewski, E. 2014a. Changes in enzyme activities as affected by green-manure catch crops and mineral nitrogen fertilization. *Zemdirbyste (Agriculture)*, 101, 139-146.
- Piotrowska-Dlugosz, A. & Wilczewski, E. 2014b. Soil phosphatase activity and phosphorus content as influenced by catch crops cultivated as green manure. *Polish Journal of Environmental Studies*, 23, 157.
- Piotrowska, A. & Wilczewski, E. 2012. Effects of catch crops cultivated for green manure and mineral nitrogen fertilization on soil enzyme activities and chemical properties. *Geoderma*, 189, 72-80.
- Poeplau, C. & Don, A. 2015. Carbon sequestration in agricultural soils via cultivation of cover crops—A meta-analysis. *Agriculture, Ecosystems & Environment*, 200, 33-41.
- Price, A., Reeves, D., Patterson, M., Gamble, B., Balkcom, K., Arriaga, F. & Monks, C. 2007. Weed control in peanut grown in a high-residue conservation-tillage system. *Peanut Science*, 34, 59-64.
- Quemada, M., Baranski, M., Nobel-de Lange, M., Vallejo, A. & Cooper, J. 2013. Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. *Agriculture, Ecosystems & Environment*, 174, 1-10.
- Ranells, N.N. & Wagger, M.G. 1996. Nitrogen release from grass and legume cover crop monocultures and bicultures. *Agronomy Journal*, 88, 777-882.
- Reeves, D. 1994. Cover crops and rotations. p. 125–172. JL Hatfield and BA Stewart (ed.) Crop residue management. Adv. Soil Sci. Lewis Publ., Boca Raton, FL. *Cover crops and rotations. p. 125–172. In JL Hatfield and BA Stewart (ed.) Crop residue management. Adv. Soil Sci. Lewis Publ., Boca Raton, FL., -*
- Reeves, D.W., Price, A.J. & Patterson, M.G. 2005. Evaluation of three winter cereals for weed control in conservation-tillage nontransgenic cotton. *Weed Technology*, 19, 731-736.
- Riaz, M., Jamil, M. & Mahmood, T.Z. 2007. Yield and yield components of maize as affected by various weed control methods under rain-fed conditions of Pakistan. *International Journal of Agriculture and Biology*, 9, 152-155.
- Rietz, D. & Haynes, R. 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. *Soil Biology and Biochemistry*, 35, 845-854.
- Rojas, M., Van Eerd, L., O'Halloran, I., Sikkema, P. & Robinson, D. 2016. Effect of herbicide residues on fall-seeded cover crops influence soil aggregate stability and mineral N. *Canadian Journal of Plant Science*, 97, 411-423.
- Roldan, A., Caravaca, F., Hernandez, M., Garci, C., Sanchez-Brito, C., Velasquez, M. & Tiscareno, M. 2003. No-tillage, crop residue additions, and legume cover cropping effects on soil quality characteristics under maize in Patzcuaro watershed (Mexico). *Soil and Tillage Research*, 72, 65-73.
- Rosario-Lebron, A., Leslie, A., Chen, G. & Hooks, C. 2018. The Effect of Barley Cover Crop Residue and Herbicide Management on the Foliar Arthropod Community in No-Till Soybeans. *Agronomy*, 8, 87.
- Ruis, S., Blanco-Canqui, H., Jasa, P.J., Ferguson, R. & Slater, G. 2017. Can cover crop use allow increased levels of corn residue removal for biofuel in irrigated and rainfed systems? *BioEnergy Research*, 10, 992-1004.
- Saha, S., Prakash, V., Kundu, S., Kumar, N. & Mina, B.L. 2008. Soil enzymatic activity as affected by long term application of farm yard manure and mineral fertilizer under a rainfed soybean–wheat system in NW Himalaya. *European Journal of Soil Biology*, 44, 309-315.
- Saini, M., Price, A.J., Van Santen, E., Arriaga, F.J., Balkcom, K.S. & Raper, R.L. 2008. Planting and termination dates affect winter cover crop biomass in a conservation-tillage corn-cotton rotation: Implications for weed control and yield. *Tifton, GA, USA. Southern Conservation Agricultural Systems*, 137-141.

- Sainju, U., Singh, B. & Whitehead, W. 2000. Cover crops and nitrogen fertilization effects on soil carbon and nitrogen and tomato yield. *Canadian Journal of Soil Science*, 80, 523-532.
- Sainju, U., Singh, B. & Whitehead, W. 2002. Long-term effects of tillage, cover crops, and nitrogen fertilization on organic carbon and nitrogen concentrations in sandy loam soils in Georgia, USA. *Soil and Tillage Research*, 63, 167-179.
- Sainju, U., Whitehead, W. & Singh, B. 2003. Cover crops and nitrogen fertilization effects on soil aggregation and carbon and nitrogen pools. *Canadian Journal of Soil Science*, 83, 155-165.
- Sainju, U.M., Whitehead, W.F. & Singh, B.P. 2005. Biculture legume–cereal cover crops for enhanced biomass yield and carbon and nitrogen. *Agronomy Journal*, 97, 1403-1412.
- Sardans, J. & Peñuelas, J. 2005. Drought decreases soil enzyme activity in a Mediterranean *Quercus ilex* L. forest. *Soil Biology and Biochemistry*, 37, 455-461.
- Sardans, J., Penuelas, J. & Estiarte, M. 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. *Applied Soil Ecology*, 39, 223-235.
- Sarrantonio, M. & Gallandt, E. 2008. The role of cover crops in North American cropping systems. *Journal of Crop Production*, 8, 37-41.
- Schomberg, H.H., Wietholter, S., Griffin, T.S., Reeves, D.W., Cabrera, M.L., Fisher, D.S., Endale, D.M., Novak, J.M., Balkcom, K.S. & Raper, R.L. 2009. Assessing indices for predicting potential nitrogen mineralization in soils under different management systems. *Soil Science Society of America Journal*, 73, 1575-1586.
- Sebiomo, A., Ogundero, V. & Bankole, S. 2011. Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *African Journal of Biotechnology*, 10, 770-778.
- Seo, J.-h., Lee, H.-j., Hur, I.-b., Kim, S.-j., Kim, C.-k. & Jo, H.-s. 2000. Use of hairy vetch green manure as nitrogen fertilizer for corn production. *Korean Journal of Crop Science*, 45, 294-299.
- Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S. & Gallo, M.E. 2008. Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, 11, 1252-1264.
- Snapp, S., Swinton, S., Labarta, R., Mutch, D., Black, J., Leep, R., Nyiraneza, J. & O'neil, K. 2005. Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agronomy Journal*, 97, 322-332.
- Soriano, M.-A., Álvarez, S., Landa, B.B. & Gómez, J.A. 2014. Soil properties in organic olive orchards following different weed management in a rolling landscape of Andalusia, Spain. *Renewable Agriculture and Food Systems*, 29, 83-91.
- Srivastava, P.K., Gupta, M., Upadhyay, R.K., Sharma, S., Singh, N., Tewari, S.K. & Singh, B. 2012. Effects of combined application of vermicompost and mineral fertilizer on the growth of *Allium cepa* L. and soil fertility. *Journal of Plant Nutrition and Soil Science*, 175, 101-107.
- Staddon, W., Duchesne, L. & Trevors, J. 1998. Acid phosphatase, alkaline phosphatase and arylsulfatase activities in soils from a jack pine (*Pinus banksiana* Lamb.) ecosystem after clear-cutting, prescribed burning, and scarification. *Biology and Fertility of Soils*, 27, 1-4.
- Stavi, I., Lal, R., Jones, S. & Reeder, R. 2012. Implications of cover crops for soil quality and geodiversity in a humid-temperate region in the Midwestern USA. *Land Degradation & Development*, 23, 322-330.
- Steele, M., Coale, F. & Hill, R. 2012. Winter annual cover crop impacts on no-till soil physical properties and organic matter. *Soil Science Society of America Journal*, 76, 2164-2173.
- Stipešević, B. & Kladičko, E.J. 2005. Effects of winter wheat cover crop desiccation times on soil moisture, temperature and early maize growth. *Plant, Soil and Environment*, 51, 255-61.
- Sullivan, D.M. & Andrews, N. 2012. *Estimating plant-available nitrogen release from cover crops*, Oregon State University Extension Service.

- Tabatabai, M. 1994. Soil enzymes. *Methods of Soil Analysis: Part 2-Microbiological and Biochemical Properties*, 775-833.
- Teasdale, J. & Mohler, C. 1993. Light transmittance, soil temperature, and soil moisture under residue of hairy vetch and rye. *Agronomy Journal*, 85, 673-680.
- Teasdale, J.R., Coffman, C.B. & Mangum, R.W. 2007. Potential long-term benefits of no-tillage and organic cropping systems for grain production and soil improvement. *Agronomy Journal*, 99, 1297-1305.
- Thilakarathna, M.S., Serran, S., Lauzon, J., Janovicek, K. & Deen, B. 2015. Management of manure nitrogen using cover crops. *Agronomy Journal*, 107, 1595-10607.
- Thorup-Kristensen, K. 2006. Effect of deep and shallow root systems on the dynamics of soil inorganic N during 3-year crop rotations. *Plant and Soil*, 288, 233-248.
- Thorup-Kristensen, K., Magid, J. & Jensen, L.S. 2003. Catch crops and green manures as biological tools in nitrogen management in temperate zones. *Advances in Agronomy*, 79, 227-302.
- Thorup-Kristensen, K. & Dresbøll, D.B. 2010. Incorporation time of nitrogen catch crops influences the N effect for the succeeding crop. *Soil Use and Management*, 26, 27-35.
- Tiemann, L., Grandy, A., Atkinson, E., Marin-Spiotta, E. & McDaniel, M. 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecology Letters*, 18, 761-771.
- Tosti, G., Benincasa, P., Farneselli, M., Pace, R., Tei, F., Guiducci, M. & Thorup-Kristensen, K. 2012. Green manuring effect of pure and mixed barley-hairy vetch winter cover crops on maize and processing tomato N nutrition. *European Journal of Agronomy*, 43, 136-146.
- Treadwell, D., Creamer, N. & Baldwin, K. 2010. An introduction to cover crop species for organic farming systems. *Cornell University Cooperative Extension* <http://www.extension.org/pages/18542/an-introduction-to-cover-cropspecies-for-organic-farming-systems>.
- Utobo, E. & Tewari, L. 2015. Soil enzymes as bioindicators of soil ecosystem status. *Applied Ecological Environmental Research*, 13, 147-169.
- van Aarle, I.M. & Plassard, C. 2010. Spatial distribution of phosphatase activity associated with ectomycorrhizal plants is related to soil type. *Soil Biology and Biochemistry*, 42, 324-330.
- Varela, M.F., Barraco, M., Gili, A., Taboada, M.A. & Rubio, G. 2017. Biomass decomposition and phosphorus release from residues of cover crops under no-tillage. *Agronomy Journal*, 109, 317-326.
- Vaughan, J.D., Hoyt, G.D. & Wollum, A.G. 2000. Cover crop nitrogen availability to conventional and no-till corn: Soil mineral nitrogen, corn nitrogen status, and corn yield. *Communications in Soil Science and Plant Analysis*, 31, 1017-1041.
- Veena, V., Poornima, P., Parvatham, R., Sivapriyadharsini & Kalaiselvi, K. 2011. Isolation and Characterization of β -glucosidase Producing Bacteria from Different Sources. *African Journal of Biotechnology*, 10, 14907-14912.
- Verdoucq, L., Czjzek, M., Moriniere, J., Bevan, D.R. & Esen, A. 2003. Mutational and Structural Analysis of Aglycone Specificity in Maize and Sorghum β -glucosidases. *Journal of Biological Chemistry*, 278, 25055-25062.
- Villamil, M., Bollero, G., Darmody, R., Simmons, F. & Bullock, D. 2006. No-till corn/soybean systems including winter cover crops. *Soil Science Society of America Journal*, 70, 1936-1944.
- Wagger, M., Cabrera, M. & Ranells, N. 1998. Nitrogen and carbon cycling in relation to cover crop residue quality. *Journal of Soil and Water Conservation*, 53, 214-218.
- Wayman, S., Cogger, C., Benedict, C., Burke, I., Collins, D. & Bary, A. 2015. The influence of cover crop variety, termination timing and termination method on mulch, weed cover and soil nitrate in reduced-tillage organic systems. *Renewable Agriculture and Food Systems*, 30, 450-460.
- Weaver, M.A., Krutz, L.J., Zablotowicz, R.M. & Reddy, K.N. 2007. Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. *Pest Management Science*, 63, 388-393.

- Wilkins, E.D. & Bellinder, R.R. 1996. Mow-kill regulation of winter cereals for spring no-till crop production. *Weed Technology*, 10, 247-252.
- Williams, M.M., Mortensen, D.A. & Doran, J.W. 2000. No-tillage soybean performance in cover crops for weed management in the western Corn Belt. *Journal of Soil and Water Conservation*, 55, 79-84.
- Wortman, S.E., Francis, C.A., Bernards, M.A., Blankenship, E.E. & Lindquist, J.L. 2013. Mechanical termination of diverse cover crop mixtures for improved weed suppression in organic cropping systems. *Weed Science*, 61, 162-170.
- Xiao-Chang, W. & Qin, L. 2006. Beta-Glucosidase activity in paddy soils of the Taihu lake region, China. *Pedosphere*, 16, 118-124.
- Yaduvanshi, N. & Sharma, D. 2008. Tillage and residual organic manures/chemical amendment effects on soil organic matter and yield of wheat under sodic water irrigation. *Soil and Tillage Research*, 98, 11-16.
- YANG, Z.-x., LIU, S.-q., ZHENG, D.-w. & FENG, S.-d. 2006. Effects of cadmium, zinc and lead on soil enzyme activities. *Journal of Environmental Sciences*, 18, 1135-1141.
- Zadoks, J.C., Chang, T.T. & Konzak, C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research*, 14, 415-421.
- Zhang, Y., Chen, L., Wu, Z. & Sun, C. 2011. Kinetic parameters of soil β -glucosidase response to environmental temperature and moisture regimes. *Revista Brasileira de Ci4ncia do Solo*, 35, 1285-1291.

CHAPTER 3

β -GLUCOSIDASE ACTIVITY, SOIL ORGANIC CARBON AND NUTRIENT IN PLANT TISSUE IN RESPONSE TO COVER CROP SPECIES AND MANAGEMENT PRACTICES

3.1 Introduction

There is a growing concern about the degradation of agricultural soils through conventional farming practices. Intensive tillage, short or no fallow, monoculture practices, inadequate biomass input and excessive inorganic fertilizer use, among other practices, result in the decline of soil organic matter and fertility in South African soils (Fourie et al., 2001). This has caused significant decline in agricultural production, biodiversity, and other ecosystem services (Blanco-Canqui et al., 2015). In recent years, responsible farming methods that preserve both nutrients and soil have become very important, hence conservation agriculture involving cover cropping are suddenly popular all over the world (Mukumbareza et al., 2015).

Cover crops proffer several benefits which include enhancing soil physical, chemical and biological properties, improving soil organic matter and nutrient release, suppressing weeds, controlling pest, as well as reducing particulate emission (Dabney et al., 2010). Cover crops may offer a cheap on-site management choice to improve soil organic matter that supplies additional carbon, which serves as energy to sustain the activities of soil microorganisms (Blanco-Canqui et al., 2015). A number of studies have reported the potential of cover crops in increasing SOC concentration in different management and agro-ecosystems (Poeplau and Don, 2015). Cover crop residue inputs, properties and level of disturbance influence SOC accumulation in agricultural soils (Govaerts et al., 2009). Therefore, SOC is one of the most important properties used to examine the effect of cover crops on soil fertility.

It is imperative to adopt the best cover crop management practices that improve organic carbon build-up and enhance the quality and productivity of agricultural soils (Lawson et al., 2013). Cover crop management can be improved through appropriate species selection, seeding rate, planting date, termination date/method and tillage system (Dabney et al., 2010, Blanco-Canqui et al., 2015). Yet, there is limited information on the influence of cover crop species, growth termination stage and termination method on SOC build-up and microbial activity under a consolidated study like this work. Most studies have focused on specific termination stages and the effects on N release. Others examined how termination methods efficiently control cover crop regrowth, but the effects that these methods and stages have on microbial population/structure and nutrient availability are poorly understood.

Increases in soil microbial populations are pointers to enhanced soil properties and general soil ecosystem services. The presence of cover crops and their residues provides cover and food to soil organisms thereby enhancing the soil microbial processes and community structure (Blanco-Canqui et al., 2015). Soil microorganisms have a great effect on soil function through enzyme activity, which catalyses numerous reactions necessary for the decomposition of organic residues, nutrient cycling, regulation of soil organic matter dynamics and soil structure (Nannipieri et al., 2012). There was a positive correlation between enzyme activity and organic carbon accumulation under soils cultivated with cover crops, compared to fallow soils (Nannipieri et al., 2012). Soil enzymes such as β -glucosidase are involved in the last phase of cellulose decomposition pathways that produce glucose, an important C source of energy for microbial activity (Merino et al., 2016). β -glucosidase activity is closely related to soil organic matter, C cycling and other soil ecosystem functions (Nannipieri et al., 2012). The soil enzyme is sensitive to environmental changes and it can provide an initial sign of management alterations long before this can be determined by other routine techniques (Lagomarsino et al., 2009, Nannipieri et al., 2012). This has greatly facilitated the adoption of β -glucosidase for soil quality testing; particularly under short-term crop management systems.

Currently, there is no reference in literature on how cover crop species, termination stage combined with termination method affect enzyme activity and fertility in South African soils. This study attempted to address this knowledge gap. A short-term greenhouse experiment was conducted to study the effect of: (1) termination stage on total C and C:N ratio content of cover crops; (2) living cover crops and residues on SOC and β -glucosidase activity; and (3) termination stage and termination method on SOC and β -glucosidase activity.

3.2 Materials and methods

3.2.1 Experimental description

The study was conducted in the research greenhouse of Agricultural Research Council (ARC) Bien Donne Experimental Farm, Western Cape Province, South Africa (33°50'26"S, 18°58'53"E), from August 2016 to November 2017. The experimental set-up was a randomized block design with 3 replications. Four experiments were conducted which consisted of 2 growth termination stages (vegetative and flowering) and 2 termination methods (slash and spray). Each experiment consisted of five cover crops namely; grazing vetch (*Vicia dasycarpa* Ten.), field pea (*Pisum sativum*), oats (*Avena sativa* L.), rye (*Secale cereal* L.) and a control (no cover crop). The test crops are suitable for cover crop management, and are among the annual legumes and grasses widely grown by farmers and researchers in the Western Cape province of South Africa for this

purpose (Fourie et al., 2001). Sandy clay loam soil was obtained from the surface layer (0 - 30 cm) of the ARC Nietvoorbij Research Farm (33°55'10"S, 18°51'58"E), in Stellenbosch, Western Cape, South Africa. The soil had sandy clay loam texture, with 61% sand, 14% silt and 25% clay, a pH (KCl) of 6.7, 0.9% C (Walkley Black), 11 mg kg⁻¹ P (Bray II), 47 mg kg⁻¹ K (ammonium acetate extraction), 5.31 mg kg⁻¹ NO₃-N and 10.36 mg kg⁻¹ NH₄-N. The soil was passed through a 2 mm mesh to remove large fragments. A total of 120 plastic pots (30 cm) were evenly filled with 10.5 kg of the soil (air - dry weight) and arranged on benches. Mesh-covered drainage holes were provided for the pots. The average minimum and maximum temperature in the greenhouse was 0.5 °C and 34 °C, respectively, and the plants were grown under natural light conditions.

All cover crops were evenly seeded by hand on August 5, 2016. Seeding rates were as follows: pea, 100 kg ha⁻¹; vetch, 90 kg ha⁻¹; oats, 160 kg ha⁻¹; rye, 160 kg ha⁻¹. Emergence was mostly complete by August 18, 2016. Fertilizer was applied at 20 kg N ha⁻¹, 50 kg P ha⁻¹ and 150 kg K ha⁻¹ at planting. Oats and rye received an additional 20 kg N ha⁻¹ at 2 - 4 leaf stage. Control pots had no cover crop and no fertilizer. At planting, pea and vetch were inoculated with *Rhizobium leguminosarium* biovar *viciae*. Each pot was irrigated to field capacity using a drip irrigation system at planting. Irrigation was repeated after each soil had lost weight equivalent to a decrease in soil water content from field capacity to 25% soil water depletion (Dohuky et al., 2011). Weeds were not controlled. Cover crops were terminated at vegetative (September 21 and 28, 2016) and flowering (October 26 and November 2, 2016) growth stages by clipping at the soil surface and spraying glyphosate (*N*-phosphonomethyl glycine) at a rate of 5 L ha⁻¹. The vetch growth stage was determined using the Mischler scale where vegetative and flowering were at stage 1 and 6, respectively (Mischler et al., 2010). The pea growth stage was measured using the Schwartz and Langham scale where vegetative and flowering stages were V4 and R3, respectively (Schwartz et al., 2009). Rye and oats growth stages were determined using the Zadoks scale where vegetative and flowering stages were 45 and 69, respectively (Zadoks et al., 1974). The cover crop residues were left on the soil surface to decompose.

3.2.2 Plant/soil sampling and analysis

Just before the termination of the cover crops, hereafter referred to as kill, aboveground biomass (two legume/four non-legume plant-stands) was cut per treatment replicate with a scissors at the ground level and oven dried at 60°C for 48 hr. Dried plant material was ground through a 1 mm screen using a Polymix Kinematica (PX-MFC 90 D) and stored until chemical analysis. Soil samples were obtained from each pot (0 - 15 cm depths) just prior to cover crop kill and at one year after kill (September 21 and November 2, 2017) following residue decomposition. Soil

samples were sieved (2 mm) and then stored at 4 °C until chemical and enzyme analyses were performed.

The total C concentration in the aboveground plant was determined from each treatment replicate by wet-combustion analysis (Dalal, 1979, Shaw, 2006). Aboveground total N concentration in plant was determined from each treatment replicate of a 0.1 g sample using TruSpec N Nitrogen Analyser (LECO, St. Joseph, MI, USA). Thereafter, the C:N ratio was calculated by using these two values. Soil organic carbon was analysed by complete oxidation of potassium dichromate ($K_2Cr_2O_7$) solution and concentrated sulphuric acid mixture, and subsequent titration of residual $K_2Cr_2O_7$ with ferrous ammonium sulphate (Okalebo et al., 2002). The difference between added and residual $K_2Cr_2O_7$, gives a measure of organic C content of the soil.

3.2.3 Soil enzyme activity assay

The activity of β -glucosidase, known to play a crucial role in C cycling was determined. β -glucosidase (EC 3.2.1.21) activity was analysed by incubating 1.0 g of a moist soil sample with *p*-nitrophenyl- β -D-glucopyranoside (as substrate) solution (pH 6.0) at 37 °C for 60 min (Eivazi and Tabatabai, 1988). The amount of *p*-nitrophenol liberated during enzymatic hydrolysis was determined at 410 nm with a digital UV–Vis spectrophotometer. A control was included per sample in which the substrate was not added. Activity of β -glucosidase was expressed as $\mu\text{g } p\text{-nitrophenol g}^{-1} \text{ soil h}^{-1}$.

3.2.4 Statistical analysis

Levene's test for homogeneity of experimental variances was verified for comparable variances (Levene, 1960). The Shapiro-Wilk test was performed on the standardized residuals from the model to verify normality (Shapiro and Wilk, 1965). Thereafter, the data were subjected to a combined analysis of variance (ANOVA) using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Observations over sampling time (kill and one year) were combined in a split-plot analysis of variance with sampling time as sub-plot factor (Little and Hills, 1972) for soil variables. Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott and Longnecker, 2015). A probability level of 5% was considered significant for all significance tests.

3.3 Results

3.3.1 Effect of termination stage on total C concentration and C:N ratio in the plant tissue of cover crops

Termination stage significantly influenced the total C concentration and C:N ratio in the plant tissue of the cover crops. Total C concentration significantly increased in the tissue of cover crops from vegetative to flowering stage with rye (50.62 - 56.42%) being the highest followed by pea (52.19 - 55.82%), oats (51.92 - 55.23%) and vetch (51.19 - 54.60%) (Table 3.1). Similarly, C:N ratios of all four cover crops varied and significantly increased from vegetative to flowering with the greatest effect for oats (22:1 – 66:1) followed by rye (21:1 – 55:1), pea (16:1 – 45:1) and vetch (13:1 – 25:1) (Table 3.1). The C:N ratios of oats and rye were higher than that of pea and vetch at vegetative and flowering stages.

Table 3.1: Interaction effects of cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) on total C and C:N ratio in the plant tissue

Cover crop	Termination stage	Total C (%)	C:N
Oats	Vegetative	51.92 (± 0.38) de	22.25 (± 0.55) de
Pea		52.19 (± 0.25) d	16.42 (± 0.26) fg
Rye		50.62 (± 0.41) f	20.52 (± 1.37) ef
Vetch		51.19 (± 0.87) ef	13.14 (± 0.39) g
Oats	Flowering	55.23 (± 0.14) bc	66.26 (± 1.81) a
Pea		55.82 (± 0.39) ab	44.67 (± 3.22) c
Rye		56.24 (± 0.17) a	54.88 (± 4.10) b
Vetch		54.60 (± 0.08) c	25.17 (± 0.89) d
LSD		0.92	5.85

Each value represents the mean ($n = 3$), and standard error values are indicated in parenthesis. Different letters within the same column indicate significant differences at $P < 0.05$ according to Fisher's least significant difference test (LSD)

3.3.2 Soil organic carbon

3.3.2.1 Effect of cover crop species and sampling time on soil organic carbon concentrations

The presence of living cover crop species had no significant effect on SOC at kill (Figure 3.1). Soil organic carbon under rye, vetch and control was slightly higher than the oats and pea treatments. At one year, SOC marginally increased with the highest under rye residue followed by oats and pea, while vetch and control remained the same.

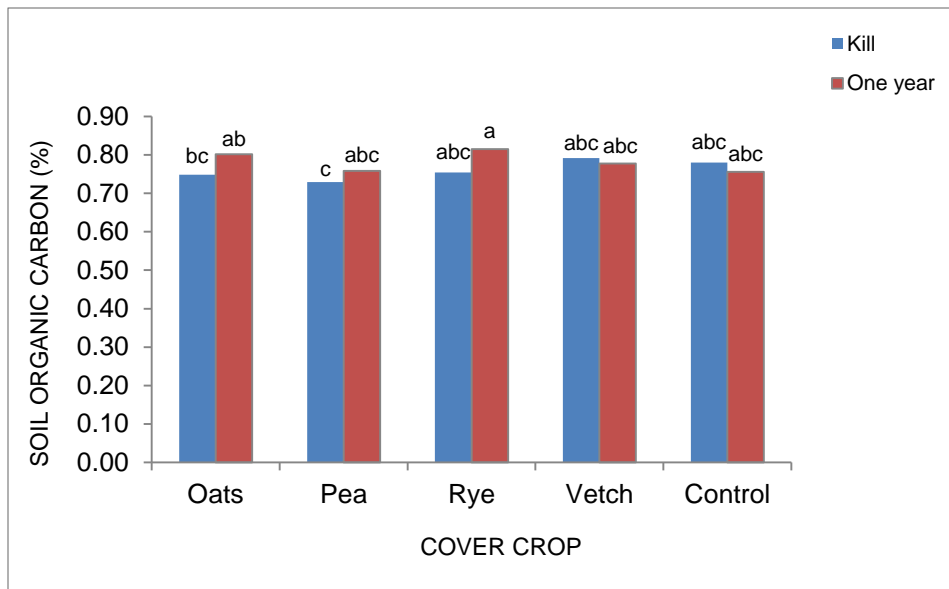


Figure 3.1: Cover crop species and sampling time effects on soil organic carbon. Bars (means - n = 3) with the same letter are not significantly different based on Fisher's LSD ($P < 0.05$)

3.3.2.2 Effect of cover crop species and termination stage on soil organic carbon at different sampling times

At kill, SOC was higher at vegetative stage than flowering stage across all cover crops (Table 3.2). At one year, SOC generally increased in flowering treatments with rye and oats being higher than the control but vegetative treatments decreased irrespective of cover crop species (Table 3.2). Overall, vegetative treatments were about 25% higher than flowering at kill, whereas, flowering treatments were about 45% higher than vegetative treatments at one year, irrespective of cover crop species (Table 3.3).

Table 3.2: Effect of cover crop species and termination stage on soil organic carbon at different sampling times (kill and one year)

Sampling time	Cover crop	Termination stage	Soil organic carbon (%)	
Kill	Oats	Vegetative	0.85 (± 0.08) bcde	
	Pea		0.84 (± 0.06) cde	
	Rye		0.87 (± 0.05) bcd	
	Vetch		0.93 (± 0.02) ab	
	Control		0.80 (± 0.07) de	
	One year	Oats	Flowering	0.64 (± 0.03) f
		Pea		0.62 (± 0.03) f
		Rye		0.64 (± 0.01) f
		Vetch		0.65 (± 0.02) f
		Control		0.76 (± 0.10) e
One year		Oats	Vegetative	0.64 (± 0.03) f
		Pea		0.62 (± 0.03) f
		Rye		0.66 (± 0.02) f
		Vetch		0.64 (± 0.02) f
		Control	0.62 (± 0.02) f	
	Oats	Flowering	0.96 (± 0.01) a	
	Pea		0.90 (± 0.04) abc	
	Rye		0.97 (± 0.02) a	
Vetch	0.92 (± 0.02) abc			
	Control	0.89 (± 0.03) abc		
LSD			0.09	

Each value represents the mean ($n = 3$), and standard error values are indicated in parenthesis. Different letters indicate significant differences at $P < 0.05$ according to Fisher's least significant difference test (LSD)

Table 3.3: Overall impact of termination stage (vegetative and flowering) on soil organic carbon (%) at different sampling times (kill and one year)

Sampling time	Termination stage	Soil organic carbon
Kill	Vegetative	0.86 (± 0.03) b
	Flowering	0.66 (± 0.02) c
One year	Vegetative	0.64 (± 0.01) c
	Flowering	0.93 (± 0.01) a
LSD		0.04

Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

3.3.2.3 Effect of termination method on soil organic carbon

At one year, termination method had a significant effect on SOC with slash treatments (0.80%) being higher than spray treatments (0.77%) when averaged over all cover crop residues (Table 3.4).

Table 3.4: Overall impact of termination method (vegetative and flowering) on soil organic carbon at one year

Termination method	Soil organic carbon (%)
Slash	0.80 (± 0.03) a
Spray	0.77 (± 0.03) b
LSD	0.03

Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

3.3.3 β -glucosidase activity

3.3.3.1 Effect of cover crop species and sampling time on β -glucosidase activity

At kill, soil β -glucosidase activity was significantly influenced by the presence of cover crops (Table 3.5). β -glucosidase activity was significantly higher in all cover crop treatments than the control. At one year, β -glucosidase activity decreased across all cover crop residue treatments with rye, oats and pea being similar and significantly higher than the control.

Table 3.5: Effect of cover crop species and sampling time on β -glucosidase activity ($\mu\text{g PNP g}^{-1}$ soil h^{-1})

Sampling time	Cover crop	β -glucosidase
Kill	Oats	127.01 (± 6.98) a
	Pea	127.53 (± 2.33) a
	Rye	133.49 (± 5.15) a
	Vetch	126.48 (± 6.09) a
	Control	118.03 (± 2.49) b
One year	Oats	76.53 (± 2.63) c
	Pea	74.64 (± 3.53) cd
	Rye	77.58 (± 3.59) c
	Vetch	67.99 (± 3.40) de
	Control	61.06 (± 1.93) e
LSD		7.92

Each value represents the mean ($n = 3$), and standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

3.3.3.2 Effect of cover crop species and termination stage on β -glucosidase activity at different sampling times

At kill, β -glucosidase activity differed significantly with flowering stage being about 17% higher than vegetative stage (Table 3.6). The higher β -glucosidase activity observed at flowering against vegetative stage was irrespective of the cover crop species, with oats and rye showing the highest

activity followed by vetch then pea (Table 3.7). β -glucosidase activity decreased at one year and termination stages showed similar indices among cover crop treatments (Table 3.7). When averaged over all cover crops, β -glucosidase activity was significantly higher in flowering (136.42 $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) compared to vegetative stage (116.60 $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) at kill, whereas no significant difference in β -glucosidase activity was observed between the vegetative (71.32 $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) and flowering (71.80 $\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) stages at one year (Table 3.6).

Table 3.6: Overall impact of termination stage (vegetative and flowering) on β -glucosidase activity ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) at different sampling times (kill and one year)

Sampling time	Termination stage	β -glucosidase
Kill	Vegetative	116.60 (± 1.88) b
	Flowering	136.42 (± 3.19) a
One year	Vegetative	71.32 (± 1.86) c
	Flowering	71.80 (± 2.53) c
LSD		5.01

Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

Table 3.7: Effect of cover crop species and termination stage on β -glucosidase activity ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) at different sampling times (kill and one year)

Sampling time	Cover crop	Termination stage	β -glucosidase	
Kill	Oats	Vegetative	106.66 (± 3.70) e	
	Pea		124.33 (± 3.36) cd	
	Rye		122.25 (± 1.56) cd	
	Vetch		115.33 (± 4.60) de	
	Control		114.42 (± 3.79) de	
	One year	Oats	Flowering	147.37 (± 5.90) a
		Pea		130.74 (± 2.92) bc
		Rye		144.74 (± 7.97) a
		Vetch		137.63 (± 9.61) ab
		Control		121.64 (± 2.78) cd
One year	Oats	Vegetative	76.06 (± 4.01) f	
	Pea		72.18 (± 2.65) fgh	
	Rye		81.65 (± 3.11) f	
	Vetch		64.52 (± 3.09) ghi	
	Control		62.20 (± 2.57) hi	
	One year	Oats	Flowering	77.00 (± 3.78) f
		Pea		77.11 (± 6.74) f
		Rye		73.51 (± 6.36) fg
		Vetch		71.46 (± 6.04) fgh
		Control		59.91 (± 3.05) i
LSD			11.20	

Each value represents the mean ($n = 3$), and standard error values are indicated in parenthesis. Different letters indicate significant differences at $P < 0.05$ according to Fisher's least significant difference test (LSD)

3.3.3.3 Effect of termination method on β -glucosidase activity

At one year, termination methods influenced β -glucosidase activity significantly with slash (76.45 $\mu\text{g PNP g}^{-1}$ soil h^{-1}) showing a more positive effect than spray (66.67 $\mu\text{g PNP g}^{-1}$ soil h^{-1}) when averaged over all cover crops (Table 3.8).

Table 3.8: Overall impact of termination method (slash and spray) on β -glucosidase activity ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) at one year

Termination method	β -glucosidase
Slash	76.45 (± 2.17) a
Spray	66.67 (± 1.88) b
LSD	4.16

Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

3.4 Discussion

This study confirmed that cover crop species, termination stage and termination method are important management approaches since they significantly influenced SOC and β -glucosidase activity in the short-term. A soil management method that involves improving the efficacy of cover cropping is crucial for enhancing soil fertility and agricultural productivity. Soil organic carbon influences soil physical, chemical and biological properties thereby playing a crucial role in soil ecosystem sustainability (Dabney et al., 2010). β -glucosidase is an important enzyme involved in the last phase of cellulose degradation that produce carbon energy for microbial activity (Merino et al., 2016). The sensitivity of β -glucosidase activity to management and consistency in separating short-term treatment effects has facilitated its adoption for soil quality testing among other C-cycle enzymes (Lagomarsino et al., 2009, Adetunji et al., 2017).

3.4.1 Cover crop tissue

As expected, the study revealed that delaying termination till flowering increased total C concentrations and C:N ratios in the plant tissue of cover crop species. The observed increase in cover crop chemical components due to postponing termination is in agreement with previous findings in the literature (Alonso-Ayuso et al., 2014, Lawson et al., 2015). Thus, delay of rye, oats and pea termination till flowering can be used as a management strategy to increase soil C input. Addition of C through cover crops increases soil organic matter, SOC and microbial activity necessary for nutrient cycling, soil fertility and soil structure improvement (Blanco-Canqui et al., 2015). Alonso-Ayuso et al. (2014) indicated that C:N ratio and the fibre content of barley and vetch residues were increased after postponing termination date from boot to inflorescence growth

stage. Similarly, late termination increased C:N ratios of rye, vetch and the mixture of both species in a study by Lawson et al. (2015). However, C:N ratios of rye and vetch was not influenced between early termination (late anthesis and 60% flowering for grain and vetch, respectively) and late termination (early milk and 100% flowering for grain and vetch, respectively) in a study by Wayman et al. (2015). Cover crops with higher total C and C:N ratios are more recalcitrant to decomposition and thus may be suitable to improve C sequestration, organic matter and protect the soil surface from erosion (Dabney et al., 2010). However, the goal of cover cropping should inform the extent of C and C:N ratio manipulation since delay in termination also involves trade-offs among the different benefits provided by cover crops (Lawson et al., 2015). Notable, these results offer evidence that growth termination stage is an important management strategy as it affected total C and C:N ratio concentration of four cover crops.

3.4.2 Soil organic carbon

There are limited reports on the impact of living cover crop on SOC and soil biology (Finney et al., 2017). Most studies have been on the association of cover crop residue with the soil ecosystem, the results which may be influenced by complex interactions between cover crops and other management factors that control microbial communities such as tillage and fertility alteration (Finney et al., 2017). It is important to separately evaluate cover crop-specific effects on SOC and soil microbial activity without other management practices in order to understand the interaction between cover crops and soil enzymes.

In this study, living cover crop species did not have a significant impact on SOC. This may be due to the high background of SOC concentrations which has been indicated to make SOC undetectable in the short-term (Lagomarsino et al., 2009). Although research on relationships between living cover crop and SOC is limited, a previous study has reported that living cover crops contribute to SOC through root exudates released via passive and active mechanisms (Dignac et al., 2017). However, Thorup-Kristensen (2006) showed that living cover crops add substrates for soil microbes via root exudates and root turnover while residue decay increases the organic matter labile fractions.

Residues of rye and oats increased SOC compared to the control after one year. This is in agreement with a study in the vineyard soil of Western Cape, South Africa which indicated that rye, oats and vetch residues increased SOC and soil organic matter irrespective of management methods (Fourie et al., 2007). The increase in SOC observed under rye and oats compared to control could be explained by higher C input from the tissue of cover crops detected in this study and probably the relationship with diverse microbial populations (Mukherjee and Lal, 2015).

Although the carbon concentration of pea biomass was also considerably high in this study, the lower C:N ratio allows for quicker decomposition compared to rye and oats. Previous studies have shown that grasses improve soil organic matter compared to legumes by supplying greater levels of organic carbon (Lithourgidis et al., 2006). Other findings have, however, shown a significant increase in SOC under a range of cover crop which includes vetch, pea, oats, crimson clover, turnip, rye, among others, compared to control (Blanco-Canqui et al., 2015, Mukumbareza et al., 2015). The current study showed that rye and oats residues increased SOC in the short-term (one year), indicating that they can rapidly contribute to the improvement of labile soil organic matter pools and biological activity.

Soil organic carbon was higher under the vegetative than flowering stage at kill in this study. This result is in contrast to a study by Hirpa (2013) which showed overall higher SOC at pod-setting growth stage than vegetative stage under four legume cover crops. On the other hand, at one year of this study, SOC increased under flowering treatments in contrast to vegetative treatments, indicating the amount and maturity effect of cover crop residue at flowering stage. Several studies have shown that delayed incorporation of cover crops led to an increase in biomass, soil organic matter and SOC concentrations (Hirpa, 2013, Ruis et al., 2017). Similarly, Koopmans and Goldstein (2001) reported greater crop residue cover and soil organic matter under delayed termination compared to early termination. It has however, been shown that quality of cover crop residues largely influences SOC availability in addition to maturity and biomass and the yield (Blanco-Canqui et al., 2015). The general lower SOC observed at vegetative growth stage at one year may be attributed to the limited aboveground biomass and rapid microbial degradation or loss of residue materials by decomposition (Hirpa, 2013). Although the current study did not include biomass measurement, we infer that cover crop termination at flowering stage may maximize aboveground biomass and residue cover that forms thick mulch, and increase C and C:N ratio thereby enhancing SOC.

Spraying of cover crops with glyphosate have been shown to negatively influence C mineralization, soluble C and β -glucosidase activity in different cropping systems (García-Orenes et al., 2010). This is in line with results observed in this study at one year. Glyphosate can be absorbed in the soil and may be toxic to soil microbes which consequently affects biochemical processes, organic matter formation and SOC levels (Abbas et al., 2014). Termination by slashing properly places cover crop residue on the soil surface and optimizes decomposition compared to spraying. This improves organic matter levels, maintains lower soil temperature and soil moisture levels thereby reduces soil evaporation; which contributes to SOC build-up, microbial activity and nutrient cycling (Liang et al., 2014). It is suggested that cover crops be slashed or mowed after

spray application for adequate residue cover and decomposition. This study indicated that the cover crop termination method involving slash fostered higher levels of SOC relative to glyphosate use.

3.4.3 β -glucosidase activity

The higher β -glucosidase activity in soils under cover crops than control, observed in this study indicated that living cover crops could increase SOC and labile C pools, which is consistent with observations from other studies (Mukumbareza et al., 2015). The increase in β -glucosidase activity during the cover crop growth period could partly be attributed to the plant root exudates that serve as the main C source for the soil biota in the rhizosphere (Boyrahmadi and Raiesi, 2018). The present study thus addressed to an extent the knowledge gap on the impact of living cover crop stands on β -glucosidase activity. It was observed that the activity of β -glucosidase generally reduced at one year, which is in agreement with studies by Liang et al. (2014) and Weerasekara et al. (2017). This may be due to changes in soil moisture and temperature as well as substrate reduction, which occurs over time (Weerasekara et al., 2017). Nevertheless, treatments with cover crop residues still maintained greater β -glucosidase activity than the control; likely due to SOC accumulation and an increase in organic matter input (Mukumbareza et al., 2015). Furthermore, surface residue retention moderates soil moisture and temperature conditions, which can increase microbial activity (Jat et al., 2013). The increased activity of β -glucosidase due to cover crop presence and residues suggests that management practices, particularly those with rye, oats and pea will enhance organic matter decomposition, carbon storage and nutrient availability in agricultural soils.

The appropriate timing of cover crop termination is a vital management consideration growers should consider to improve soil biological activity and crop productivity (Balkcom et al., 2015). In this study, the higher β -glucosidase activity observed at kill of cover crop may be as a result of accumulated plant root exudates and greater microbial biomass associated with killing cover crop at flowering compared to the vegetative stage (Boyrahmadi and Raiesi, 2018). Residue quality and higher biomass input that improves carbon pools might have contributed to the slightly greater β -glucosidase activity observed at vegetative under rye and oats residues and flowering under pea and oats residues, after one year (Mukumbareza et al., 2015). In addition, vegetative stage marginally favoured β -glucosidase activity the most under rye and oats probably due to the persistence ability of the crop residues on the soil surface, which in turn regulates temperature and improves moisture conservation (Liang et al., 2014). Evaluation of soil enzyme level at cover crop growth termination stage can inform better decisions in fallow periods and crop rotation

systems particularly with regards to timing cash crop cultivation. Previous studies have shown that β -glucosidase activity is linked to tillage, SOC, organic matter, quality of crop residues and litter accrued on the soil surface in areas under zero tillage (de Almeida et al., 2015). In this study, β -glucosidase activity discriminated between cover crop vegetative and flowering growth stages within one year; demonstrating the efficacy of this enzyme in predicting early changes in soil alteration (Lagomarsino et al., 2009, Adetunji et al., 2017).

The current results showed that slash was better than spray operation in stimulating β -glucosidase activity probably due to changes in soil properties related to the termination methods. Slashing and spraying cover crop stands differ in the position where cover crop residues are placed after termination, and the extent to which the methods change soil moisture, temperature and biology (Liang et al., 2014). Contrary to slashing, spraying kept cover crop residue intact, rather than cut into smaller parts to cover the soil surface. Several studies have shown that, cover crop residues left on the soil surface as mulch increased β -glucosidase activity (Mukumbareza et al., 2015) and reduced diurnal soil temperature fluctuations (Sarkar et al., 2007). Furthermore, it enhanced water infiltration, reduced soil evaporation, and consequently conserved soil moisture (Murungu et al., 2011) which in turn elevates β -glucosidase activity (Zhang et al., 2011). In a study reported by Zhang et al. (2007), mulching on dry lands raised soil water storage by up to 8% and decreased soil evaporation by up to 13%. Application of herbicides including glyphosate has been widely reported to have negative impact on soil microbes and β -glucosidase activity in the short and long-term (García-Orenes et al., 2010, Liang et al., 2014). The population of Acidobacteria, which is greatly involved in the biogeochemical processes of cellulose degradation decreased, in response to glyphosate application (Newman et al., 2016). The general lower activity of β -glucosidase in spray compared with the slash treatment in this study, indicates that the long-term accumulation of glyphosate may potentially impair microbial activity and soil nutrient status. A number of studies have, however, reported that the addition of glyphosate stimulated soil microbial activity and diversity in the short and long-term as consequence of its microbial degradation (Mijangos et al., 2009, Arango et al., 2014).

3.5 Conclusion

The results from this study showed that delaying termination till flowering stage increased total C and C:N ratio concentration of all the four cover crops tested. This is an indication that total C and C:N ratios of oats, pea, vetch and rye can be manipulated for specific cover crop management goals. Rye and oats residues are the most promising of the cover crop species with regard to SOC build-up. The living stands of rye, oats, pea and vetch, as well as their respective residues stimulated soil β -glucosidase activity more than the control. Soil organic carbon levels varied with

termination stages with the vegetative stage being higher than the flowering stage at kill, and the flowering stage being higher than the vegetative stage at one year. Flowering contributed more significantly to the stimulation of β -glucosidase activity compared to vegetative. Cover crop termination by slash had a positive impact on SOC and β -glucosidase activity compared to spray. In general, there was high significant interaction between sampling time, cover crop and termination stage, indicating that cover crop and growth termination stage have a greater impact on SOC and β -glucosidase activity relative to termination methods. Nonetheless, longer-term studies are required at field-scale to see whether or not these trends are temporary.

References

- Abbas, Z., Akmal, M. & Khan, K.S. 2014. Effect of buctril super (Bromoxynil) herbicide on soil microbial biomass and bacterial population. *Brazilian Archives of Biology and Technology*, 57, 9-14.
- Adetunji, A.T., Lewu, F.B., Mulidzi, R. & Ncube, B. 2017. The biological activities of β -glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of Soil Science and Plant Nutrition*, 17, 794-807.
- Alonso-Ayuso, M., Gabriel, J.L. & Quemada, M. 2014. The kill date as a management tool for cover cropping success. *PloS one*, 9, e109587.
- Arango, L., Buddrus-Schiemann, K., Opelt, K., Lueders, T., Haesler, F., Schmid, M., Ernst, D. & Hartmann, A. 2014. Effects of glyphosate on the bacterial community associated with roots of transgenic Roundup Ready® soybean. *European Journal of Soil Biology*, 63, 41-48.
- Balkcom, K.S., Duzy, L.M., Kornecki, T.S. & Price, A.J. 2015. Timing of cover crop termination: Management considerations for the Southeast. *Crop, Forage & Turfgrass Management*, 1.
- Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A. & Hergert, G.W. 2015. Cover crops and ecosystem services: Insights from studies in temperate soils. *Agronomy Journal*, 107, 2449-2474.
- Boyrahmadi, M. & Raiesi, F. 2018. Plant roots and species moderate the salinity effect on microbial respiration, biomass, and enzyme activities in a sandy clay soil. *Biology and Fertility of Soils*, 54, 509-521.
- Dabney, S.M., Delgado, J.A., Meisinger, J.J., Schomberg, H.H., Liebig, M.A., Kaspar, T., Mitchell, J. & Reeves, W. 2010. Using cover crops and cropping systems for nitrogen management. In: Delgado, J.A. & Follett, R. (eds.) *Advances in nitrogen management for water quality*. Ankeny, Iowa: Soil water conservation society
- Dalal, R. 1979. Simple procedure for the determination of total carbon and its radioactivity in soils and plant materials. *Analyst*, 104, 151-154.
- de Almeida, R.F., Naves, E.R. & da Mota, R.P. 2015. Soil quality: Enzymatic activity of soil β -glucosidase. *Global Journal of Agricultural Research and Reviews*, 3, 146-150.
- Dignac, M.-F., Derrien, D., Barré, P., Barot, S., Cécillon, L., Chenu, C., Chevallier, T., Freschet, G.T., Garnier, P. & Guenet, B. 2017. Increasing soil carbon storage: mechanisms, effects of agricultural practices and proxies. A review. *Agronomy for Sustainable Development*, 37, 14.
- Dohuky, M., Abdel, C. & Khalid, N. 2011. A Greenhouse Study on Growth, Yield and Anatomical Parameters of Three Pea Cultivars: under Different Irrigation Levels and Growth Regulators. *American Journal of Experimental Agriculture*, 1, 121.
- Eivazi, F. & Tabatabai, M. 1988. Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, 20, 601-606.
- Finney, D., Buyer, J. & Kaye, J. 2017. Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*, 72, 361-373.

- Fourie, J., Agenbag, G. & Louw, P. 2007. Cover crop management in a Chardonnay/99 Richter vineyard in the coastal region, South Africa. 3. Effect of different cover crops and cover crop management practices on organic matter and macro-nutrient content of a medium-textured soil. *South African Journal for Enology and Viticulture*, 28, 61.
- Fourie, J., Louw, P. & Agenbag, G. 2001. Effect of seeding date on the performance of grasses and broadleaf species evaluated for cover crop management in two wine grape regions of South Africa. *South African Journal of Plant and Soil*, 18, 118-127.
- García-Orenes, F., Guerrero, C., Roldán, A., Mataix-Solera, J., Cerdà, A., Campoy, M., Zornoza, R., Bárcenas, G. & Caravaca, F. 2010. Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. *Soil and Tillage Research*, 109, 110-115.
- Govaerts, B., Verhulst, N., Castellanos-Navarrete, A., Sayre, K., Dixon, J. & Dendooven, L. 2009. Conservation agriculture and soil carbon sequestration: between myth and farmer reality. *Critical Reviews in Plant Sciences*, 28, 97-122.
- Hirpa, T. 2013. Effect of stage at termination of legume green manures on soil organic carbon, yield and economic performance of subsequent maize crop. *International Journal of Current Research and Academic Review*, 1, 84-101.
- Jat, M., Gathala, M., Saharawat, Y., Tatarwal, J. & Gupta, R. 2013. Double no-till and permanent raised beds in maize–wheat rotation of north-western Indo-Gangetic plains of India: Effects on crop yields, water productivity, profitability and soil physical properties. *Field Crops Research*, 149, 291-299.
- Koopmans, C. & Goldstein, W. 2001. Soil organic matter budgeting in sustainable farming with applications to southeastern Wisconsin and northern Illinois. *Bulletin*.
- Lagomarsino, A., Moscatelli, M., Di Tizio, A., Mancinelli, R., Grego, S. & Marinari, S. 2009. Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic C in a Mediterranean environment. *Ecological Indicators*, 9, 518-527.
- Lawson, A., Cogger, C., Bary, A. & Fortuna, A.-M. 2015. Influence of seeding ratio, planting date, and termination date on rye-hairy vetch cover crop mixture performance under organic management. *PloS one*, 10, e0129597.
- Lawson, A., Fortuna, A.M., Cogger, C., Bary, A. & Stubbs, T. 2013. Nitrogen contribution of rye-hairy vetch cover crop mixtures to organically grown sweet corn. *Renewable Agriculture and Food Systems*, 28, 59-69.
- Levene, H. 1960. Robust tests for equality of variances, p 278–292. *Contributions to probability and statistics: essays in honor of Harold Hotelling*. Stanford University Press, Palo Alto, CA.
- Liang, S., Grossman, J. & Shi, W. 2014. Soil microbial responses to winter legume cover crop management during organic transition. *European Journal of Soil Biology*, 65, 15-22.
- Lithourgidis, A., Vasilakoglou, I., Dhima, K., Dordas, C. & Yiakoulaki, M. 2006. Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. *Field Crops Research*, 99, 106-113.
- Little, T.M. & Hills, F. 1972. *Statistical Methods in Agricultural Research* UCD Book Store, University of California Davis.
- Merino, C., Godoy, R. & Matus, F. 2016. Soil enzymes and biological activity at different levels of organic matter stability. *Journal of Soil Science and Plant Nutrition*, 16, 14-30.
- Mijangos, I., Becerril, J.M., Albizu, I., Epelde, L. & Garbisu, C. 2009. Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and-independent methodologies. *Soil Biology and Biochemistry*, 41, 505-513.
- Mischler, R., Duiker, S.W., Curran, W.S. & Wilson, D. 2010. Hairy vetch management for no-till organic corn production. *Agronomy Journal*, 102, 355-362.
- Mukherjee, A. & Lal, R. 2015. Short-term effects of cover cropping on the quality of a Typic Argiaquolls in Central Ohio. *Catena*, 131, 125-129.

- Mukumbareza, C., Muchaonyerwa, P. & Chiduzza, C. 2015. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. *South African Journal of Plant and Soil*, 32, 189-197.
- Murungu, F., Chiduzza, C., Muchaonyerwa, P. & Mkeni, P. 2011. Mulch effects on soil moisture and nitrogen, weed growth and irrigated maize productivity in a warm-temperate climate of South Africa. *Soil and Tillage Research*, 112, 58-65.
- Nannipieri, P., Giagnoni, L., Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, M.C. & Marinari, S. 2012. Soil enzymology: classical and molecular approaches. *Biology and Fertility of Soils*, 48, 743-762.
- Newman, M.M., Hoilett, N., Lorenz, N., Dick, R.P., Liles, M.R., Ramsier, C. & Kloepper, J.W. 2016. Glyphosate effects on soil rhizosphere-associated bacterial communities. *Science of the Total Environment*, 543, 155-160.
- Okalebo, J., Gathua, K. & Woomer, P. 2002. Laboratory methods of soil and plant analysis. *A working manual*, 2, 29-68.
- Ott, R.L. & Longnecker, M.T. 2015. *An introduction to statistical methods and data analysis*, Nelson Education.
- Poeplau, C. & Don, A. 2015. Carbon sequestration in agricultural soils via cultivation of cover crops—A meta-analysis. *Agriculture, Ecosystems & Environment*, 200, 33-41.
- Ruis, S., Blanco-Canqui, H., Jasa, P.J., Ferguson, R. & Slater, G. 2017. Can cover crop use allow increased levels of corn residue removal for biofuel in irrigated and rainfed systems? *BioEnergy Research*, 10, 992-1004.
- Sarkar, S., Paramanick, M. & Goswami, S. 2007. Soil temperature, water use and yield of yellow sarson (*Brassica napus* L. var. *glauca*) in relation to tillage intensity and mulch management under rainfed lowland ecosystem in eastern India. *Soil and Tillage Research*, 93, 94-101.
- Schwartz, H.F., Langham, M.A., Golod, J., Tolin, S.A., LaForest, J. & Cardwell, K.F. 2009. Legume ipmPIPE: the next evolution of web-based interactive tools for disease management and extension outreach. *APSnet* (<http://www.apsnet.org/online/feature/ipmPIPE/>).
- Shapiro, S. & Wilk, M. 1965. An analysis of variance test for normality. *Biometrika*, 52, 591-611.
- Shaw, K. 2006. Determination of organic carbon in soil and plant material. *European Journal of Soil Science*, 10, 316-326.
- Thorup-Kristensen, K. 2006. Effect of deep and shallow root systems on the dynamics of soil inorganic N during 3-year crop rotations. *Plant and Soil*, 288, 233-248.
- Wayman, S., Cogger, C., Benedict, C., Burke, I., Collins, D. & Bary, A. 2015. The influence of cover crop variety, termination timing and termination method on mulch, weed cover and soil nitrate in reduced-tillage organic systems. *Renewable Agriculture and Food Systems*, 30, 450-460.
- Weerasekara, C.S., Udawatta, R.P., Gantzer, C.J., Kremer, R.J., Jose, S. & Veum, K.S. 2017. Effects of Cover Crops on Soil Quality: Selected Chemical and Biological Parameters. *Communications in Soil Science and Plant Analysis*, 48, 2074-2082.
- Zadoks, J.C., Chang, T.T. & Konzak, C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research*, 14, 415-421.
- Zhang, S., Lövdahl, L., Grip, H., Jansson, P.-E. & Tong, Y. 2007. Modelling the effects of mulching and fallow cropping on water balance in the Chinese Loess Plateau. *Soil and Tillage Research*, 93, 283-298.
- Zhang, Y., Chen, L., Wu, Z. & Sun, C. 2011. Kinetic parameters of soil β -glucosidase response to environmental temperature and moisture regimes. *Revista Brasileira de Ciência do Solo*, 35, 1285-1291.

CHAPTER 4

THE IMPACT OF COVER CROP SPECIES, TERMINATION STAGE AND TERMINATION METHOD ON SOIL NITROGEN AND ENZYME ACTIVITIES

4.1 Introduction

Soil degradation can be averted by managing and improving soil fertility in a more sustainable way. The integration of cover crops provides a reliable means of increasing organic matter and N supply to the soil (Lawson et al., 2013). This has enabled the wide inclusion of legumes in the cover cropping systems since N released from non-legumes may not synchronize with the N demands of a subsequent crop and thus, do not offer significant N credit (Thilakarathna et al., 2015). Legumes biologically fix N and have been shown to contribute a considerable amount of N into the soil, resulting in increased yield of the cash crop (Thilakarathna et al., 2015). Legume residues are of superior quality with lower C:N ratio and lignocellulose content than non-legumes. These chemical properties have been shown to be the main factor controlling the magnitude and rate of decomposition (Liang et al., 2014, Coombs et al., 2017). Non-legume cover crops on the other hand, not only increase soil C and organic matter (Fourie et al., 2007), but also trap and recycle residual available N that might have leached below the rooting zone (Fortuna et al., 2008). Cover crops improve soil physical properties, chemical properties, biological processes, weed suppression and pest control (Dabney et al., 2010, Blanco-Canqui et al., 2015). Therefore, it is crucial to manage cover crops in order to maximize their benefits for soil quality improvement. The selection of suitable cover crop species, termination stage and termination method may improve soil N availability, microbial activity and soil fertility in agricultural cropping systems (Kornecki et al., 2013, Wayman et al., 2015).

A dynamic microbial community structure and function can be a reliable signal of a healthy and improved soil status (Karaca et al., 2010). Soil biological quality measurements are therefore becoming increasingly important in evaluating soil management practices and the sustainability of land-use in agricultural soils (Mganga et al., 2016). Soil microbes produce enzymes that catalyse numerous biochemical reactions which bring about the decomposition and recycling of nutrients from organic matter, N fixation, nitrification and stabilization of soil structure (Baležentienė, 2012). Soil enzymes such as urease and phosphatase play a crucial role in N and P cycling, respectively (García-Ruiz et al., 2008). They are highly sensitive to environmental factors and soil management changes (García-Ruiz et al., 2008, Adetunji et al., 2017). Additionally, phosphatase activity has been shown to respond to organic and inorganic N inputs under various cropping systems (Lemanowicz, 2011, Maseko and Dakora, 2013). Therefore, the activities of urease and

phosphatase have been considered a more reliable index for the early evaluation of quality changes due to soil management (García-Ruiz et al., 2008, Adetunji et al., 2017).

A number of studies have examined the effect of cover crop termination stage and termination method on biomass production, weed control, soil moisture and soil N mineralization (Lawson et al., 2015, Wayman et al., 2015, Keene et al., 2017). Other studies explored specific growth termination stages and how termination method effectively killed cover crops and prevented regrowth (Mirsky et al., 2009). Yet, the impact of these management approaches on soil microbial processes and enzyme activities is still poorly understood. The evaluation of the management impact on urease and phosphatase activities will aid better decision in choosing appropriate cover crop varieties, termination methods and termination stages that best improve soil N levels, fertility, productivity and sustainability of agricultural soils.

The objectives of this study were to determine the effect of: (1) termination stage on N and C:N ratio content of four cover crops; (2) living cover crops and residues on soil pH, total N and urease and phosphatase activities; and (3) termination stage and termination method on soil N level and urease and phosphatase activities under greenhouse conditions.

4.2 Materials and methods

The experimental description and plant/soil sample collection and analysis are reported in sections 3.2.1 and 3.2.2. The Total N concentration was measured in the soil samples (0.1 g) using a TruSpec N Nitrogen Analyser (LECO, St. Joseph, MI, USA). Soil pH was analysed in a 1:2.5 Soil:KCl mixture (1 M KCl solution) using a glass electrode pH meter.

4.2.1 Soil enzyme activity assay

Urease activity (EC 3.5.1.5) was analysed by 2 hour incubation of a reaction mixture of 5.0 g of field-moist soils and 2.5 mL of 80 mM urea solution at 37°C (Kandeler and Gerber, 1988). Deionized water was added to the controls. The ammonium released was extracted with 50 mL KCl solution and product was measured at 690 nm with a digital UV–Vis spectrophotometer against the reagent blank. Urease activity was expressed as $\mu\text{g ammonium g}^{-1} \text{ soil } 2 \text{ h}^{-1}$. Acid phosphatase (EC 3.1.3.2) was assayed by a colorimetric method of Tabatabai and Bremner (1969) with the exception of using a homogenized reaction of 1.0 mL 25 mM *p*-nitrophenol phosphate (substrate), 4.0 mL Modified Universal Buffer and 0.25 mL toluene, and that the product *p*-nitrophenol was extracted with 4.0 mL of 0.5 M NaOH at pH 6.5. The *p*-nitrophenol released was measured at 410 nm with a digital UV–Vis spectrophotometer and activity expressed as μg

p-nitrophenol g⁻¹ soil h⁻¹. Two replicates and one control from each soil were analysed for the urease and acid phosphatase assays. Enzyme activity was expressed on a moisture-free basis. Soil moisture content was determined from the loss in weight after drying at 105°C for 24 h.

4.2.2 Statistical analysis

The statistical procedures are reported in section 3.2.4.

4.3 Results

4.3.1 Combined statistical analysis

It was observed from ANOVA significant sampling time, cover crop and termination stage interaction effects on total soil nitrogen ($P = 0.0001$) and phosphatase activity ($P = 0.0382$) (Table 4.1). There were significant interaction effects of sampling time and cover crop treatments on urease activity ($P = 0.0002$).

Table 4.1: Analysis of variance (Pr > F) for total soil nitrogen, urease activity and phosphatase activity data in combined sampling times as affected by cover crop species, termination stage, and termination method

Source	DF	Nitrogen	Urease	Phosphatase
Method (M)	1	0.7084	0.5566	0.4364
Stage (S)	1	<.0001	0.4793	<.0001
M x S	1	0.6594	0.338	0.1606
Block (Method*Stage)	8	0.2793	0.1066	0.8503
Cover crop (CC)	4	<.0001	<.0001	0.0552
CC x M	4	0.2097	0.5832	0.0785
CC x S	4	0.0652	0.0107	0.1463
CC x M x S	4	0.4951	0.2035	0.2403
Block (Method*Stage*Cover Crop)	32	-	-	-
Sampling Time (ST)	1	0.1452	<.0001	<.0001
ST x M	1	0.4509	0.6105	0.0203
ST x S	1	<.0001	0.1578	<.0001
ST x M x S	1	0.6381	0.0029	0.9004
ST x CC	4	0.0003	0.0002	0.4262
ST x CC x M	4	0.1857	0.5813	0.5265
ST x CC x S	4	0.0001	0.0645	0.0382
ST x CC x M x S	4	0.7346	0.2615	0.7217
Error	40	-	-	-
Corrected Total	119	-	-	-

Method and Stage refer to termination method and termination stage respectively

4.3.2 Effect of termination stage on total N concentration and C:N ratio in the plant tissue of cover crops

The total N concentration and C:N ratio in the plant tissue of cover crops was significantly affected by termination stage. The total nitrogen concentration significantly decreased from vegetative to flowering stage across all cover crop tissues with vetch (3.9 - 2.2%) being the highest followed by pea (3.2 - 1.3%), rye (2.5 - 1.1%) and oats (2.3 - 0.8%) (Table 4.2). On the contrary, C:N ratio considerably increased from vegetative to flowering stage across all cover crops with the highest concentration observed under oats (22:1 – 66:1) followed by rye (21:1 - 55:1), pea (16:1 – 45:1) and vetch (13:1 – 25:1) (Table 4.2). The C:N ratios of vetch and pea were lower than that of rye and oats at vegetative and flowering stages.

Table 4.2: Interaction effects of cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) at kill on tissue N concentration and C:N ratio

Cover crop	Termination stage	N (%)	C:N
Oats	Vegetative	2.3 d	22.3 de
Pea		3.2 b	16.4 fg
Rye		2.5 c	20.5 ef
Vetch		3.9 a	13.1 g
Oats	Flowering	0.8 h	66.3 a
Pea		1.3 f	44.7 c
Rye		1.1 g	54.9 b
Vetch		2.2 e	25.2 d
LSD		5.9	5.9

Each value represents the mean (n = 3). Different letters within the same column indicate significant differences at P<0.05 according to Fisher's least significant difference test (LSD)

4.3.3 Soil pH as affected by cover crop species and sampling time

At kill, there was no significant difference in soil pH among living pea (7.19), rye (7.17), vetch (7.17) and control (7.17) except oats (7.06) which had significantly lower soil pH (Table 4.3). However, soil pH significantly increased across all treatments at one year with control (7.31) and oats (7.30) being the highest followed by rye (7.27) and the lowest being pea (7.22) and vetch (7.20) compared to the initial measured pH of 6.7 (Table 4.3).

Table 4.3: Cover crop species and sampling time effects on soil pH and nitrogen

Sampling time	Cover crop	Soil pH	Soil nitrogen (%)
Kill	Oats	7.06 (± 0.06) d	0.22 (± 0.01) d
	Pea	7.19 (± 0.03) c	0.25 (± 0.02) bcd
	Rye	7.17 (± 0.03) c	0.27 (± 0.01) abc
	Vetch	7.17 (± 0.09) c	0.27 (± 0.02) abc
	Control	7.17 (± 0.03) c	0.26 (± 0.01) abcd
One year	Oats	7.30 (± 0.02) a	0.22 (± 0.03) d
	Pea	7.22 (± 0.01) bc	0.29 (± 0.02) a
	Rye	7.27 (± 0.02) ab	0.23 (± 0.03) cd
	Vetch	7.20 (± 0.05) bc	0.29 (± 0.01) ab
	Control	7.31 (± 0.02) a	0.17 (± 0.02) e
LSD		0.07	0.04

Each value represents the mean ($n = 3$) and standard error values are indicated in parenthesis. Different letters within a column indicate significant differences at $P < 0.05$ according to Fisher's least significant difference test (LSD)

4.3.4 Total soil nitrogen

4.3.4.1 Total soil nitrogen concentration as affected by cover crop species and sampling time

At kill, there was no significant difference in soil N concentration between living cover crop species and the control soils (Table 4.3). There was a marginal increase in N level in rye (0.27%) and vetch (0.27%) soils than the control (0.26%) soils. At one year, pea and vetch residues significantly increased soil N by about 16% and about 7%, respectively, whereas soil N decreased under rye and control (Table 4.3). Oats soils maintained the same soil N concentration.

4.3.4.2 Total soil nitrogen concentration as affected by cover crop species and termination stage at different sampling times

Soil N concentration at kill was significantly higher at the vegetative stage than flowering under vetch, whereas no significant differences were observed between the termination stages for pea, rye, oats and control soils (Table 4.4). When averaged over all cover crops, termination stage had no significant effect on soil N concentration at kill (Table 4.5). At one year, vegetative stage had greater soil N concentration than flowering for all the cover crop residues except vetch which was similar at both termination stages (Table 4.4). Overall, there were significant interaction effects of sampling time, cover crop and termination stage on soil N concentration (Table 4.1; $P = 0.0001$).

Table 4.4: Cover crop species and termination stage effects on soil nitrogen at different sampling times (kill and one year)

Sampling time	Cover crop	Termination stage	Soil nitrogen (%)	
Kill	Oats	Vegetative	0.21 (± 0.02) g	
	Pea		0.26 (± 0.03) defg	
	Rye		0.27 (± 0.02) bcdef	
	Vetch		0.30 (± 0.02) abcd	
	Control		0.26 (± 0.01) cdefg	
	One year	Oats	Flowering	0.23 (± 0.01) fg
		Pea		0.24 (± 0.03) efg
		Rye		0.27 (± 0.02) bcdefg
		Vetch		0.24 (± 0.02) efg
		Control		0.25 (± 0.01) defg
One year		Oats	Vegetative	0.31 (± 0.01) abc
		Pea		0.32 (± 0.04) ab
		Rye		0.33 (± 0.00) a
		Vetch		0.28 (± 0.01) abcde
		Control		0.24 (± 0.01) efg
	One year	Oats	Flowering	0.13 (± 0.02) h
		Pea		0.25 (± 0.02) defg
		Rye		0.13 (± 0.01) h
		Vetch		0.29 (± 0.01) abcde
		Control		0.10 (± 0.02) h
LSD			0.06	

Each value represents the mean (n = 3) and standard error values are indicated in parenthesis. Different letters within a column indicate significant differences at $P < 0.05$ according to Fisher's least significant difference test (LSD)

Table 4.5: The overall effect of termination stage (vegetative and flowering) on total soil nitrogen at different sampling times (kill and one year)

Sampling time	Termination stage	Soil nitrogen (%)
Kill	Vegetative	0.26 (± 0.01) b
	Flowering	0.24 (± 0.01) b
One year	Vegetative	0.30 (± 0.01) a
	Flowering	0.18 (± 0.02) c
LSD		0.03

Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

4.3.4.3 Total soil nitrogen concentration as affected by termination method

At one year, slash and spray treatments had no significant effect on soil N levels when averaged over all cover crop residues (Table 4.6).

Table 4.6: The overall effect of termination method (slash and spray) on total soil nitrogen at one year

Termination method	Soil nitrogen (%)
Slash	0.23 (± 0.02) a
Spray	0.24 (± 0.02) a
LSD	0.02

Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

4.3.5 Soil enzyme activity

4.3.5.1 Soil urease and phosphatase activities as affected by cover crop species and sampling time

At kill, urease activity was significantly higher in living cover crop soils than in control with rye ($48.71 \mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) and oats ($43.75 \mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) showing greater activity than vetch ($34.70 \mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) and pea ($34.60 \mu\text{g NH}_4^+ \text{g}^{-1} \text{soil } 2 \text{ h}^{-1}$) (Table 4.7). There was also significantly higher phosphatase activity in living pea, rye and vetch soils than the control soils, at kill (Table 4.7). Phosphatase activity in living oats soil was marginally higher than the control soil.

Urease activity decreased over time with no significant differences between cover crop residue soils and the control, at one year (Table 4.7). The phosphatase activity also decreased across all cover crop residue soils, with oats ($80.04 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) and pea residues ($79.90 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) being the highest and significantly greater than the control ($61.84 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) (Table 4.7). However, there were no significant differences in phosphatase activity under vetch ($66.37 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$), rye ($67.10 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) and the control ($61.84 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) soils (Table 4.7).

Table 4.7: The activities of urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil 2 h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) as affected by cover crop species and sampling time

Sampling time	Cover crop	Urease	Phosphatase
Kill	Oats	43.75 (± 2.60) a	104.21 (± 13.23) ab
	Pea	34.60 (± 1.56) b	108.27 (± 19.37) a
	Rye	48.71 (± 4.29) a	106.68 (± 13.33) a
	Vetch	34.70 (± 3.18) b	106.21 (± 15.40) a
	Control	25.93 (± 1.77) c	91.75 (± 15.93) bc
One year	Oats	10.36 (± 1.35) d	80.04 (± 9.90) cd
	Pea	9.73 (± 1.33) d	79.90 (± 6.23) cd
	Rye	11.25 (± 1.38) d	67.10 (± 3.08) de
	Vetch	9.55 (± 0.80) d	66.37 (± 4.65) de
	Control	5.87 (± 1.07) d	61.84 (± 4.22) e
LSD		5.42	14.29

Sampling time is kill and one year. Each value represents the mean ($n = 3$) and standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

4.3.5.2 Soil urease and phosphatase activities as affected by cover crop species and termination stage

At kill, the flowering treatment showed significantly higher urease activity than vegetative treatment under rye and oats while no significant differences were observed in termination stage among the other cover crops and the control (Table 4.8). Also, the activity of phosphatase was higher at the vegetative stage than the flowering stage irrespective of cover crop type (Table 4.8). However, there was no significant difference in urease activity between the vegetative and flowering stages across all cover crop residues at one year (Table 4.8).

Generally, the activity of urease was not significantly affected by termination stages at both sampling times (Table 4.9 and Table 4.1; $P = 0.0645$). Greater phosphatase activity was observed at the vegetative compared to the flowering stage regardless of the cover crop residue type (Table 4.8), at one year. Overall, the termination stage greatly affected phosphatase activity regardless of the sampling time, with the vegetative stage being about 168% and about 29% greater than flowering, at kill and one year, respectively (Table 4.9 and Table 4.1; $P = 0.0382$).

Table 4.8: Cover crop species and termination stage effects on urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil 2 h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) activities at different sampling times (kill and one year)

Sampling time	Cover crop	Termination stage	Urease	Phosphatase
Kill	Oats	Vegetative	38.28 (± 1.42) cd	146.62 (± 6.09) b
	Pea		36.13 (± 1.28) cde	167.78 (± 13.58) a
	Rye		41.67 (± 1.85) bc	144.70 (± 8.75) b
	Vetch		35.48 (± 5.80) cde	155.56 (± 5.88) ab
	Control		29.63 (± 2.25) ef	138.77 (± 12.21) b
	Oats	Flowering	49.22 (± 3.97) ab	61.79 (± 3.61) efg
	Pea		33.08 (± 2.85) de	48.75 (± 7.08) fg
	Rye		55.75 (± 7.60) a	68.66 (± 11.28) ef
	Vetch		33.92 (± 3.27) de	56.86 (± 5.85) efg
Control	22.24 (± 1.78) f	44.72 (± 9.08) g		
One year	Oats	Vegetative	10.42 (± 2.05) gh	101.88 (± 12.71) c
	Pea		9.68 (± 2.11) gh	90.64 (± 8.30) cd
	Rye		11.18 (± 2.56) g	73.01 (± 3.05) de
	Vetch		9.15 (± 0.95) gh	74.37 (± 5.96) de
	Control		8.45 (± 1.21) gh	61.10 (± 5.73) efg
	Oats	Flowering	10.29 (± 1.94) gh	58.20 (± 3.32) efg
	Pea		9.78 (± 1.82) gh	69.17 (± 7.46) e
	Rye		11.32 (± 1.36) g	61.19 (± 4.30) efg
	Vetch		9.95 (± 1.36) gh	58.38 (± 5.85) efg
	Control		3.30 (± 0.96) h	62.58 (± 6.72) efg
LSD		7.67	20.21	

Each value represents the mean ($n = 3$) and standard error values are indicated in parenthesis. Different letters within a column indicate significant differences at $P < 0.05$ according to Fisher's least significant difference test (LSD)

Table 4.9: The overall effect of termination stage (vegetative and flowering) on the activities of soil urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil 2 h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) at different sampling times (kill and one year)

Sampling time	Termination stage	Urease	Phosphatase
Kill	Vegetative	36.24 (± 1.45) a	150.69 (± 4.49) a
	Flowering	38.84 (± 2.88) a	56.16 (± 3.63) c
One year	Vegetative	9.78 (± 0.80) b	80.20 (± 4.21) b
	Flowering	8.93 (± 0.83) b	61.90 (± 2.49) c
LSD		3.43	9.04

Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

4.3.5.3 Soil urease and phosphatase activities as affected by termination method

At one year, termination methods did not have a significant effect on the activities of urease and phosphatase when averaged across all cover crops (Table 4.10).

Table 4.10: The overall effect of termination method (slash and spray) on the activities of urease ($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil 2 h}^{-1}$) and phosphatase ($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) at one year

Termination method	Urease	Phosphatase
Slash	8.68 (± 0.79) a	73.40 (± 3.97) a
Spray	10.02 (± 0.82) a	68.70 (± 3.69) a
LSD	1.60	8.41

Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

4.4 Discussion

4.4.1 Cover crop tissue

Results from this study revealed that termination stage considerably affected cover crop chemical composition. This is consistent with other studies which indicated that late termination increased cover crop C:N ratio while tissue N concentration decreased from early termination to late termination (Alonso-Ayuso et al., 2014, Lawson et al., 2015, Coombs et al., 2017). Results showed that termination of vetch, pea, rye and oats at the vegetative stage can serve as a management approach to optimize biomass N accumulation and quality, as well as soil N input. According to Whittinghill et al. (2012), cover crop C:N ratio and lignocellulose index are biochemical indicators that may serve as quantitative parameters for modelling soil and microbial activities. They may also serve as qualitative variables when examining residue effects on soil microbial properties and processes (Liang et al., 2014). For example, residues with low C:N ratios (less than 35) are generally of better quality than residues with high C:N ratios (greater than 35) and they have, therefore, been used as pointer to rapid and optimum microbial decomposition and N mineralization (Coombs et al., 2017). Thus, at vegetative stage, the termination of pea, vetch and most importantly oats and rye, will enhance soil microbial and enzymatic activity resulting in rapid decomposition, N cycling and nutrient release for the subsequent cash crop. Alonso-Ayuso et al. (2014) reported that postponement of termination date led to cover crop biomass increase and more residues with higher fibre content and C:N ratio that, however do not easily decay and, thus, are more suitable to protect the soil and enhance the slow release of the N pool. The choice of cover crop termination stage can be targeted towards reducing N immobilization, improving N input and microbial processes.

4.4.2 Soil pH

Living cover crops and their residues do not appear to have significant impact on soil pH as the sampling time did; as shown by this study. The observed increase in soil pH across all cover crop

residue treatments including control at one year is in agreement with a study by Balota and Chaves (2011), which showed an increase in soil pH from about 4 to 6 after 10 years under seven summer legumes (*L. leucocephala*, *C. spectabilis*, *C. brevipflora*, *M. pruriens*, *M. deeringiana*, *A. hypogaea*, *V. unguiculata* and control). However, Mukherjee and Lal (2015) indicated that in one season turnip and pea residues significantly decreased soil pH from about 6.7 to 5.7 compared to the control. Similarly, hairy vetch and crimson clover decreased soil pH by 11% and it was attributed to greater exchangeable ions (Al and Mn) under cover crops compared to those under fallow (McVay et al., 1989). In the present study, despite the general increase in soil pH, pea and vetch were the least, and this is consistent with previous studies that showed lower soil pH under legumes compared to non-legumes (Nuruzzaman et al., 2006, Li et al., 2007, Maltais-Landry, 2015). The observed lower soil pH under legumes has been indicated to probably be a result of the effect of soil N fixation (Nuruzzaman et al., 2006, Maltais-Landry, 2015). Nevertheless, we suggest future sampling and more research to better understand the impact of living cover crops and their residues on pH since soil acidity or alkalinity affects organic acids and enzymatic activity to some extent (Maltais-Landry, 2015).

4.4.3 Total soil nitrogen

There is not much information documenting the impact of living cover crop stands on soil nutrient availability, microbial processes and soil enzyme activities (Qian et al., 2015, Finney et al., 2017). Most research has been on examining cover crop residues in relation to soil ecosystem and fertility improvement, the outcome which is usually controlled by complex interactions between cover crops and other management factors (Wortman et al., 2013, Lienhard et al., 2014).

Findings from this study indicate that living cover crop stands of vetch and rye slightly increased soil N concentrations. Other authors have reported similar superior total soil N indices under clover and crown vetch (XU et al., 2013, Qian et al., 2015). Living cover crops may affect soil nutrient levels and availability by enhancing biological activity thereby improving mineralization and nutrient levels (Yao et al., 2005), root exudation, which provide substrates and labile carbon leading to optimum nutrient release (Hoagland et al., 2008), and sequestering of nutrients thereby reducing availability (Qian et al., 2015). The observed slight increase in soil N level under living vetch may be as a result of biological N fixation processes that add extra N and may also stimulate soil enzyme activity in legume roots (Steenwerth and Belina, 2008, Maseko and Dakora, 2013). The marginally higher N concentrations under living rye soils may be explained by root exudates effects which provide substrate and boost soil soluble compounds for soil microbial activity and nutrient release (Thorup-Kristensen, 2006, Mukumbareza et al., 2015). However, Qian et al.

(2015) reported that soil mineral N was lower in non-legume living ryegrass compared to no cover crop treatments in apple orchard soils. This may be due to scavenging ability and N use competition between ryegrass and the apple trees (Qian et al., 2015). At one year, residues of pea and vetch increased soil N level compared to the control and rye, which is consistent with reports from previous studies (Balkcom et al., 2015, Blanco-Canqui et al., 2015, Coombs et al., 2017). The increased soil N concentration observed under pea and vetch soils in contrast to the other treatments could be due to higher tissue N input detected in this study and the biological N fixation associated with the legume crops. Furthermore, lower C:N ratios of pea and vetch residues compared to rye and oats might have contributed to enhanced microbial decomposition and enzyme activity that resulted in optimum N cycling and mineralization. This is consistent with the findings of Balkcom et al. (2015) and Coombs et al. (2017). Several studies have shown that legumes, including alfalfa, vetch, clover, sunhemp, soybean, pea considerably increased soil N compared to grasses and no cover crop under different management methods (Blanco-Canqui et al., 2011, Jani et al., 2016). Nonetheless, there are reports of little or no N increase in vetch treated soils (Benincasa et al., 2010, Alonso-Ayuso et al., 2014). Non-legumes including rye and oats do not fix N but rather sequester and scavenge N (Balkcom et al., 2015, Thilakarathna et al., 2015) and this explains why no increase in soil N concentration was observed under their respective soils in this study at one year. It was also observed that pea and vetch residues markedly enhanced soil N level in the short-term (one year). This showed that pea and vetch can rapidly improve soil fertility, reduce N fertilizer requirement for subsequent cash crop and mitigate potential field loss.

Generally, legumes add more nitrogen to the soil compared to grasses, while early termination is associated with quality residues, rapid decomposition and optimum N release (Alonso-Ayuso et al., 2014, Lawson et al., 2015). The observed significantly higher soil N level at vegetative stage relative to flowering under vetch, at kill, is in agreement with other studies (Alonso-Ayuso et al., 2014, Coombs et al., 2017, Boyrahmadi and Raiesi, 2018). Other authors found that postponing vetch termination time for eight days (Cline and Silvernail, 2001), fourteen days (Clark et al., 1995) or till flowering (Drinkwater et al., 2000) augmented vetch biomass rather than increasing N contribution from N fixation. As stated by Sullivan and Andrews (2012), plant available N of legumes rises as growth advances, but peaks at bud growth stage and gradually decreases as the flowering growth stage continues. The current results showed that termination at vegetative stage compared to flowering not only significantly increased soil N under pea soils, but also considerably elevated N levels under non-legume residues of rye and oats soils at one year. Termination at vegetative stage maintains lower C:N ratio and reduces N uptake by cover crops especially grasses and allows more time for N release from cover crop residues (Alonso-Ayuso et

al., 2014). In a study reported by Thorup-Kristensen and Dresbøll (2010), it was noted that there was a reduction in net N mineralization, and availability of nutrient to the following cash crop when rye termination was delayed. This further supports the current findings that cover crop termination stage is an important soil management strategy for manipulating C:N ratio for reduced N immobilization, optimum decomposition and N cycling, as well as increased soil N availability for the succeeding cash crop.

Generally, termination method had no impact on soil N concentration when averaged over all the tested cover crops. Termination methods are driven towards the same goal, but the main difference between slashing, disking, roller-crimping and spraying a cover crop stand is the point of placement of residues after termination and the extent of alteration on soil ecological properties (Liang et al., 2014). Previous research found that disking of cover crops led to rapid shoot decay and higher mineral N availability in contrast to herbicide use (Jani et al., 2016). It has also been reported that mowing of the rye and vetch mixture (Snapp et al., 2005) and roller crimping of vetch (Parr et al., 2011) resulted in enhanced N mineralization and increased soil N levels. Haney et al. (2002) reported that cover crop termination with glyphosate was also indicated to enhance N mineralization under different soil types.

4.4.4 Soil enzymes

In agricultural soils, urease and phosphatase play crucial roles in N and P cycling respectively. Their activities are sensitive to environmental factors and rapidly respond to soil management changes, making them a more reliable biological soil quality indicator (Zhang et al., 2015, Adetunji et al., 2017). However, there are still limited studies on the use of soil enzyme assays to monitor soil management changes in cover cropping systems.

At kill, the observed higher urease and phosphatase activities under living cover crops relative to control may be as a result of the stimulant effect of plant root activity (Boyrahmadi and Raiesi, 2018). Cover crops produce enzymes such as phosphatase and urease from living roots (Follmer, 2008), and root exudates from the plants serve as C substrate and energy source for microbial activity (Sanaullah et al., 2011). Furthermore, living cover crops enhance soil physical and chemical properties which may result in higher microbial population and activity that improves enzyme activity (Diouf et al., 2010). Previous studies have reported significant increase in urease activity under white clover, crown vetch and alfalfa living mulches compared to perennial ryegrass in vineyard soils (Xi et al., 2011, Qian et al., 2015). Adamavičienė et al. (2012) similarly reported that only living legume mulches increased urease activity in a cornfield, indicating that higher urease activity can be traced back to the N fixing ability of legumes (Qian et al., 2015). A number

of studies have reported that legumes namely *Aspalathus*, cowpea, chickpea, and *Cyclopia* release more phosphatase enzymes than non-legumes (Liu et al., 2004, Makoi et al., 2010, Maseko and Dakora, 2013). This is because legumes make use of more P in the symbiotic N fixation process than grasses (Makoi and Ndakidemi, 2008). Phosphatase activity has been linked to organic matter and N availability through inorganic and organic N amendments in various studies (Lemanowicz, 2011, Kalembasa and Symanowicz, 2012). Consistent with other studies (Liang et al., 2014, Weerasekara et al., 2017), urease and phosphatase activities decreased over time (at one year); and this may be as a result of a reduction by decomposition of cover crop residue materials and substrates (Weerasekara et al., 2017). However, while phosphatase maintained higher activity under cover crop residues than the control, urease activity did not respond to cover crop residues at one year. The observed higher phosphatase activity under cover crop residue soils is consistent with previous findings which indicated that cover crop residues do not only add labile C, organic matter and substrate that stimulate phosphatase synthesis, but also moderate soil moisture and temperature conditions that enhance microbial processes (Balota and Chaves, 2010, Jat et al., 2013).

The cover crop termination stage is a crucial soil management technique that can affect N availability for subsequent cash crops and fallow land (Balkcom et al., 2015). Despite the importance, there is limited or no report of the effect of cover crop termination timing on urease and phosphatase activities in South African soils. In the present study, the higher urease activity observed under rye and oats at flowering relative to vegetative stage may be a result of higher organic matter and over synthesis of this enzyme by microbes due to delay in termination. Nevertheless, when averaged over all the cover crops, termination stage generally had no significant impact on urease activity across the two sampling times (Kill and one year). However, there was significant interaction of cover crops, termination stage and sampling time effects on phosphatase activity, with vegetative stage showing a positive effect in contrast to flowering. This is an indication that phosphatase activity responds more to cover crop residue and substrate quality than quantity. Termination at vegetative stage favours lower C:N ratio which enhances microbial decomposition and N mineralization in agricultural soils (Alonso-Ayuso et al., 2014). The present results, therefore, suggest that cover crops should be terminated at vegetative stage for improved soil phosphatase activity, microbial decomposition, N and P cycling.

It was also observed that spraying slightly favoured urease activity more than slash, while it was the other way around for phosphatase activity. There was a decrease in microbial biomass C, urease and phosphatase activities under oats soils terminated with glyphosate in a study by García-Orenes et al. (2010). It has been reported that herbicides such as glyphosate could

accumulate in the soil and be toxic to soil microbes, consequently affecting biogeochemical reactions, nutrient cycling and soil fertility (Abbas et al., 2014). Furthermore, glyphosate application adversely affected soil bacterial diversity and rhizobacterial community structure under maize (Barriuso and Mellado, 2012) and grass (Druille et al., 2016). In contrast to spraying, cover crop termination by slashing promoted shoot-soil contact as well as surface mulch cover, which enhanced soil moisture, temperature regulation and biological decomposition (Liang et al., 2014). However, there have been conflicting reports of the impact of glyphosate use on soil ecology with other findings indicating no substantial effect on diversity and activity of soil beneficial microbes (Weaver et al., 2007, Lane et al., 2012). According to Haney et al. (2002), glyphosate application stimulated microbial processes and inorganic N release under different soils. The present study indicated that termination methods did not have a significant impact on urease and phosphatase activity when averaged over all the cover crops. Therefore, there is a need for further research on the impact of cover crop termination methods on soil enzyme activities and N availability under different soils in the short and long-term.

4.5 Conclusion

This study indicates that early cover crop termination at the vegetative stage maintains higher tissue N and lower C:N ratio concentration, irrespective of cover crop species. Cover crop presence had no effect on soil pH but sampling time did. Pea and vetch followed by rye are better cover crop options to farmers in increasing soil N levels. Living cover crops stimulated urease and phosphatase activities, whereas their dead residues considerably increased only phosphatase activity at one year. Termination methods had no significant effect on soil N levels and the activities of urease and phosphatase, at one year. Termination at vegetative stage raised soil N levels and phosphatase activity irrespective of cover crop species and sampling time. This study, therefore, suggests that cover crop chemical composition/quality and termination stage should be considered when selecting management methods that improve soil N level and microbial activity. Further research with longer time spans should be done at field level including examining the N input of the cover crops to the subsequent cash crop.

References

- Abbas, Z., Akmal, M. & Khan, K.S. 2014. Effect of buctril super (Bromoxynil) herbicide on soil microbial biomass and bacterial population. *Brazilian Archives of Biology and Technology*, 57, 9-14.
- Adamavičienė, A., Romaneckas, K., Pilipavičius, V., Avižienytė, D., Šarauskis, E. & Sakalauskas, A. 2012. Interaction of maize and living mulch: Soil chemical properties and bioactivity. *Journal of Food, Agriculture and Environment*, 10, 1219-1223.

- Adetunji, A.T., Lewu, F.B., Mulidzi, R. & Ncube, B. 2017. The biological activities of β -glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of Soil Science and Plant Nutrition*, 17, 794-807.
- Alonso-Ayuso, M., Gabriel, J.L. & Quemada, M. 2014. The kill date as a management tool for cover cropping success. *PloS one*, 9, e109587.
- Baležentienė, L. 2012. Hydrolases related to C and N cycles and soil fertility amendment: Responses to different management styles of agro-ecosystems. *Polish Journal of Environmental Studies*, 21, 1153-1159.
- Balkcom, K.S., Duzy, L.M., Kornecki, T.S. & Price, A.J. 2015. Timing of cover crop termination: Management considerations for the Southeast. *Crop, Forage & Turfgrass Management*, 1.
- Balota, E.L. & Chaves, J.C.D. 2010. Enzymatic activity and mineralization of carbon and nitrogen in soil cultivated with coffee and green manures. *Revista Brasileira de Ciência do Solo*, 34, 1573-1583.
- Balota, E.L. & Chaves, J.C.D. 2011. Microbial activity in soil cultivated with different summer legumes in coffee crop. *Brazilian Archives of Biology and Technology*, 54, 35-44.
- Barriuso, J. & Mellado, R.P. 2012. Relative effect of glyphosate on glyphosate-tolerant maize rhizobacterial communities is not altered by soil properties. *Journal of Microbiology and Biotechnology*, 22, 159-165.
- Benincasa, P., Tosti, G., Tei, F. & Guiducci, M. 2010. Actual N availability from winter catch crops used for green manuring in maize cultivation. *Journal of Sustainable Agriculture*, 34, 705-723.
- Blanco-Canqui, H., Mikha, M.M., Presley, D.R. & Claassen, M.M. 2011. Addition of cover crops enhances no-till potential for improving soil physical properties. *Soil Science Society of America Journal*, 75, 1471-1482.
- Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A. & Hergert, G.W. 2015. Cover crops and ecosystem services: Insights from studies in temperate soils. *Agronomy Journal*, 107, 2449-2474.
- Boyrahmadi, M. & Raiesi, F. 2018. Plant roots and species moderate the salinity effect on microbial respiration, biomass, and enzyme activities in a sandy clay soil. *Biology and Fertility of Soils*, 54, 509-521.
- Clark, A.J., Decker, A.M., Meisinger, J.J., Mulford, F.R. & McIntosh, M.S. 1995. Hairy vetch kill date effects on soil water and corn production. *Agronomy Journal*, 87, 579-585.
- Cline, G.R. & Silvernail, A.F. 2001. Residual nitrogen and kill date effects on winter cover crop growth and nitrogen content in a vegetable production system. *HortTechnology*, 11, 219-225.
- Coombs, C., Lauzon, J.D., Deen, B. & Van Eerd, L.L. 2017. Legume cover crop management on nitrogen dynamics and yield in grain corn systems. *Field Crops Research*, 201, 75-85.
- Dabney, S.M., Delgado, J.A., Meisinger, J.J., Schomberg, H.H., Liebig, M.A., Kaspar, T., Mitchell, J. & Reeves, W. 2010. Using cover crops and cropping systems for nitrogen management. In: Delgado, J.A. & Follett, R. (eds.) *Advances in nitrogen management for water quality*. Ankeny, Iowa: Soil water conservation society
- Diouf, M., Baudoin, E., Dieng, L., Assigbetsé, K. & Brauman, A. 2010. Legume and gramineous crop residues stimulate distinct soil bacterial populations during early decomposition stages. *Canadian Journal of Soil Science*, 90, 289-293.
- Drinkwater, L., Janke, R. & Rossoni-Longnecker, L. 2000. Effects of tillage intensity on nitrogen dynamics and productivity in legume-based grain systems. *Plant and Soil*, 227, 99-113.
- Druille, M., García-Parisi, P.A., Golluscio, R., Cavagnaro, F.P. & Omacini, M. 2016. Repeated annual glyphosate applications may impair beneficial soil microorganisms in temperate grassland. *Agriculture, Ecosystems & Environment*, 230, 184-190.
- Finney, D., Buyer, J. & Kaye, J. 2017. Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation*, 72, 361-373.
- Follmer, C. 2008. Insights into the role and structure of plant ureases. *Phytochemistry*, 69, 18-28.

- Fortuna, A., Blevins, R., Frye, W., Grove, J. & Cornelius, P. 2008. Sustaining soil quality with legumes in no-tillage systems. *Communications in Soil Science and Plant Analysis*, 39, 1680-1699.
- Fourie, J., Agenbag, G. & Louw, P. 2007. Cover crop management in a Chardonnay/99 Richter vineyard in the coastal region, South Africa. 3. Effect of different cover crops and cover crop management practices on organic matter and macro-nutrient content of a medium-textured soil. *South African Journal for Enology and Viticulture*, 28, 61.
- García-Orenes, F., Guerrero, C., Roldán, A., Mataix-Solera, J., Cerdà, A., Campoy, M., Zornoza, R., Bárcenas, G. & Caravaca, F. 2010. Soil microbial biomass and activity under different agricultural management systems in a semiarid Mediterranean agroecosystem. *Soil and Tillage Research*, 109, 110-115.
- García-Ruiz, R., Ochoa, V., Hinojosa, M.B. & Carreira, J.A. 2008. Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. *Soil Biology and Biochemistry*, 40, 2137-2145.
- Haney, R., Senseman, S. & Hons, F. 2002. Effect of Roundup Ultra on microbial activity and biomass from selected soils. *Journal of Environmental Quality*, 31, 730-735.
- Hoagland, L., Carpenter-Boggs, L., Granatstein, D., Mazzola, M., Smith, J., Peryea, F. & Reganold, J.P. 2008. Orchard floor management effects on nitrogen fertility and soil biological activity in a newly established organic apple orchard. *Biology and Fertility of Soils*, 45, 11.
- Jani, A.D., Grossman, J., Smyth, T.J. & Hu, S. 2016. Winter legume cover-crop root decomposition and N release dynamics under disking and roller-crimping termination approaches. *Renewable Agriculture and Food Systems*, 31, 214-229.
- Jat, M., Gathala, M., Saharawat, Y., Tatarwal, J. & Gupta, R. 2013. Double no-till and permanent raised beds in maize–wheat rotation of north-western Indo-Gangetic plains of India: Effects on crop yields, water productivity, profitability and soil physical properties. *Field Crops Research*, 149, 291-299.
- Kalembasa, S. & Symanowicz, B. 2012. Enzymatic activity of soil after applying various waste organic materials, ash, and mineral fertilizers. *Polish Journal of Environmental Studies*, 21, 1635-1641.
- Kandeler, E. & Gerber, H. 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biology and Fertility of Soils*, 6, 68-72.
- Karaca, A., Cetin, S.C., Turgay, O.C. & Kizilkaya, R. 2010. Soil enzymes as indication of soil quality. *Soil enzymology*. Berlin, Heidelberg: Springer.
- Keene, C., Curran, W., Wallace, J., Ryan, M., Mirsky, S., VanGessel, M. & Barbercheck, M. 2017. Cover crop termination timing is critical in organic rotational no-till systems. *Agronomy Journal*, 109, 272-282.
- Kornecki, T.S., Arriaga, F.F., Price, A.J. & Balkcom, K.S. 2013. Effects of Recurrent Rolling/Crimping Operations on Cover Crop Termination, Soil Moisture, and Soil Strength for Conservation Organic Systems. *Applied Engineering in Agriculture*, 29, 841-850.
- Lane, M., Lorenz, N., Saxena, J., Ramsier, C. & Dick, R.P. 2012. Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate. *Pedobiologia*, 55, 153-159.
- Lawson, A., Cogger, C., Bary, A. & Fortuna, A.-M. 2015. Influence of seeding ratio, planting date, and termination date on rye-hairy vetch cover crop mixture performance under organic management. *PloS one*, 10, e0129597.
- Lawson, A., Fortuna, A.M., Cogger, C., Bary, A. & Stubbs, T. 2013. Nitrogen contribution of rye-hairy vetch cover crop mixtures to organically grown sweet corn. *Renewable Agriculture and Food Systems*, 28, 59-69.
- Lemanowicz, J. 2011. Phosphatases activity and plant available phosphorus in soil under winter wheat (*Triticum aestivum* L.) fertilized minerally. *Polish Journal of Agronomy*, 4, 12-15.

- Li, L., Li, S.-M., Sun, J.-H., Zhou, L.-L., Bao, X.-G., Zhang, H.-G. & Zhang, F.-S. 2007. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. *Proceedings of the National Academy of Sciences*, 104, 11192-11196.
- Liang, S., Grossman, J. & Shi, W. 2014. Soil microbial responses to winter legume cover crop management during organic transition. *European Journal of Soil Biology*, 65, 15-22.
- Lienhard, P., Terrat, S., Prévost-Bouré, N.C., Nowak, V., Régnier, T., Sayphoummie, S., Panyasiri, K., Tivet, F., Mathieu, O. & Levêque, J. 2014. Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. *Agronomy for Sustainable Development*, 34, 525-533.
- Liu, Y., Mi, G., Chen, F., Zhang, J. & Zhang, F. 2004. Rhizosphere effect and root growth of two maize (*Zea mays* L.) genotypes with contrasting P efficiency at low P availability. *Plant Science*, 167, 217-223.
- Makoi, J.H., Chimphango, S.B. & Dakora, F.D. 2010. Elevated levels of acid and alkaline phosphatase activity in roots and rhizosphere of cowpea (*Vigna unguiculata* L. Walp.) genotypes grown in mixed culture and at different densities with sorghum (*Sorghum bicolor* L.). *Crop and Pasture Science*, 61, 279-286.
- Makoi, J.H. & Ndakidemi, P.A. 2008. Selected soil enzymes: examples of their potential roles in the ecosystem. *African Journal of Biotechnology*, 7, 181-191.
- Maltais-Landry, G. 2015. Legumes have a greater effect on rhizosphere properties (pH, organic acids and enzyme activity) but a smaller impact on soil P compared to other cover crops. *Plant and Soil*, 394, 139-154.
- Maseko, S. & Dakora, F. 2013. Rhizosphere acid and alkaline phosphatase activity as a marker of P nutrition in nodulated *Cyclopia* and *Aspalathus* species in the Cape fynbos of South Africa. *South African Journal of Botany*, 89, 289-295.
- McVay, K., Radcliffe, D. & Hargrove, W. 1989. Winter legume effects on soil properties and nitrogen fertilizer requirements. *Soil Science Society of America Journal*, 53, 1856-1862.
- Mganga, K.Z., Razavi, B.S. & Kuzyakov, Y. 2016. Land use affects soil biochemical properties in Mt. Kilimanjaro region. *Catena*, 141, 22-29.
- Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R. & Shumway, D.L. 2009. Control of cereal rye with a roller/crimper as influenced by cover crop phenology. *Agronomy Journal*, 101, 1589-1596.
- Mukherjee, A. & Lal, R. 2015. Short-term effects of cover cropping on the quality of a Typic Argiaquolls in Central Ohio. *Catena*, 131, 125-129.
- Mukumbareza, C., Muchaonyerwa, P. & Chiduzza, C. 2015. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. *South African Journal of Plant and Soil*, 32, 189-197.
- Nuruzzaman, M., Lambers, H., Bolland, M.D. & Veneklaas, E.J. 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. *Plant and Soil*, 281, 109-120.
- Parr, M., Grossman, J., Reberg-Horton, S., Brinton, C. & Crozier, C. 2011. Nitrogen delivery from legume cover crops in no-till organic corn production. *Agronomy Journal*, 103, 1578-1590.
- Qian, X., Gu, J., Pan, H.-j., Zhang, K.-y., Sun, W., Wang, X.-j. & Gao, H. 2015. Effects of living mulches on the soil nutrient contents, enzyme activities, and bacterial community diversities of apple orchard soils. *European Journal of Soil Biology*, 70, 23-30.
- Sanaullah, M., Blagodatskaya, E., Chabbi, A., Rumpel, C. & Kuzyakov, Y. 2011. Drought effects on microbial biomass and enzyme activities in the rhizosphere of grasses depend on plant community composition. *Applied Soil Ecology*, 48, 38-44.
- Snapp, S., Swinton, S., Labarta, R., Mutch, D., Black, J., Leep, R., Nyiraneza, J. & O'neil, K. 2005. Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agronomy Journal*, 97, 322-332.
- Steenwerth, K. & Belina, K. 2008. Cover crops and cultivation: Impacts on soil N dynamics and microbiological function in a Mediterranean vineyard agroecosystem. *Applied Soil Ecology*, 40, 370-380.

- Sullivan, D.M. & Andrews, N. 2012. *Estimating plant-available nitrogen release from cover crops*, Oregon State University Extension Service.
- Tabatabai, M. & Bremner, J. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, 1, 301-307.
- Thilakarathna, M.S., Serran, S., Lauzon, J., Janovicek, K. & Deen, B. 2015. Management of manure nitrogen using cover crops. *Agronomy Journal*, 107, 1595-10607.
- Thorup-Kristensen, K. 2006. Effect of deep and shallow root systems on the dynamics of soil inorganic N during 3-year crop rotations. *Plant and Soil*, 288, 233-248.
- Thorup-Kristensen, K. & Dresbøll, D.B. 2010. Incorporation time of nitrogen catch crops influences the N effect for the succeeding crop. *Soil Use and Management*, 26, 27-35.
- Wayman, S., Cogger, C., Benedict, C., Burke, I., Collins, D. & Bary, A. 2015. The influence of cover crop variety, termination timing and termination method on mulch, weed cover and soil nitrate in reduced-tillage organic systems. *Renewable Agriculture and Food Systems*, 30, 450-460.
- Weaver, M.A., Krutz, L.J., Zablutowicz, R.M. & Reddy, K.N. 2007. Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil. *Pest Management Science*, 63, 388-393.
- Weerasekara, C.S., Udawatta, R.P., Gantzer, C.J., Kremer, R.J., Jose, S. & Veum, K.S. 2017. Effects of Cover Crops on Soil Quality: Selected Chemical and Biological Parameters. *Communications in Soil Science and Plant Analysis*, 48, 2074-2082.
- Whittinghill, K.A., Currie, W.S., Zak, D.R., Burton, A.J. & Pregitzer, K.S. 2012. Anthropogenic N Deposition Increases Soil C Storage by Decreasing the Extent of Litter Decay: Analysis of Field Observations with an Ecosystem Model. *Ecosystems*, 15, 450-461.
- Wortman, S.E., Francis, C.A., Bernards, M.A., Blankenship, E.E. & Lindquist, J.L. 2013. Mechanical termination of diverse cover crop mixtures for improved weed suppression in organic cropping systems. *Weed Science*, 61, 162-170.
- Xi, Z.-m., Tao, Y.-s., Zhang, L. & Li, H. 2011. Impact of cover crops in vineyard on the aroma compounds of *Vitis vinifera* L. cv Cabernet Sauvignon wine. *Food Chemistry*, 127, 516-522.
- XU, L.-f., Peng, Z., Han, Q.-f., Li, Z.-h., Yang, B.-p. & Nie, J.-f. 2013. Spatial distribution of soil organic matter and nutrients in the pear orchard under clean and sod cultivation models. *Journal of Integrative Agriculture*, 12, 344-351.
- Yao, S., Merwin, I.A., Bird, G.W., Abawi, G.S. & Thies, J.E. 2005. Orchard floor management practices that maintain vegetative or biomass groundcover stimulate soil microbial activity and alter soil microbial community composition. *Plant and Soil*, 271, 377-389.
- Zhang, L., Chen, W., Burger, M., Yang, L., Gong, P. & Wu, Z. 2015. Changes in soil carbon and enzyme activity as a result of different long-term fertilization regimes in a greenhouse field. *PLoS one*, 10, e0118371.

CHAPTER 5

COVER CROP SPECIES AND MANAGEMENT EFFECT ON SOIL NITROGEN, ORGANIC CARBON AND ENZYME ACTIVITIES

5.1 Introduction

Conventional farming practices and misuse of soil resources are among the key factors increasing soil degradation and seriously impairing biodiversity and global agricultural production (Delgado and Gantzer, 2015). The cultivation and proper management of cover crops can serve as a viable approach to maintain and improve soil biology and quality (Dabney et al., 2001).

Cover crops improve soil organic matter, aggregate stability, nutrient supply, water retention capacity and reduce soil erosion, thereby enhancing soil physical, chemical and biological properties (Dabney et al., 2001, Thorup-Kristensen et al., 2003, Blanco-Canqui et al., 2015). Legume cover crops reduce nitrogen (N) fertilization requirement by contributing significant amounts of N to the subsequent cash crops through biological fixation of atmospheric N in the soil (Ketterings et al., 2015, Thilakarathna et al., 2015). Non-legumes and grasses, on the other hand, supply a considerable amount of carbon (C) and sequester, as well as recycle inorganic N that may leach below the rooting zone (Cline and Silvernail, 2002). A number of studies have reported that cover crops including vetch, pea, rye, oats and crimson clover grown during fallow periods or rotation systems, considerably increased soil organic carbon (SOC) and N concentrations in contrast to plots without cover crops (Dabney et al., 2001, Sainju et al., 2002, Mukherjee and Lal, 2015).

Cover crop benefits can be enhanced by appropriate selection of cover crop species, growth termination stage and termination method (Wortman et al., 2013, Wayman et al., 2015). It has been reported that cover crop species and growth termination stage affected biomass cover and the release of plant available N (Sullivan and Andrews, 2012, Alonso-Ayuso et al., 2014, Benedict et al., 2014). Late termination of cover crops increased biomass and development of thick mulch that resulted in improved C input, water conservation and weed control (Saini et al., 2008). Furthermore, the delay in growth termination date of vetch-barley mixture (Alonso-Ayuso et al., 2014), sole vetch (Decker et al., 1994, Drinkwater et al., 2000) and sole rye (Thorup-Kristensen and Dresbøll, 2010) led to increases in aboveground biomass and a decrease in soil N levels. Thus, soil quality can be enhanced by manipulating residue quality and quantity through the proper selection of cover crop species and growth termination stage under different soil ecosystems. There is limited research on the impact of cover crop termination methods on soil N and organic C accumulation. Previous research has mostly reported on how termination methods successfully

killed/controlled cover crops and weeds (Mirsky et al., 2009, Keene et al., 2017) rather than their effect on soil biology and nutrient build-up.

Microbial population, structure and activity reflect the condition and health status of the soil (Schaller, 2009). Cover crops and other organic residues are sources of C which make up organic matter that serves as substrates for soil microbial communities (Blanco-Canqui et al., 2015). Microorganisms synthesize and release enzymes responsible for organic matter decomposition, nutrient cycling and mineralization in the soil (Balota and Chaves, 2010). Thus the amount and composition of organic matter affects enzyme activity in the soil. Soil enzyme activities respond rapidly to slight environmental and land management alterations, making them a more reliable tool for early evaluation of soil quality change (García-Ruiz et al., 2008). Soil enzymes such as β -glucosidase, urease and phosphatase are widely distributed and are involved in the soil C, N and P cycle, respectively (Adetunji et al., 2017). Several studies have indicated higher β -glucosidase, urease and phosphatase activities in cover crop and organic residue treated soils compared to the fallow soils (Liang et al., 2014, Meyer et al., 2015, Mukumbareza et al., 2015). However, the impact of cover crop species, termination stage and termination method on the activities of these soil enzymes is poorly understood, especially in Western Cape in South African cropping systems where cover crops are used in vines. A better understanding of the impacts of these management options on soil organic C and enzyme activities will aid proper selection and development of management approaches that will result in the restoration of healthy microbial communities and crop productivity in the region.

Field studies at separate sites in the Western Cape, South Africa were conducted to determine the short-term effect of: (1) termination stage on the biomass, total C, total N and C:N ratio content of cover crops; (2) living cover crops and their residues on soil pH, total N, organic C, β -glucosidase, urease and phosphatase activities; and (3) cover crop termination stage and termination method on soil pH, total N, organic C, β -glucosidase, urease and phosphatase activities.

5.2 Materials and methods

5.2.1 Study sites

The study was conducted from 2017 through 2018 at two fallow sites, 42 km apart; at the Agricultural Research Council (ARC) Nietvoorbij Research Farm (33°55'10"S, 18°51'57"E) and ARC Bien Donne Research Farm (33°50'30"S, 18°58'59"E), in the Western Cape Province, South

Africa. The soil at the Nietvoorbij site was a sandy clay loam, while the soil at Bien Donne was sandy loam. Initial physical and chemical analyses were done for both sites for soils sampled in the 0 - 15 cm depth. The sites are under Mediterranean climate, with mean annual temperature of about 18°C, and a January mean of about 24°C and a July mean of about 13°C. However, the Nietvoorbij site had higher average rainfall (597 mm) than the Bien Donne site (479 mm) during the experimental period.

5.2.2 Experimental design

The experimental set-up was a randomized block design with 3 replications, at both sites. Four experiments were conducted which consisted of 2 growth termination stages (vegetative and flowering) and 2 termination methods (slash and spray). Each experiment consisted of five cover crops namely; grazing vetch (*Vicia dasycarpa* Ten.), field pea (*Pisum sativum*), oats (*Avena sativa* L.), rye (*Secale cereal* L.) and control (no cover crop). The test crops were chosen because they are well adapted to the Western Cape soils and are among the annual winter legumes and non-legumes widely grown by farmers and researchers in the region (Fourie et al., 2001). Row spacing was 2 m at both sites while plot size for each replicate was 2 m by 2 m at Nietvoorbij and 3 m by 2 m at Bien Donne. Cover crops were broadcast by hand and buried (about 3 cm) with a shallow rake, on 9 June 2017 and 11 July 2017 at Bien Donne and Nietvoorbij, respectively. At both sites, seeding rates were as follows: pea, 80 kg ha⁻¹; vetch, 50 kg ha⁻¹; oats, 100 kg ha⁻¹; rye, 100 kg ha⁻¹ (Fourie et al., 2001). At Nietvoorbij, fertilizer was applied at 7 kg N ha⁻¹ and 10 kg P ha⁻¹ on each plot, at planting. At Bien Donne, oats and rye plots received 7 kg N ha⁻¹ and 5 kg P ha⁻¹ while pea and vetch plots received only 5 kg P ha⁻¹ at 3 - 4 leaf stage. Control plots had no cover crop and no fertilizer at both sites. At planting, pea and vetch were inoculated with *Rhizobium leguminosarium* biovar *viciae*. Weeds were not controlled and plots were not irrigated at both sites. Cover crops were terminated at vegetative and flowering growth stages by slashing at the soil surface and spraying glyphosate (*N*-phosphonomethyl glycine) at a rate of 5 L ha⁻¹. The termination dates at each growth stage for Nietvoorbij and Bien Donne sites are presented in Table 5.1. The vetch growth stage was determined using the Mischler scale where vegetative and flowering were stage 1 and 6, respectively (Mischler et al., 2010). Pea growth stage was measured using the Schwartz and Langham scale where vegetative and flowering stages were V4 and R3, respectively (Schwartz et al., 2009). Rye and oats growth stages were determined using the Zadoks scale where vegetative and flowering stages were 45 and 69, respectively (Zadoks et al., 1974). The residues were left on the soil surface to decay.

Table 5.1: Cover crop growth termination date and sampling times for four cover crops at Nietvoorbij and Bien Donne study sites

Cover crop	Nietvoorbij				Bien Donne			
	Kill (2017)		One year (2018)		Kill (2017)		One year (2018)	
	Veg	Flow	Veg	Flow	Veg	Flow	Veg	Flow
Pea and Vetch	27 Sept	18 Oct	4 Oct	22 Oct	8 Sept	29 Sept	14 Sept	5 Oct
Oats and Rye	4 Oct	20 Oct	4 Oct	22 Oct	14 Sept	5 Oct	14 Sept	5 Oct

Veg is vegetative and Flow is flowering

5.2.3 Cover crop sampling and analysis

Just before cover crop kill, a 0.25 m² quadrat was used to collect aboveground biomass per treatment plot with a cordless grass shear at the ground level, at both sites. Biomass samples were oven dried at 60°C for 48 hours and were weighed to determine dry biomass before subsamples were ground through a 1 mm screen using a Polymix Kinematica (PX-MFC 90 D). Total N concentration was determined in the 0.1 g dried and ground samples using TruSpec N Nitrogen Analyser (LECO, St. Joseph, MI, USA). The total C concentration was measured from the dried and ground samples by wet-combustion analysis (Dalal, 1979, Shaw, 2006). Thereafter, the C:N ratio was calculated by using these two values.

5.2.4 Soil sampling and chemical analysis

Soil samples were randomly obtained from 3 points per treatment plot (0 - 15 cm depths) just prior to cover crop termination and at one year after termination. The composite soil samples were passed through 2 mm sieve and stored at 4°C until enzyme analysis after subsamples have been collected and air-dried for soil pH, organic C and total N analysis. Soil pH was analysed in 1:2.5 Soil:KCl mixture (1 M KCl solution) using a glass electrode pH meter. Soil organic C was analysed by complete oxidation of the potassium dichromate (K₂Cr₂O₇) solution and concentrated sulphuric acid mixture, and subsequent titration of residual K₂Cr₂O₇ with ferrous ammonium sulphate (Okalebo et al., 2002). The difference between added and residual K₂Cr₂O₇ gives a measure of organic C content of the soil. Total soil N was measured from a 0.1 g sample using TruSpec N Nitrogen Analyser (LECO, St. Joseph, MI, USA).

5.2.5 Soil enzyme activity assay

The activities of soil β-glucosidase, phosphatase and urease known to play crucial roles in C, P, N cycling, respectively, were determined using the procedures in sections 3.2.3 and 4.2.1.

5.2.6 Statistical analysis

The trials were conducted at two sites. The data from the sites were analysed separately. Levene's test for homogeneity of experimental variances was verified for comparable variances (Levene, 1960). The data were then subjected to a combined analysis of variance (ANOVA) using the General Linear Models Procedure (PROC GLM) of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Observations over sampling time (kill and one year after) were combined in a split-plot analysis of variance with sampling time as sub-plot factor (Little and Hills, 1972) for soil variables. The Shapiro-Wilk test was performed on the standardized residuals from the model to verify normality (Shapiro and Wilk, 1965). The Fisher's least significant difference was calculated at the 5% level to compare treatment means (Ott and Longnecker, 2015). A probability level of 5% was considered significant for all significance tests.

5.3 Results

5.3.1 Initial soil characteristics

Table 5.2 shows the initial characteristics of the soils collected from the ARC Nietvoorbij and Bien Donne sites. The soil pH was conducive for plant growth, although the fertility level was low as shown by the low N, P and K levels, at both sites. However, the Bien Donne site had higher P (50 mg/kg), compared to the Nietvoorbij site (12 mg/kg).

Table 5.2: Initial physiochemical characteristics of Nietvoorbij and Bien Donne soils

Soil property	Nietvoorbij value	Bien Donne value
pH (KCl)	5.5	5.8
P (mg/kg) Bray II	12	50
K (mg/kg)	25	20
C%	1.29	1.04
NO ₃ -N (mg/kg)	2.09	4.63
NH ₄ -N (mg/kg)	9.03	5.15
Clay (%)	31	19
Silt (%)	16	6
Sand (%)	53	75

5.3.2 Effect of termination stage on cover crop biomass, total C, total N and C:N ratio

Termination stage significantly affected cover crop biomass, total C, total N and C:N ratio, at both sites. Biomass varied and increased from vegetative stage to flowering under all cover crops with rye and oats being the highest followed by pea and vetch, at both sites (Table 5.3). Cover crop biomass at the Nietvoorbij site varied from 207 - 4480 kg ha⁻¹ while at the Bien Donne site it varied from 835 – 9891 kg ha⁻¹. At both sites, total C concentration of all cover crops significantly

increased from vegetative stage to flowering, except vetch and rye which marginally increased at Nietvoorbij (Table 5.3). Total C concentrations were greatest at flowering stage in rye (55.50%) and oats (55.45%) at the Nietvoorbij site and in rye (56.24%) and pea (55.82%) at the Bien Donne site. However, tissue N concentration significantly decreased in all cover crops from vegetative to flowering with vetch being the highest followed by pea, rye and oats, at both sites (Table 5.3). At both sites, the C:N ratio significantly increased from vegetative to flowering across all cover crops with the highest concentration observed in oats followed by rye, pea and vetch. The C:N ratios of vetch and pea were lower than that of rye and oats at vegetative and flowering stages, at both sites (Table 5.3).

Table 5.3: Interaction effects of cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) on cover crop biomass, total C, total N and C:N ratio at Nietvoorbij and Bien Donne study sites

Site	Cover crop	Termination stage	BM (kg ha ⁻¹)	C (%)	N (%)	C:N
Nietvoorbij	Oats	Vegetative	751.94 cde	53.13 c	1.91 d	30.29 d
			1024.75 bcd	53.18 c	2.50 b	21.88 ef
			206.72 e	54.29 abc	2.29 bc	24.13 e
			605.46 de	53.76 abc	3.07 a	17.77 f
	Oats	Flowering	4479.66 a	55.45 a	0.84 f	65.84 a
			1475.59 b	55.15 ab	1.36 e	40.55 c
			4029.87 a	55.50 a	1.05 f	52.43 b
			1277.27 bc	53.57 bc	2.18 cd	24.35 e
	LSD		604.65	1.87	0.30	5.53
Bien Donne	Oats	Vegetative	1475.2 e	51.75 e	2.00 de	25.15 d
			5795.73 cd	53.72 dc	2.69 b	19.85 e
			835.2 e	53.12 d	2.18 c	23.76 de
			1995.2 e	51.53 e	4.09 a	12.90 f
	Oats	Flowering	8271.74 ab	55.23 b	0.64 f	86.82 a
			6895.36 bc	55.82 a	1.89 e	30.06 c
			9890.67 a	56.24 a	0.70 f	81.20 b
			4461.87 d	54.59 bc	2.10 cd	25.91 cd
	LSD		1754.8	1.06	0.16	4.38

BM denotes biomass. Each value represents the mean (n = 3). Values within the same column (separated by site) followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's least significant difference test (LSD)

5.3.3 Effect of cover crop species and sampling time on soil pH

Living cover crops and their residues affected soil pH at both sites. At kill, the soil pH in the control (5.12) plot was significantly higher than the living vetch (4.99) plot and marginally higher than the pea (5.05), oats (5.02) and rye (5.02) plots, respectively, at the Nietvoorbij site (Table 5.4). Similarly, at the Bien Donne site, the soil pH in the control (6.23) plot was significantly greater than the living pea (6.01), rye (6.04) and vetch (6.0) plots, respectively (Table 5.4). At one year, soil pH

at both sites was reduced across all treatments with the control still being greater than the cover crop residue plots, except vetch plot which was similar at Nietvoorbij (Table 5.4).

Table 5.4: Cover crop species and sampling time effects on soil pH at Nietvoorbij and Bien Donne study sites

Sampling time	Cover crop	Nietvoorbij	Bien Donne
		Soil pH	Soil pH
Kill	Oats	5.02 (± 0.07) abc	6.11 (± 0.07) ab
	Pea	5.05 (± 0.06) ab	6.01 (± 0.10) bc
	Rye	5.02 (± 0.08) abc	6.04 (± 0.08) bc
	Vetch	4.99 (± 0.03) bc	6.00 (± 0.10) bcd
	Control	5.12 (± 0.12) a	6.23 (± 0.07) a
One year	Oats	4.92 (± 0.03) cd	5.96 (± 0.07) cde
	Pea	4.95 (± 0.05) bcd	5.88 (± 0.06) def
	Rye	4.84 (± 0.05) d	5.86 (± 0.09) ef
	Vetch	4.89 (± 0.03) cd	5.83 (± 0.09) f
	Control	4.95 (± 0.03) bcd	6.06 (± 0.05) bc
LSD		0.13	0.12

Each value represents the mean ($n = 3$). Standard error values are indicated in parenthesis. Values within the same column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's least significant difference test (LSD)

5.3.4 Soil organic carbon and total soil nitrogen

5.3.4.1 Effect of cover crop species and sampling time on soil organic C and total soil N concentrations

At kill, SOC was significantly higher in living vetch (1.33%) plots compared to oats (1.20%), pea (1.08%) and rye (1.14%) plots, at the Nietvoorbij site (Figure 5.1A). However, living rye and pea had a significantly negative effect on SOC compared to the control. At the Bien Donne site, living cover crops considerably affected SOC with pea (1.02%) and rye (1.00%) plots being significantly greater, and oats (0.94%) and vetch (0.94%) plots being marginally greater than the control (0.89%) plots, respectively (Figure 5.1B). At one year, SOC levels increased under oats and pea residues at the Nietvoorbij site (Figure 5.1A) and under oats residues at the Bien Donne site (Figure 5.1B). Soil organic C level was greater in oats residue plots than the control plots, at both sites (Figure 5.1A and B).

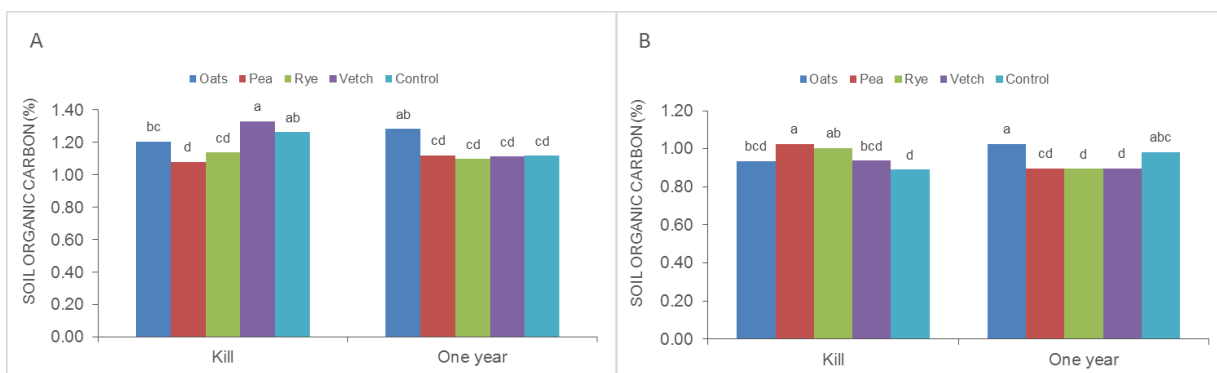


Figure 5.1: Cover crop species and sampling time effects on soil organic carbon (A) Nietvoorbij site and (B) Bien Donne site. Bars (means - n = 3) with the same letter are not significantly different based on Fisher's LSD ($P < 0.05$)

At kill, the total soil N concentration at the Nietvoorbij site was significantly higher under the living cover crop plots than the control plots (0.24%), except living pea (0.26%), which was marginally higher (Figure 5.2A). Also, at the Bien Donne site, the total soil N concentration was significantly greater under all the living cover crop plots than the control with pea being the highest (0.24%), followed by vetch (0.23%), oats (0.21%) and rye (0.13%), respectively (Figure 5.2B). At one year, total soil N levels at the Nietvoorbij site were significantly increased by about 36%, 54%, 28% and 21% under oats, pea, rye and control residue plots, respectively (Figure 5.2A). At the Bien Donne site, pea and control residues significantly increased total soil N levels while vetch slightly increased the level with oats and rye having no effect at one year (Figure 5.2B). However, total soil N accumulated at one year remained significantly greater in pea (0.33%), vetch (0.25%) and oats (0.21%) residue plots than the control (0.16%) and rye (0.14%) plots, at Bien Donne site (Figure 5.2B).

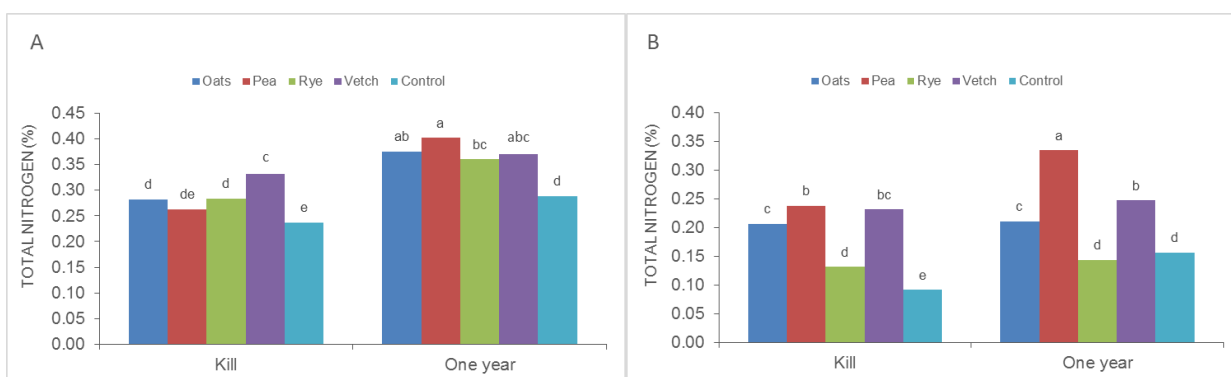


Figure 5.2: Cover crop species and sampling time effects on soil organic carbon (A) Nietvoorbij site and (B) Bien Donne site. Bars (means - n = 3) with the same letter are not significantly different based on Fisher's LSD ($P < 0.05$)

5.3.4.2 Effect of cover crop termination stage on soil organic C and total soil N concentrations at different sampling times

At kill, SOC was significantly higher at the flowering stage than vegetative, when averaged over all living cover crop plots, at both sites (Table 5.6). At one year, SOC at flowering stage (1.11%) remained significantly greater than vegetative (0.79%) at the Bien Donne site, whereas no significant differences were observed between termination stages at the Nietvoorbij site (Table 5.6).

At kill, total soil N was significantly greater at the flowering stage than the vegetative stage, when averaged over all living cover crop plots, at both sites (Table 5.6). However, at one year, total soil N at the vegetative stage was significantly and marginally greater than the flowering stage at both Nietvoorbij and Bien Donne sites, respectively (Table 5.6).

Table 5.5: Average effect of cover crop termination stage (vegetative and flowering) on soil organic carbon and total soil nitrogen at different sampling times (kill and one year) at Nietvoorbij and Bien Donne study sites

Sampling time	Termination stage	Nietvoorbij		Bien Donne	
		SOC (%)	Soil N (%)	SOC (%)	Soil N (%)
Kill	Vegetative	1.02 (± 0.05) c	0.18 (± 0.01) d	0.90 (± 0.02) c	0.17 (± 0.01) c
	Flowering	1.24 (± 0.03) a	0.36 (± 0.01) b	1.02 (± 0.03) b	0.20 (± 0.02) b
One year	Vegetative	1.21 (± 0.05) ab	0.40 (± 0.02) a	0.79 (± 0.03) d	0.22 (± 0.02) a
	Flowering	1.14 (± 0.02) b	0.33 (± 0.01) c	1.11 (± 0.02) a	0.21 (± 0.01) ab
LSD		0.10	0.03	0.05	0.02

Standard error values are indicated in parenthesis. Values within the same column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's least significant difference test (LSD)

5.3.4.3 Effect of cover crop termination method on soil organic C and total soil N concentrations

At one year, there was no significant difference in SOC between slash and spray plots, when averaged over all living cover crops, at Nietvoorbij site (Table 5.7). However, at the Bien Donne site, SOC was significantly greater under slash plots (0.98%) than spray (0.84%) (Table 5.7).

At one year, termination method had no significant effect on the total soil N concentration when averaged over all cover crop residue plots, at the Nietvoorbij site (Table 5.7). However, at Bien Donne, significantly higher total soil N level was observed under spray (0.24%) treatment than slash (0.21%) treatment (Table 5.7).

Table 5.6: Average effect of cover crop termination method (slash and spray) on soil organic carbon and total soil nitrogen at one year at Nietvoorbij and Bien Donne study sites

Termination method	Nietvoorbij		Bien Donne	
	SOC (%)	Soil N (%)	SOC (%)	Soil N (%)
Slash	1.19 (± 0.04) a	0.36 (± 0.02) a	0.98 (± 0.04) a	0.21 (± 0.02) b
Spray	1.13 (± 0.02) a	0.36 (± 0.01) a	0.84 (± 0.04) b	0.24 (± 0.02) a
LSD	0.08	0.03	0.06	0.02

Standard error values are indicated in parenthesis. Values within the same column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's least significant difference test (LSD)

5.3.5 Soil enzyme activity

5.3.5.1 Effect of cover crop species and sampling time on the activities of soil β -glucosidase, urease and phosphatase

At both sampling times (kill and one year), β -glucosidase, urease and phosphatase activities did not significantly respond to the living cover crops and residues tested, at both sites (Table 5.8). At kill, β -glucosidase activity was slightly higher in the living cover crop plots than the control plots, at Nietvoorbij (Table 5.8). Also, there was a marginal increase in the activity of phosphatase in living pea ($811.87 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) plots than the control ($701.42 \mu\text{g PNP g}^{-1} \text{soil h}^{-1}$) plots at Nietvoorbij (Table 5.8). Living rye and vetch plots indicated slightly higher urease activity than the control, at Bien Donne site (Table 5.8). At one year, oats residue plots had slightly higher β -glucosidase and phosphatase activities than the control plots, at Nietvoorbij (Table 5.8). Pea residue plots also had slightly higher β -glucosidase and phosphatase activities than the control plots, at Bien Donne (Table 5.8). There were substantial differences in soil enzyme activity levels

between sites, with the activities of β -glucosidase, urease and phosphatase generally being higher at the Nietvoorbij site than the Bien done site (Table 5.8).

Table 5.7: Cover crop species and sampling time effects on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}), urease ($\mu\text{g NH}_4^+ \text{g}^{-1}$ soil 2 h^{-1}) and phosphatase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) at Nietvoorbij and Bien Donne study sites

Site	Sampling time	Cover crop	β -glucosidase	Urease	Phosphatase	
Nietvoorbij	Kill	Oats	201.75 ab	31.74 b	628.52 b	
		Pea	192.78 ab	29.41 b	811.87 a	
		Rye	188.30 ab	30.29 b	563.66 b	
		Vetch	204.55 ab	31.84 b	644.09 ab	
		Control	164.75 b	28.30 b	701.42 ab	
	One year	Oats	220.61 a	44.10 a	672.77 ab	
		Pea	162.46 b	31.65 b	593.40 b	
		Rye	183.57 ab	36.46 ab	688.80 ab	
		Vetch	169.47 ab	32.97 b	642.42 ab	
		Control	171.05 ab	34.50 ab	672.22 ab	
	Bien Donne	LSD		53.88	9.61	175.16
		Kill	Oats	95.20 cd	21.14 b	252.35 c
Pea			103.91 bcd	20.37 b	266.01 bc	
Rye			91.90 d	18.71 b	292.28 abc	
Vetch			93.77 cd	18.53 b	294.45 abc	
Control			108.84 abcd	21.97 b	264.47 bc	
One year		Oats	118.97 ab	29.06 a	303.74 abc	
		Pea	123.39 a	29.04 a	335.16 a	
		Rye	117.64 ab	29.49 a	284.73 abc	
		Vetch	111.55 abc	26.12 a	316.34 ab	
		Control	119.24 ab	27.30 a	304.77 ab	
LSD			18.15	3.92	52.32	

Each value represents the mean ($n = 3$). Values within a column (separate by site) followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's least significant difference test (LSD)

5.3.5.2 Effect of cover crop termination stage on the activities of soil β -glucosidase, urease and phosphatase

At kill, cover crop termination stage significantly affected soil enzyme activities at the Nietvoorbij and Bien Donne sites, when averaged across all living cover crop plots. The activities of β -glucosidase, urease and phosphatase were significantly higher at flowering than vegetative stage, at both sites (Table 5.9). At one year, while termination stage had no significant effect on β -glucosidase and urease activities, flowering stage ($736.23 \mu\text{g PNP g}^{-1}$ soil h^{-1}) maintained significantly higher phosphatase activity than vegetative stage ($571.61 \mu\text{g PNP g}^{-1}$ soil h^{-1}), at Nietvoorbij (Table 5.9). It was observed at the Bien Donne site that β -glucosidase and urease activities were greater at flowering stage than vegetative, at one year, whereas no significant difference was noticed in phosphatase activities between termination stages (Table 5.9).

Table 5.8: Average effect of cover crop termination stage (vegetative and flowering) on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}), urease ($\mu\text{g NH}_4^+ \text{g}^{-1}$ soil 2 h^{-1}) and phosphatase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) at different sampling times (kill and one year) at Nietvoorbij and Bien Donne study sites

Site	Sampling time	Termination stage	β -glucosidase	Urease	Phosphatase
Nietvoorbij	Kill	Vegetative	123.80 c	18.11 c	584.00 b
		Flowering	257.05 a	42.52 a	755.82 a
	One year	Vegetative	169.94 b	36.40 b	571.61 b
		Flowering	192.92 b	35.47 b	736.23 a
	LSD		34.08	6.08	110.78
Bien Donne	Kill	Vegetative	75.80 c	14.20 c	195.96 c
		Flowering	121.65 ab	26.09 b	351.86 a
	One year	Vegetative	110.54 b	26.59 b	300.19 b
		Flowering	125.78 a	29.81 a	317.71 b
	LSD		11.48	2.48	33.09

Values within a column (separated by site) followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's least significant difference test (LSD)

5.3.5.3 Effect of cover crop termination method on the activities of soil β -glucosidase, urease and phosphatase

At the Nietvoorbij site, β -glucosidase, urease and phosphatase activities were 23.5%, 44.7% and 18.4%, respectively, greater in slash than spray plots, when averaged overall cover crops, at one year (Table 5.10). Nevertheless, at the Bien Donne site, termination method had no significant effect on β -glucosidase, urease and phosphatase activities at one year (Table 5.10). There was a site main effect owing to the response of β -glucosidase, urease and phosphatase activities to termination methods at the Nietvoorbij site relative to the Bien Donne site.

Table 5.9: Average effect of cover crop termination method (slash and spray) on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}), urease ($\mu\text{g NH}_4^+ \text{g}^{-1}$ soil 2 h^{-1}) and phosphatase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) at one year at Nietvoorbij and Bien Donne study sites

Site	Termination Method	β -glucosidase	Urease	Phosphatase
Nietvoorbij	Slash	200.49 (± 12.31) a	42.50 (± 2.62) a	709.10 (± 41.05) a
	Spray	162.37 (± 12.97) b	29.37 (± 1.47) b	598.74 (± 31.88) b
	LSD	36.45	6.05	105.06
Bien Donne	Slash	123.03 (± 7.29) a	29.72 (± 1.31) a	317.25 (± 18.27) a
	Spray	113.29 (± 3.50) a	26.68 (± 0.91) a	300.65 (± 15.33) a
	LSD	17.70	3.13	48.98

Standard error values are indicated in parenthesis. Values within a column (separated by site) followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's least significant difference test (LSD)

5.4 Discussion

5.4.1 Cover crop

This study confirmed that cover crop termination stage is a crucial management approach as it affected cover crop biomass, total C, total N and C:N ratio concentration, at Nietvoorbij and Bien Donne sites. The observed higher biomass under non-legume than legume plots and the general increase in biomass due to delaying termination till flowering stage at both sites, is in agreement with other studies (Mirsky et al., 2011, Alonso-Ayuso et al., 2014, Keene et al., 2017). Also, the general increase in tissue total C and C:N ratio from vegetative to flowering and the differing relationship between legumes and non-legumes detected at both sites, is in agreement with the previous greenhouse experiment (chapter 3) and the trends reported in other studies (Alonso-Ayuso et al., 2014, Lawson et al., 2015, Coombs et al., 2017). Findings from this study also support previous studies (Alonso-Ayuso et al., 2014, Lawson et al., 2015) that termination of vetch and pea and most importantly oats and rye at the vegetative stage will improve soil N levels. Thus, early or late termination of rye, oats, pea and vetch can serve as management methods to improve C input, SOC, C sequestration, soil surface cover, microbial activity, N mineralization and reduce N immobilization in cropping systems (Blanco-Canqui et al., 2013, Du et al., 2014).

5.4.2 Soil pH

The acidity and alkalinity of the soil have been shown to affect nutrient availability, organic acids and enzyme activities to some extent (Maltais-Landry, 2015). The results from this study showed that living cover crops and their residues reduced soil pH at both sites in the short-term, which is consistent with previous studies (Maltais-Landry, 2015, Mukherjee and Lal, 2015). Mukherjee and Lal (2015) reported that, in one year the presence of pea and turnip decreased soil pH from about 6.7 to 5.7 compared to the control. Additionally, Maltais-Landry (2015) indicated that legumes such as faba bean, vetch and pea had the lower soil pH compared to no cover crop and cereal soils. This was also noticed in this study with living vetch having the lowest soil pH at both sites including its (vetch) residue at Bien Donne plots. However, under greenhouse conditions, cover crops had no impact on soil pH (chapter 4), the observed increase in pH within one year was probably a sampling time factor. Although the reduction in soil pH at both sites was observed to be associated with cover crop presence in the present study, further/future sampling is needed to investigate if the effect is temporary or permanent.

5.4.3 Soil organic carbon and total nitrogen

The significant impact of living cover crops on SOC levels was only observed at Bien Donne and not at the Nietvoorbij site. The observed non-responsiveness of SOC to living cover crop at Nietvoorbij site is in agreement with the previous greenhouse study that was done using similar soil (chapter 3). This may be as a result of the higher initial SOC levels observed at the Nietvoorbij site than Bien Donne (Table 5.2), which has been reported to make SOC undetectable in the short-term (Lagomarsino et al., 2009). The significantly higher SOC in living pea and rye plots compared to the control at the Bien Donne site, is an indication that these cover crops contribute to build up SOC through their root exudates and root turnover (Dignac et al., 2017). The detected higher SOC levels under oats residue plots compared to the control, at both sites, is consistent with the greenhouse study (chapter 3) and a study in the vineyard soil in the Western Cape, South Africa, which indicated that oats residue improved SOC and soil organic matter regardless of management methods (Fourie et al., 2007). The higher oats biomass and total C input observed in this study might have also contributed to the higher SOC level compared to the control, at both sites. However, previous cover crop studies observed no significant effect on SOC in the short-term (Calderon et al., 2016), while others detected effects after four (Lagomarsino et al., 2009), seven (Bowman et al., 1999) and nine (Olson et al., 2010) years, respectively. Thus, longer research period may be needed to detect changes in SOC levels under the cover crop plots where no responses were observed in this study.

The observed higher total soil N under the living cover crop plots compared to the control at both sites is consistent with other research which reported greater soil N levels under crown vetch and clover (XU et al., 2013, Qian et al., 2015). However, a slightly higher total soil N was noticed in the living vetch and rye plots in the previous greenhouse study (chapter 4). Living legume cover crops may enhance soil N by biologically fixing N and stimulating soil enzyme activity in the roots (Steenwerth and Belina, 2008, Maseko and Dakora, 2013). Living grasses may also enhance soil N by root exudates effects, which provide substrate and improve biological activity leading to nutrient release (Thorup-Kristensen, 2006, Mukumbareza et al., 2015). Findings from this study indicate that plots with cover crop residues had higher total soil N than the control at one year, which is in line with previous studies (Blanco-Canqui et al., 2015, Coombs et al., 2017) including the greenhouse results (chapter 4). The biological N fixation, lower C:N ratio, which improves N mineralization and higher tissue N input observed in this study are among the factors that contributed to higher total N accumulated under pea and vetch plots than the control. However, non-legumes such as rye and oats do not fix N (Balkcom et al., 2015), but probably improved total N by scavenging extra inorganic N and making it available through decomposition (Balkcom et al.,

2015, Thilakarathna et al., 2015). The observed increase in total soil N at one year under the control plots, at both sites, may be attributed to sampling time and weed effects.

The results showed that, at kill, termination of cover crops at flowering stage significantly elevated SOC than the vegetative stage, at both study sites. This is in agreement with the greenhouse results (chapter 3) and a study by Hirpa (2013) which indicated overall greater SOC at pod-setting growth stage than vegetative under *Vigna unguiculata*, *Glycine max* and *Phaseolus vulgaris* plots. At one year, the superior SOC level observed at flowering stage relative to vegetative at Bien Donne can be attributed to the maturity, higher biomass and total carbon input from cover crop residues as shown in the current results and previous studies (Njunie et al., 2004, Sainju et al., 2005, Hirpa, 2013, Ruis et al., 2017). This study, therefore, indicates that cover crop termination at flowering stage may promote C sequestration and increase SOC in the short-term.

The observed higher total soil N at kill under the flowering stage than vegetative at both sites is in contrast to previous studies (Alonso-Ayuso et al., 2014, Coombs et al., 2017, Boyrahmadi and Raiesi, 2018). On the other hand, at one year of this study, total soil N increased and was greater under vegetative treatments than flowering treatments, indicating the effect of residue quality (lower C:N ratio), rapid decomposition and optimum N mineralization associated with the vegetative stage (Alonso-Ayuso et al., 2014, Lawson et al., 2015). Additionally, early termination decreases N uptake by cover crops especially grasses and allows more time for N release from cover crop residues (Alonso-Ayuso et al., 2014). This study, therefore, shows that termination stage can be managed to improve cover crop quality, N release and reduce N immobilization in the cropping system.

At one year, termination methods had no significant effect on SOC level at Nietvoorbij, which is not in agreement with the previous greenhouse study with similar soil (chapter 3); which showed positive effects under slash treatments against spray. However, cover crop termination by slash significantly elevated SOC compared to spray, at the Bien Donne site, thus corroborating the greenhouse study. This is consistent with several other studies which reported that application of herbicides such as glyphosate could be toxic to soil microbes and negatively affect biochemical processes, organic matter formation and SOC build-up (García-Orenes et al., 2010, Abbas et al., 2014).

Although termination methods in cover cropping systems are directed towards a common goal, the variation between them is the point of placement of residues after termination and the magnitude of soil biological stimulation (Liang et al., 2014). This study found that termination methods had no impact on total soil N at the Nietvoorbij site, which was also observed in the

greenhouse study (chapter 4). On the other hand, spray application accumulated more soil N than the slash at Bien Donne site, showing site influence, at one year. Cover crop termination with glyphosate was also indicated to improve N release in a study by Haney et al. (2002).

5.4.4 Soil enzymes

Three enzymes were chosen for this study, β -glucosidase, urease and phosphatase, which are widely spread and offer insights into the soil C, N and P cycling, respectively. However, the amount of research on the effect of cover crop species and management on the activity of these soil enzymes is still limited.

The results indicate that living cover crops had no substantial effect on the activities of the three enzymes, at both sites, which is in contrast with the findings from the previous greenhouse pot experiment which showed a considerable effect on β -glucosidase and urease activities (chapter 3 and 4). Literature has shown differences in crop and soil expression between pot and field studies due to buffering effects of the soil (Irvine et al., 2004, Limpens et al., 2012). The contrasting results between the field and greenhouse pot experiment may be a result of the topography or larger soil surface area in the field which probably caused a slow rate of change in enzyme activities in response to the presence of living cover crops. However, studies evaluating the impact of living cover crops on enzyme activity are very few. It has been shown that living cover crop roots secrete extracellular enzymes and carbon substrates which significantly stimulate microbial growth and enzyme activities in the soil (Follmer, 2008, Boyrahmadi and Raiesi, 2018). This may explain the marginally higher β -glucosidase activity under all living cover crop plots compared to the control at Nietvoorbij. The slightly greater phosphatase activity at the Nietvoorbij living pea plots may be as a result of extra phosphatase secretion due to initial lower soil P concentration (Table 5.2) and higher P requirement by legumes during symbiotic N fixation process compared to grasses (Makoi and Ndakidemi, 2008, Maseko and Dakora, 2013). At one year, oats residues slightly increase urease and β -glucosidase activities, respectively, at Nietvoorbij site; indicating the addition of labile C, organic matter and substrate by non-legumes which promotes enzyme synthesis and microbial processes (Balota and Chaves, 2010, Jat et al., 2013, Muzangwa et al., 2019). A number of short-term studies (Acosta-Martinez et al., 2011, Liang et al., 2014), including similar treatments from the previous greenhouse study (chapter 3 and 4), have reported significant and positive effect in soil enzyme activities due to cover crops. Nevertheless, other field studies did not detect differences in soil enzyme activities in the short-term (Calderon et al., 2016).

Termination stage is a crucial cover crop management approach that can be used to improve biological processes, carbon build-up and N release in agricultural soils (Liang et al., 2014).

However, the impact of this method on soil enzyme activities is not well understood in the South African cropping systems. The observed higher β -glucosidase, urease and phosphatase activities at kill under flowering plots relative to vegetative, at both sites may be due to the effects of higher biomass, total C input and SOC detected at flowering stage in this study. It may also be due to the accumulated plant root exudates and over synthesis of these enzymes by microbes (Cheng, 2009, Boyrahmadi and Raiesi, 2018) as a result of the delay in termination. At kill, the observed higher β -glucosidase activity at flowering stage against vegetative was also indicated in the earlier greenhouse study when averaged over all cover crops (chapter 3). At one year, flowering stage maintained higher β -glucosidase and urease activities at Bien Donne and higher phosphatase at Nietvoorbij, showing the persistent effect of higher cover crop residues on the soil surface, which in turn regulates temperature and improves moisture conservation. Soil enzymes such as β -glucosidase, urease and phosphatase are also sensitive to environmental factors, SOC, tillage and other management methods, making them an important soil quality indicator (Zhang et al., 2015). This study indicates that the selected soil enzymes discriminated between cover crop vegetative and flowering growth stages to some extent, in the short-term. However, a further field study is required to monitor and better evaluate the impact of the cover crop termination stages on soil enzyme activities, SOC build-up and N level in the long-term.

The observed significantly higher β -glucosidase, urease and phosphatase activities at one year, under slash plots compared to spray at Nietvoorbij plots is an indication that cover crop termination by slash stimulates biological processes compared to spraying glyphosate. While β -glucosidase indices from the previous greenhouse study (Chapter 3) are consistent with this observation, urease and phosphatase activities were not significantly affected. This study indicates that these three enzymes were not significantly affected by termination methods when averaged over all cover crops in the Bien Donne plots. Unlike killing cover crop by spraying glyphosate, slashing or disking favours shoot-soil contact and surface mulch coverage, which consequently improves moisture, regulates temperature and promotes biological activities in the soil (Liang et al., 2014, Jani et al., 2016). The accrual of glyphosate in the soil could be toxic to soil microorganisms thus, negatively affecting microbial processes and nutrient cycling. However, reports of glyphosate impact on soil microbial activity have been inconsistent with some studies indicating positive or no considerable impact in the short- and long-term (Lane et al., 2012, Arango et al., 2014). The results indicate site effect since no significant impact was observed at the Bien Donne field whereas cover crop termination by slash significantly and positively affected β -glucosidase, urease and phosphatase compared to spraying, at Nietvoorbij, in the short-term (one year). A continuous and long-term study is necessary to examine the impact of termination methods on soil enzyme activities, as well as N and C build-up under different soils/ecosystems.

5.5 Conclusion

This study suggests that delaying termination till flowering stage increase biomass, total C and C:N ratio and reduce total N, irrespective of cover crop species. Cover crop presence reduced the soil pH. Oats residues performed best with regards to elevating SOC in the short-term. Though there were variations in effects, all tested living cover crops and residues appeared to raise total soil N. The presence of living cover crops and residues had no main effect on the activities of the three enzymes, in the short-term. Cover crop termination at flowering stage favoured SOC and the activities of β -glucosidase, urease and phosphatase compared to the vegetative stage. However, the effect of termination stage on total soil N varied with sampling times, with flowering stage having greater total soil N at kill and the other way round at one year. Termination by slashing promoted SOC while spraying elevated total soil N at the Bien Donne site, whereas no impact was observed between termination methods at Nietvoorbij. The impact of termination methods on enzyme activities was only felt at the Nietvoorbij site with slash stimulating β -glucosidase, urease and phosphatase activities contrary to spray. Site effect was noticeable in this study, especially with regards to termination methods. The detected high significant interaction between sampling time, cover crop, termination stage and termination method on total soil N at both sites, is an indication that these management approaches should be considered for improving cover crop benefits in South African soils. However, the results of this field study suggest that longer time spans may be needed to explore the impact of cover crops on soil enzyme activities and SOC levels in this Mediterranean environment.

References

- Abbas, Z., Akmal, M. & Khan, K.S. 2014. Effect of buctril super (Bromoxynil) herbicide on soil microbial biomass and bacterial population. *Brazilian Archives of Biology and Technology*, 57, 9-14.
- Acosta-Martinez, V., Mikha, M.M., Sistani, K.R., Stahlman, P.W., Benjamin, J.G., Vigil, M.F. & Erickson, R. 2011. Multi-location study of soil enzyme activities as affected by types and rates of manure application and tillage practices. *Agriculture*, 1, 4-21.
- Adetunji, A.T., Lewu, F.B., Mulidzi, R. & Ncube, B. 2017. The biological activities of β -glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of Soil Science and Plant Nutrition*, 17, 794-807.
- Alonso-Ayuso, M., Gabriel, J.L. & Quemada, M. 2014. The kill date as a management tool for cover cropping success. *PloS one*, 9, e109587.
- Arango, L., Buddrus-Schiemann, K., Opelt, K., Lueders, T., Haesler, F., Schmid, M., Ernst, D. & Hartmann, A. 2014. Effects of glyphosate on the bacterial community associated with roots of transgenic Roundup Ready® soybean. *European Journal of Soil Biology*, 63, 41-48.
- Balkcom, K.S., Duzy, L.M., Kornecki, T.S. & Price, A.J. 2015. Timing of cover crop termination: Management considerations for the Southeast. *Crop, Forage & Turfgrass Management*, 1.

- Balota, E.L. & Chaves, J.C.D. 2010. Enzymatic activity and mineralization of carbon and nitrogen in soil cultivated with coffee and green manures. *Revista Brasileira de Ciência do Solo*, 34, 1573-1583.
- Benedict, C., Cogger, C.G. & Andrews, N. 2014. *Methods for successful cover crop management in your home garden*, Washington State University Extension.
- Blanco-Canqui, H., Holman, J.D., Schlegel, A.J., Tatarko, J. & Shaver, T.M. 2013. Replacing fallow with cover crops in a semiarid soil: Effects on soil properties. *Soil Science Society of America Journal*, 77, 1026-1034.
- Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A. & Hergert, G.W. 2015. Cover crops and ecosystem services: Insights from studies in temperate soils. *Agronomy Journal*, 107, 2449-2474.
- Bowman, R., Vigil, M., Nielsen, D. & Anderson, R. 1999. Soil organic matter changes in intensively cropped dryland systems. *Soil Science Society of America Journal*, 63, 186-191.
- Boyrahmadi, M. & Raiesi, F. 2018. Plant roots and species moderate the salinity effect on microbial respiration, biomass, and enzyme activities in a sandy clay soil. *Biology and Fertility of Soils*, 54, 509-521.
- Calderon, F.J., Nielsen, D., Acosta-Martinez, V., Vigil, M.F. & Drew, L. 2016. Cover crop and irrigation effects on soil microbial communities and enzymes in semiarid agroecosystems of the central Great Plains of North America. *Pedosphere*, 26, 192-205.
- Cheng, W. 2009. Rhizosphere priming effect: its functional relationships with microbial turnover, evapotranspiration, and C-N budgets. *Soil Biology and Biochemistry*, 41, 1795-1801.
- Cline, G.R. & Silvernail, A.F. 2002. Effects of cover crops, nitrogen, and tillage on sweet corn. *HortTechnology*, 12, 118-125.
- Coombs, C., Lauzon, J.D., Deen, B. & Van Eerd, L.L. 2017. Legume cover crop management on nitrogen dynamics and yield in grain corn systems. *Field Crops Research*, 201, 75-85.
- Dabney, S., Delgado, J. & Reeves, D. 2001. Using winter cover crops to improve soil and water quality. *Communications in Soil Science and Plant Analysis*, 32, 1221-1250.
- Dalal, R. 1979. Simple procedure for the determination of total carbon and its radioactivity in soils and plant materials. *Analyst*, 104, 151-154.
- Decker, A.M., Clark, A.J., Meisinger, J.J., Mulford, F.R. & McIntosh, M.S. 1994. Legume cover crop contributions to no-tillage corn production. *Agronomy Journal*, 86, 126-135.
- Delgado, J.A. & Gantzer, C.J. 2015. The 4Rs for cover crops and other advances in cover crop management for environmental quality. *Journal of Soil and Water Conservation*, 70, 142A-145A.
- Dignac, M.-F., Derrien, D., Barré, P., Barot, S., Cécillon, L., Chenu, C., Chevallier, T., Freschet, G.T., Garnier, P. & Guenet, B. 2017. Increasing soil carbon storage: mechanisms, effects of agricultural practices and proxies. A review. *Agronomy for Sustainable Development*, 37, 14.
- Drinkwater, L., Janke, R. & Rossoni-Longnecker, L. 2000. Effects of tillage intensity on nitrogen dynamics and productivity in legume-based grain systems. *Plant and Soil*, 227, 99-113.
- Du, Z., Xie, Y., Hu, L., Hu, L., Xu, S., Li, D., Wang, G. & Fu, J. 2014. Effects of fertilization and clipping on carbon, nitrogen storage, and soil microbial activity in a natural grassland in southern China. *PLoS one*, 9, e99385.
- Follmer, C. 2008. Insights into the role and structure of plant ureases. *Phytochemistry*, 69, 18-28.
- Fourie, J., Agenbag, G. & Louw, P. 2007. Cover crop management in a Chardonnay/99 Richter vineyard in the coastal region, South Africa. 3. Effect of different cover crops and cover crop management practices on organic matter and macro-nutrient content of a medium-textured soil. *South African Journal for Enology and Viticulture*, 28, 61.
- Fourie, J., Louw, P. & Agenbag, G. 2001. Effect of seeding date on the performance of grasses and broadleaf species evaluated for cover crop management in two wine grape regions of South Africa. *South African Journal of Plant and Soil*, 18, 118-127.
- García-Orenes, F., Guerrero, C., Roldán, A., Mataix-Solera, J., Cerdà, A., Campoy, M., Zornoza, R., Bárcenas, G. & Caravaca, F. 2010. Soil microbial biomass and activity under different

- agricultural management systems in a semiarid Mediterranean agroecosystem. *Soil and Tillage Research*, 109, 110-115.
- García-Ruiz, R., Ochoa, V., Hinojosa, M.B. & Carreira, J.A. 2008. Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. *Soil Biology and Biochemistry*, 40, 2137-2145.
- Haney, R., Senseman, S. & Hons, F. 2002. Effect of Roundup Ultra on microbial activity and biomass from selected soils. *Journal of Environmental Quality*, 31, 730-735.
- Hirpa, T. 2013. Effect of stage at termination of legume green manures on soil organic carbon, yield and economic performance of subsequent maize crop. *International Journal of Current Research and Academic Review*, 1, 84-101.
- Irvine, R.L., Crone, E.E., Jackson, L.J. & MacIsaac, E.A. 2004. Does scale affect ecological model predictions? A test with lake responses to fertilization. *Ecological Applications*, 14, 1178-1188.
- Jani, A.D., Grossman, J., Smyth, T.J. & Hu, S. 2016. Winter legume cover-crop root decomposition and N release dynamics under disking and roller-crimping termination approaches. *Renewable Agriculture and Food Systems*, 31, 214-229.
- Jat, M., Gathala, M., Saharawat, Y., Tatarwal, J. & Gupta, R. 2013. Double no-till and permanent raised beds in maize–wheat rotation of north-western Indo-Gangetic plains of India: Effects on crop yields, water productivity, profitability and soil physical properties. *Field Crops Research*, 149, 291-299.
- Keene, C., Curran, W., Wallace, J., Ryan, M., Mirsky, S., VanGessel, M. & Barbercheck, M. 2017. Cover crop termination timing is critical in organic rotational no-till systems. *Agronomy Journal*, 109, 272-282.
- Ketterings, Q.M., Swink, S.N., Duiker, S.W., Czymmek, K.J., Beegle, D.B. & Cox, W.J. 2015. Integrating cover crops for nitrogen management in corn systems on northeastern US dairies. *Agronomy Journal*, 107, 1365-1376.
- Lagomarsino, A., Moscatelli, M., Di Tizio, A., Mancinelli, R., Grego, S. & Marinari, S. 2009. Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic C in a Mediterranean environment. *Ecological Indicators*, 9, 518-527.
- Lane, M., Lorenz, N., Saxena, J., Ramsier, C. & Dick, R.P. 2012. Microbial activity, community structure and potassium dynamics in rhizosphere soil of soybean plants treated with glyphosate. *Pedobiologia*, 55, 153-159.
- Lawson, A., Cogger, C., Bary, A. & Fortuna, A.-M. 2015. Influence of seeding ratio, planting date, and termination date on rye-hairy vetch cover crop mixture performance under organic management. *PloS one*, 10, e0129597.
- Levene, H. 1960. Robust tests for equality of variances, p 278–292. *Contributions to probability and statistics: essays in honor of Harold Hotelling*. Stanford University Press, Palo Alto, CA.
- Liang, S., Grossman, J. & Shi, W. 2014. Soil microbial responses to winter legume cover crop management during organic transition. *European Journal of Soil Biology*, 65, 15-22.
- Limpens, J., Granath, G., Aerts, R., Heijmans, M.M., Sheppard, L.J., Bragazza, L., Williams, B.L., Rydin, H., Bubier, J. & Moore, T. 2012. Glasshouse vs field experiments: do they yield ecologically similar results for assessing N impacts on peat mosses? *New Phytologist*, 195, 408-418.
- Little, T.M. & Hills, F. 1972. *Statistical Methods in Agricultural Research* UCD Book Store, University of California Davis.
- Makoi, J.H. & Ndakidemi, P.A. 2008. Selected soil enzymes: examples of their potential roles in the ecosystem. *African Journal of Biotechnology*, 7, 181-191.
- Maltais-Landry, G. 2015. Legumes have a greater effect on rhizosphere properties (pH, organic acids and enzyme activity) but a smaller impact on soil P compared to other cover crops. *Plant and Soil*, 394, 139-154.

- Maseko, S. & Dakora, F. 2013. Rhizosphere acid and alkaline phosphatase activity as a marker of P nutrition in nodulated *Cyclopia* and *Aspalathus* species in the Cape fynbos of South Africa. *South African Journal of Botany*, 89, 289-295.
- Meyer, A.H., Wooldridge, J. & Dames, J.F. 2015. Variation in urease and β -glucosidase activities with soil depth and root density in a 'Cripp's Pink'/M7 apple orchard under conventional and organic management. *South African Journal of Plant and Soil*, 32, 227-234.
- Mirsky, S., Curran, W., Mortenseny, D., Ryany, M. & Shumway, D. 2011. Timing of cover-crop management effects on weed suppression in no-till planted soybean using a roller-crimper. *Weed Science*, 59, 380-389.
- Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R. & Shumway, D.L. 2009. Control of cereal rye with a roller/crimper as influenced by cover crop phenology. *Agronomy Journal*, 101, 1589-1596.
- Mischler, R., Duiker, S.W., Curran, W.S. & Wilson, D. 2010. Hairy vetch management for no-till organic corn production. *Agronomy Journal*, 102, 355-362.
- Mukherjee, A. & Lal, R. 2015. Short-term effects of cover cropping on the quality of a Typic Argiaquolls in Central Ohio. *Catena*, 131, 125-129.
- Mukumbareza, C., Muchaonyerwa, P. & Chiduzza, C. 2015. Effects of oats and grazing vetch cover crops and fertilisation on microbial biomass and activity after five years of rotation with maize. *South African Journal of Plant and Soil*, 32, 189-197.
- Muzangwa, L., Mnkeni, P.N.S. & Chiduzza, C. 2019. The use of residue retention and inclusion of legumes to improve soil biological activity in maize-based no-till systems of the Eastern Cape Province, South Africa. *Agricultural Research*, 1-11.
- Njunie, M., Wagger, M. & Luna-Orea, P. 2004. Residue decomposition and nutrient release dynamics from two tropical forage legumes in a Kenyan environment. *Agronomy Journal*, 96, 1073-1081.
- Okalebo, J., Gathua, K. & Woomey, P. 2002. Laboratory methods of soil and plant analysis. *A working manual*, 2, 29-68.
- Olson, K.R., Ebelhar, S.A. & Lang, J.M. 2010. Cover crop effects on crop yields and soil organic carbon content. *Soil Science*, 175, 89-98.
- Ott, R.L. & Longnecker, M.T. 2015. *An introduction to statistical methods and data analysis*, Nelson Education.
- Qian, X., Gu, J., Pan, H.-j., Zhang, K.-y., Sun, W., Wang, X.-j. & Gao, H. 2015. Effects of living mulches on the soil nutrient contents, enzyme activities, and bacterial community diversities of apple orchard soils. *European Journal of Soil Biology*, 70, 23-30.
- Ruis, S., Blanco-Canqui, H., Jasa, P.J., Ferguson, R. & Slater, G. 2017. Can cover crop use allow increased levels of corn residue removal for biofuel in irrigated and rainfed systems? *BioEnergy Research*, 10, 992-1004.
- Saini, M., Price, A.J., Van Santen, E., Arriaga, F.J., Balkcom, K.S. & Raper, R.L. 2008. Planting and termination dates affect winter cover crop biomass in a conservation-tillage corn-cotton rotation: Implications for weed control and yield. *Tifton, GA, USA. Southern Conservation Agricultural Systems*, 137-141.
- Sainju, U., Singh, B. & Whitehead, W. 2002. Long-term effects of tillage, cover crops, and nitrogen fertilization on organic carbon and nitrogen concentrations in sandy loam soils in Georgia, USA. *Soil and Tillage Research*, 63, 167-179.
- Sainju, U.M., Whitehead, W.F. & Singh, B.P. 2005. Biculture legume-cereal cover crops for enhanced biomass yield and carbon and nitrogen. *Agronomy Journal*, 97, 1403-1412.
- Schaller, K. 2009. Soil enzymes—valuable indicators of soil fertility and environmental impacts. *Bulletin UASVM Horticulture*, 66, 911-916.
- Schwartz, H.F., Langham, M.A., Golod, J., Tolin, S.A., LaForest, J. & Cardwell, K.F. 2009. Legume ipmPIPE: the next evolution of web-based interactive tools for disease management and extension outreach. *APSnet* (<http://www.apsnet.org/online/feature/ipmPIPE/>).
- Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika*, 591-611.

- Shaw, K. 2006. Determination of organic carbon in soil and plant material. *European Journal of Soil Science*, 10, 316-326.
- Steenwerth, K. & Belina, K. 2008. Cover crops and cultivation: Impacts on soil N dynamics and microbiological function in a Mediterranean vineyard agroecosystem. *Applied Soil Ecology*, 40, 370-380.
- Sullivan, D.M. & Andrews, N. 2012. *Estimating plant-available nitrogen release from cover crops*, Oregon State University Extension Service.
- Thilakarathna, M.S., Serran, S., Lauzon, J., Janovicek, K. & Deen, B. 2015. Management of manure nitrogen using cover crops. *Agronomy Journal*, 107, 1595-10607.
- Thorup-Kristensen, K. 2006. Effect of deep and shallow root systems on the dynamics of soil inorganic N during 3-year crop rotations. *Plant and Soil*, 288, 233-248.
- Thorup-Kristensen, K., Magid, J. & Jensen, L.S. 2003. Catch crops and green manures as biological tools in nitrogen management in temperate zones. *Advances in Agronomy*, 79, 227-302.
- Thorup-Kristensen, K. & Dresbøll, D.B. 2010. Incorporation time of nitrogen catch crops influences the N effect for the succeeding crop. *Soil Use and Management*, 26, 27-35.
- Wayman, S., Cogger, C., Benedict, C., Burke, I., Collins, D. & Bary, A. 2015. The influence of cover crop variety, termination timing and termination method on mulch, weed cover and soil nitrate in reduced-tillage organic systems. *Renewable Agriculture and Food Systems*, 30, 450-460.
- Wortman, S.E., Francis, C.A., Bernards, M.A., Blankenship, E.E. & Lindquist, J.L. 2013. Mechanical termination of diverse cover crop mixtures for improved weed suppression in organic cropping systems. *Weed Science*, 61, 162-170.
- XU, L.-f., Peng, Z., Han, Q.-f., Li, Z.-h., Yang, B.-p. & Nie, J.-f. 2013. Spatial distribution of soil organic matter and nutrients in the pear orchard under clean and sod cultivation models. *Journal of Integrative Agriculture*, 12, 344-351.
- Zadoks, J.C., Chang, T.T. & Konzak, C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research*, 14, 415-421.
- Zhang, L., Chen, W., Burger, M., Yang, L., Gong, P. & Wu, Z. 2015. Changes in soil carbon and enzyme activity as a result of different long-term fertilization regimes in a greenhouse field. *PloS one*, 10, e0118371.

CHAPTER 6

GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary and conclusions

It is important to adopt reliable soil management practices that improve soil fertility and crop yield. Cover crop management is an essential and a sustainable approach to provide soil organic C and N, and improve soil physical, chemical and biological properties. However, proper management is required to realize many of the benefits associated with cover crops. The impacts of cover crop management particularly species selection, termination stage and termination methods on soil enzyme activities and fertility are still not well understood. This study reports the impact of two termination stages on the biomass, total C, total N and C:N ratios of four cover crops. It also reports the short-term effect of living cover crops and residues, termination stages and termination methods on soil pH, organic C, total soil N and the activities of glucosidase, urease and phosphatase. The first part of the thesis reports results of a greenhouse experiment. The second part of the thesis reports the results of a field experiment which was carried out at two sites. This study constitutes a new report on national and global understanding of the integration of different cover crops and management practices in a single consolidated research; and therefore extends our knowledge of the appropriate soil fertility management practices in grape-based farming system of the Western Cape Province. This study could find relevance internationally if applied in similar soil types and Mediterranean climate. The prominent results are summarized in this chapter followed by suggestions for future works.

In the greenhouse study, it was observed that delaying termination till flowering stage increased total C and C:N ratio, and decreased total N concentration of all the tested cover crop species. This is an indication that C, N and C:N ratios of oats, pea, vetch and rye can be manipulated for specific cover crop management goals. Cover crop presence had no effect on soil pH but sampling time did. Rye and oats residues are the most promising of the cover crop species with regard to SOC build-up. Pea and vetch followed by rye are better cover crop options to farmers in increasing soil N levels. The living rye, oats, pea and vetch, stimulated soil β -glucosidase, urease and phosphatase activities, whereas their respective residues considerably increased only β -glucosidase and phosphatase activities at one year. Soil organic carbon levels varied with sampling time and termination stages with vegetative being higher than flowering at kill; and flowering being higher than vegetative at one year. Cover crop termination at flowering stage contributed more significantly to the stimulation of β -glucosidase activity compared to vegetative. However, termination at vegetative stage raised soil N levels and phosphatase activity irrespective of cover crop species and sampling time. While cover crop termination by slash had a positive

impact on SOC and β -glucosidase activity compared to spray, termination methods had no significant effect on soil N levels and the activities of urease and phosphatase, at one year. In general, there was high significant interaction between sampling time, cover crop and termination stage, indicating that cover crop and growth termination stage have greater impact on SOC, soil total N, β -glucosidase and urease activities relative to termination methods. This greenhouse study suggests that cover crop chemical composition/quality and termination stage should be considered when selecting management methods that improve SOC, soil N level and microbial activity.

The field study affirms that postponing termination till flowering stage increase plant biomass, total C and C:N ratio and reduce total N concentration, irrespective of cover crop species. The results of this study also indicate that the levels of these cover crop biomass and chemical components vary with species. The presence of cover crops led to reduction of soil pH within one year. Oats residues performed best with regards to elevating SOC in the short-term. Though there were variations in effects, all tested living cover crops and residues increased total soil N. However, β -glucosidase, urease and phosphatase did not seem to respond to living cover crops and residues, in the short-term. Cover crop termination at flowering stage promoted SOC and the activities of β -glucosidase, urease and phosphatase than the vegetative stage. Termination by slash raised SOC while spray elevated total soil N at the Bien Donne site, whereas no impact was observed between termination methods at Nietvoorbij. The impact of termination methods on enzyme activities was only observed at the Nietvoorbij site with slash stimulating the activities of the three enzymes contrary to spray. Site effects (micro climate and soil types) was noticeable in this study, especially with regards to termination methods. Cover crop and growth termination stage have greater impact on SOC, soil total N, β -glucosidase and urease activities relative to termination methods. Notably, the observed high significant interaction between sampling time, cover crop, termination stage and termination method on total soil N at both sites, indicates that these management methods are important for maximizing the cover crop benefits in agricultural systems. However, findings from the field study indicate that longer time spans may have been needed to examine the response of soil enzyme and SOC to cover crops in this Mediterranean environment. The entire soil physicochemical and biological properties of pot experiment are contained in a relatively small area whom activities are confined compared to field situation where there is extended surface area. This phenomenon causes delay in observation/detection on the field compared with pot experiment.

6.2 Recommendations

Although this thesis reports the successful evaluation of the short-term impact of cover crop species, termination stages and termination methods on important soil chemical and biological properties; longer-term studies should be done in the field to see whether or not the observed trends are permanent. Further research should be done to evaluate the impact of the different cover crop management approaches, including soil organic carbon and nitrogen contribution to the subsequent cash crop. It is important to also investigate the effect of termination stages and termination methods on soil enzyme activities using cover crop mixtures composed of the most promising species highlighted in this study.

APPENDICES

Appendix A: Chapter 3 analysis of variance (Pr > F) for soil organic carbon and β -glucosidase activity in combined sampling times as influenced by cover crop species, termination stage, and termination method

Source	DF	SOC	β -glucosidase
Method (M)	1	<.0001	0.0008
Stage (S)	1	<.0001	<.0001
M x S	1	0.0080	0.0023
Block (Method*Stage)	8	0.5316	0.6695
Cover crop (CC)	4	0.1360	0.0003
CC x M	4	0.0002	0.5139
CC x S	4	0.0270	0.0479
CC x M x S	4	0.6749	0.2385
Block (Method*Stage*Cover Crop)	32	-	-
Sampling Time (ST)	1	0.1420	<.0001
ST x M	1	<.0001	0.198
ST x S	1	<.0001	<.0001
ST x M x S	1	<.0001	0.4176
ST x CC	4	0.2262	0.6093
ST x CC x M	4	0.0602	0.5232
ST x CC x S	4	0.0363	0.0083
ST x CC x M x S	4	0.0577	0.0572
Error	40	-	-
Corrected Total	119	-	-

Appendix B: Chapter 3 Effect of cover crop species and termination method (slash and spray) on soil organic carbon (%) and β -glucosidase activity ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$) at one year

Sampling time	Treatment		SOC	β -glucosidase
	Cover crop	Method		
One year	Oats	Slash	0.84 (± 0.06) a	79.01 (± 3.58) ab
		Spray	0.76 (± 0.08) bc	74.06 (± 3.90) abc
	Pea	Slash	0.77 (± 0.06) bc	82.91 (± 4.87) a
		Spray	0.75 (± 0.08) c	66.38 (± 1.96) cd
	Rye	Slash	0.82 (± 0.07) ab	82.89 (± 3.12) a
		Spray	0.81 (± 0.08) abc	72.27 (± 5.97) bc
	Vetch	Slash	0.81 (± 0.06) ab	75.37 (± 5.18) abc
		Spray	0.74 (± 0.07) c	60.61 (± 1.50) d
	Control	Slash	0.74 (± 0.06) c	62.08 (± 2.08) d
		Spray	0.77 (± 0.07) bc	60.03 (± 3.42) d
	LSD		0.06	9.23

Each value represents the mean ($n = 3$), and standard error values are indicated in parenthesis. Different letters indicate significant differences at $P < 0.05$ according to Fisher's least significant difference test (LSD)

Appendix C: Chapter 4 Cover crop species and termination method (slash and spray) effects on soil nitrogen and the activities of urease and phosphatase at one year

Treatment		Soil nitrogen	Urease	Phosphatase
Cover crop	Method	(%)	($\mu\text{g NH}_4^+ \text{g}^{-1} \text{soil 2 h}^{-1}$)	($\mu\text{g PNP g}^{-1} \text{soil h}^{-1}$)
Oats	Slash	0.24 (± 0.04) cd	9.78 (± 2.03) abc	84.02 (± 16.23) a
	Spray	0.21 (± 0.04) de	10.93 (± 1.93) ab	76.06 (± 9.68) ab
Pea	Slash	0.26 (± 0.02) bcd	9.39 (± 1.94) bc	83.16 (± 2.44) ab
	Spray	0.32 (± 0.04) a	10.07 (± 1.99) abc	76.64 (± 12.67) ab
Rye	Slash	0.23 (± 0.04) cd	9.33 (± 1.79) bc	69.15 (± 2.82) abc
	Spray	0.23 (± 0.05) cd	13.16 (± 1.93) a	65.05 (± 5.66) bc
Vetch	Slash	0.29 (± 0.01) ab	9.68 (± 1.52) abc	77.37 (± 5.54) ab
	Spray	0.28 (± 0.01) abc	9.42 (± 0.72) bc	55.38 (± 4.02) c
Control	Slash	0.16 (± 0.04) f	5.21 (± 1.30) d	53.29 (± 4.37) c
	Spray	0.18 (± 0.03) ef	6.54 (± 1.78) d	70.38 (± 5.47) abc
LSD		0.05	3.58	18.81

Each value represents the mean (n = 3) and standard error values are indicated in parenthesis. Different letters within a column indicate significant differences at P<0.05 according to Fisher's least significant difference test (LSD)

Appendix D: Chapter 5 analysis of variance (Pr > F) for SOC and total soil nitrogen data in combined sampling times as affected by cover crop species, termination stage, and termination method at Nietvoorbij and Bien Donne sites

Source	DF	Nietvoorbij		Bien Donne	
		SOC	Soil nitrogen	SOC	Soil nitrogen
Method (M)	1	0.7579	0.151	0.0003	<.0001
Stage (S)	1	0.012	<.0001	<.0001	0.4252
M x S	1	0.0023	0.7038	0.0139	0.675
Block (Method*Stage)	8	0.8273	0.1556	0.2045	0.0321
Cover crop (CC)	4	0.0014	0.0014	0.4271	<.0001
CC x M	4	0.3251	0.5132	0.0216	<.0001
CC x S	4	0.7756	0.0051	0.186	0.3224
CC x M x S	4	0.0156	0.4719	0.2499	0.3415
Block (Method*Stage*Cover Crop)	32	-	-	-	-
Sampling Time (ST)	1	0.0328	<.0001	0.3066	<.0001
ST x M	1	0.0962	0.0408	0.0856	0.7407
ST x S	1	0.0007	<.0001	<.0001	0.0005
ST x M x S	1	0.0034	0.6636	0.0184	0.4934
ST x CC	4	0.0036	0.0104	0.0005	<.0001
ST x CC x M	4	0.35	0.5121	0.0034	<.0001
ST x CC x S	4	0.8984	<.0001	0.0167	0.3397
ST x CC x M x S	4	0.4154	0.0005	0.0083	0.0064
Error	40	-	-	-	-
Corrected Total	119	-	-	-	-

Appendix E: Chapter 5 analysis of variance (Pr > F) for soil β -glucosidase, urease and phosphatase activities data in combined sampling times as affected by cover crop species, termination stage, and termination method at Nietvoorbij and Bien Donne sites

Source	DF	Nietvoorbij			Bien Donne		
		β -glucosidase	Urease	Phosphatase	β -glucosidase	Urease	Phosphatase
Method (M)	1	0.3886	0.0027	0.1654	0.5658	0.1423	0.9763
Stage (S)	1	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001
M x S	1	0.0994	0.1346	0.0085	0.0103	0.0089	0.1182
Block (Method*Stage)	8	0.9885	0.7388	0.6211	0.5519	0.6015	0.4234
Cover crop (CC)	4	0.6034	0.2467	0.7363	0.7261	0.5602	0.8876
CC x M	4	0.6379	0.1622	0.7409	0.9174	0.4504	0.0436
CC x S	4	0.8072	0.6798	0.9516	0.7991	0.5184	0.5643
CC x M x S	4	0.7633	0.4579	0.9804	0.9674	0.8035	0.3738
Block (Method*Stage*Cover Crop)	32	-	-	-	-	-	-
Sampling Time (ST)	1	0.455	0.0116	0.6821	<.0001	<.0001	0.0043
ST x M	1	0.0608	0.0066	0.1763	0.1431	0.1279	0.1458
ST x S	1	<.0001	<.0001	0.9264	0.0005	<.0001	<.0001
ST x M x S	1	0.0008	0.0029	0.292	0.299	0.8867	0.6673
ST x CC	4	0.557	0.496	0.091	0.7772	0.4033	0.292
ST x CC x M	4	0.6345	0.9581	0.4691	0.5487	0.4929	0.3384
ST x CC x S	4	0.7407	0.9485	0.5497	0.7356	0.1235	0.5283
ST x CC x M x S	4	0.4816	0.2317	0.3824	0.4224	0.3316	0.0161
Error	40	-	-	-	-	-	-
Corrected Total	119	-	-	-	-	-	-

Appendix F: Chapter 5 cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) effects on SOC and total soil nitrogen at different sampling times (kill and one year) at Nietvoorbij and Bien Donne sites

Sampling time	Cover crop	Termination stage	Nietvoorbij SOC (%)	Soil nitrogen (%)	Bien Donne SOC (%)	Soil nitrogen (%)
Kill	Oats	Vegetative	1.03 de	0.16 j	0.80 hij	0.19 gh
	Pea		1.02 de	0.17 ij	1.04 cde	0.23 def
	Rye		0.99 e	0.17 ij	0.94 efg	0.13 ij
	Vetch		1.05 cde	0.24 h	0.89 fghi	0.20 fg
	Control		1.02 de	0.14 j	0.83 ghij	0.08 k
	Oats	Flowering	1.24 abcd	0.38 bcde	1.08 bc	0.23 cdef
	Pea		1.09 bcde	0.34 def	1.01 cdef	0.25 bcde
	Rye		1.17 abcde	0.37 cde	1.07 bcd	0.14 ij
	Vetch		1.38 a	0.40 bc	1.00 cdef	0.27 bc
	Control		1.31 ab	0.31 fg	0.95 defg	0.11 jk
One year	Oats	Vegetative	1.38 a	0.39 bcd	0.90 fgh	0.21 fg
	Pea		1.18 abcde	0.47 a	0.75 j	0.39 a
	Rye		1.09 bcde	0.48 a	0.77 ij	0.14 ij
	Vetch		1.20 abcde	0.43 ab	0.76 j	0.23 def
	Control		1.18 abcde	0.22 hi	0.76 j	0.15 hi
	Oats	Flowering	1.27 abc	0.36 cdef	1.16 ab	0.22 efg
	Pea		1.11 bcde	0.35 cdef	1.06 bcd	0.26 bcd
	Rye		1.10 bcde	0.27 gh	1.03 cde	0.15 hi
	Vetch		1.10 bcde	0.32 efg	1.05 bcde	0.27 b
	Control		1.11 bcde	0.34 def	1.23 a	0.16 hi
LSD			0.23	0.06	0.12	0.04

Each value represents the mean (n = 3). Different letters within a column indicate significant differences at P<0.05 according to Fisher's least significant difference test (LSD)

Appendix G: Chapter 5 effects of cover crop species (oats, pea, rye and vetch) and termination stage (vegetative and flowering) on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}), urease ($\mu\text{g NH}_4^+ \text{g}^{-1}$ soil 2 h^{-1}) and phosphatase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) over two sampling times at Nietvoorbij and Bien Donne study sites

Sampling time	Cover crop	Termination stage	Nietvoorbij			Bien Donne			
			β -glucosidase	Urease	Phosphatase	β -glucosidase	Urease	Phosphatase	
Kill	Oats	Vegetative	119.50 gh	17.86 fg	566.54 c	66.03 g	12.18 e	175.41 gh	
			Pea	133.40 fgh	18.23 fg	683.28 bc	92.44 def	16.29 e	215.14 fgh
			Rye	109.64 h	15.81 g	505.96 c	69.65 fg	13.18 e	192.80 gh
			Vetch	128.97 fgh	19.83 defg	610.24 bc	72.90 fg	15.06 e	240.07 efg
			Control	127.49 fgh	18.79 efg	553.99 c	77.97 efg	14.29 e	156.37 h
	Oats	Flowering	284.00 a	45.61 ab	690.50 bc	124.37 abc	30.10 a	329.29 abcd	
			Pea	252.16 abc	40.58 abc	940.47 a	115.39 abcd	24.45 bcd	316.87 bcd
			Rye	266.97 ab	44.76 ab	621.36 bc	114.15 abcd	24.23 bcd	391.77 a
			Vetch	280.12 a	43.84 abc	677.94 bc	114.64 abcd	21.99 d	348.82 abc
			Control	202.01 bcdef	37.81 abc	848.84 ab	139.71 a	29.66 ab	372.57 ab
One year	Oats	Vegetative	210.11 abcde	46.32 a	611.20 bc	109.26 bcd	26.70	306.71 bcde	
			Pea	169.65 defgh	32.27 bcde	535.45 c	118.78 abc	27.04 abcd	343.47 abcd
			Rye	163.90 defgh	33.25 abcd	553.96 c	110.17 bcd	28.09 abc	282.79 cdef
			Vetch	148.40 efgh	33.78 abc	543.04 c	102.86 cde	24.02 cd	297.07 cde
			Control	157.62 defgh	36.38 abc	614.42 bc	111.61 bcd	27.11 abcd	271.92 def
	Oats	Flowering	231.10 abcd	41.89 abc	734.34 abc	128.69 ab	31.42 a	300.77 bcde	
			Pea	155.27 defgh	31.02 cdef	651.34 c	128.00 abc	31.04 a	326.85 abcd
			Rye	203.24 bcdef	39.67 abc	823.63 ab	125.10 abc	30.89 a	287.67 cdef
			Vetch	190.53 cdefg	32.15 bcde	741.80 abc	120.25 abc	28.23 abc	335.62 abcd
			Control	184.5 cdefgh	32.62 bcd	730.0 abc	126.87 abc	27.49 abcd	337.61 abcd
LSD			76.20	13.59	247.72	25.67	5.54	74.00	

Each value represents the mean ($n = 3$). Different letters within a column indicate significant differences at $P < 0.05$ according to Fisher's least significant difference test (LSD)

Appendix H: Chapter 5 effects of cover crop species (oats, pea, rye and vetch) and termination method (slash and spray) on soil organic C and total N at one year in Nietvoorbij and Bien Donne study sites

Sampling time	Cover crop	Termination method	Nietvoorbij SOC (%)	Soil nitrogen (%)	Bien Donne SOC (%)	Soil nitrogen (%)	
One year	Oats	Slash	1.34 a	0.32 ab	1.00 a	0.18 c	
	Pea		1.11 bc	0.32 ab	0.96 ab	0.31 a	
	Rye		1.13 bc	0.33 ab	1.02 a	0.14 d	
	Vetch		1.19 bc	0.35 ab	0.93 ab	0.19 c	
	Control		1.14 bc	0.25 c	0.95 ab	0.12 d	
	Oats	Spray	1.22 abc	0.34 ab	0.92 ab	0.26 b	
	Pea		1.10 c	0.36 a	0.95 ab	0.24 b	
	Rye		1.11 bc	0.31 abc	0.75 c	0.14 d	
	Vetch		1.23 ab	0.36 a	0.87 b	0.32 a	
	Control		1.20 bc	0.29 bc	0.89 b	0.19 d	
	LSD			0.13	0.06	0.11	0.03

Each value represents the mean (n = 3). Values within the same column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's least significant difference test (LSD)

Appendix I: Chapter 5 effects of cover crop species (oats, pea, rye and vetch) and termination method (slash and spray) on the activities of soil β -glucosidase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}), urease ($\mu\text{g NH}_4^+ \text{g}^{-1}$ soil 2 h^{-1}) and phosphatase ($\mu\text{g PNP g}^{-1}$ soil h^{-1}) at one year in Nietvoorbij and Bien Donne study sites

Sampling time	Cover crop	Termination method	Nietvoorbij			Bien Donne			
			β -glucosidase	Urease	Phosphatase	β -glucosidase	Urease	Phosphatase	
One year	Oats	Slash	222.21 ab	51.35 a	717.05 a	127.08 a	32.47 a	301.30 ab	
			Pea	176.26 abc	34.99 bcd	660.16 a	129.44 a	30.14 ab	371.66 a
			Rye	235.76 a	46.71 ab	749.54 a	122.88 a	31.94 a	321.07 ab
			Vetch	189.90 abc	38.61 abcd	715.81 a	116.34 a	27.89 ab	307.77 ab
			Control	178.32 abc	40.83 abc	702.95 a	119.43 a	26.16 ab	284.44 ab
	Oats	Spray	219.01 ab	36.85 bcd	628.50 a	110.86 a	25.64 ab	306.18 ab	
			Pea	148.67 bc	28.30 cd	526.63 a	117.34 a	27.95 ab	298.66 ab
			Rye	131.38 c	26.21 d	628.05 a	112.40 a	27.04 ab	248.39 b
			Vetch	149.03 bc	27.33 cd	569.03 a	106.77 a	24.36 b	324.91 ab
			Control	163.78 abc	28.18 cd	641.49 a	119.06 a	28.44 ab	325.09 ab
	LSD			81.50	13.53	234.92	39.58	6.99	109.53

Each value represents the mean (n = 3). Values within the same column followed by the same letter are not significantly different at $P < 0.05$ according to Fisher's least significant difference test

Appendix J: Chapter 3 and 4 analysis of variance (Pr > F) for soil organic matter (SOM) and soil alteration index three (AI3) data in combined sampling times as affected by cover crop species, termination stage, and termination method

Source	DF	SOM (%)	Soil AI3
Method (M)	1	<.0001	0,0415
Stage (S)	1	<.0001	<.0001
M x S	1	0.0080	0,5348
Block (Method*Stage)	8	0.5320	0,1545
Cover crop (CC)	4	0.1363	<.0001
CC x M	4	0.0002	0,2394
CC x S	4	0.0269	0,0313
CC x M x S	4	0.6767	0,2152
Block (Method*Stage*Cover Crop)	32		
Sampling Time (ST)	1	0.1417	<.0001
ST x M	1	<.0001	0,6236
ST x S	1	<.0001	<.0001
ST x M x S	1	<.0001	0,0152
ST x CC	4	0.2280	0,0007
ST x CC x M	4	0.0597	0,3466
ST x CC x S	4	0.0361	0,0516
ST x CC x M x S	4	0.0580	0,2619
Error	40	-	-
Corrected Total	119	-	-

The AI3 values were calculated with the following equation: AI3 = 7.87 β -glucosidase - 8.22 phosphatase - 0.49 urease

Appendix K: Chapter 3 and 4 cover crop species and sampling time effects on soil organic matter (SOM) and soil alteration index three (AI3) at different sampling times (kill and one year)

Sampling time	Cover crop	SOM (%)	AI3
Kill	Oats	1,29 (± 0.09) bc	-9,69 (± 0.87) d
	Pea	1,26 (± 0.08) c	-7,66 (± 1.39) c
	Rye	1,30 (± 0.08) abc	-10,68 (± 0.93) d
	Vetch	1,36 (± 0.08) abc	-7,62 (± 1.48) c
	Control	1,34 (± 0.10) abc	-5,10 (± 1.31) b
One year	Oats	1,38 (± 0.09) ab	-2,94 (± 0.57) a
	Pea	1,31 (± 0.09) abc	-2,88 (± 0.53) a
	Rye	1,40 (± 0.08) a	-2,33 (± 0.36) a
	Vetch	1,34 (± 0.08) abc	-2,42 (± 0.29) a
	Control	1,30 (± 0.07) abc	-1,64 (± 0.40) a
LSD		0.11	1.56

The AI3 values were calculated with the following equation: $AI3 = 7.87 \beta\text{-glucosidase} - 8.22 \text{phosphatase} - 0.49 \text{urease}$. Each value represents the mean ($n = 3$) and standard error values are indicated in parenthesis. Different letters within a column indicate significant differences at $P < 0.05$ according to Fisher's least significant difference test (LSD)

Appendix L: Chapter 3 and 4 cover crop species and termination stage effects on soil organic matter (SOM) and soil alteration index three (AI3) at different sampling times (kill and one year)

Sampling time	Cover crop	Termination stage	SOM (%)	Soil AI3
Kill	Oats	Vegetative	1,47 (±0.14) cdef	-12,01 (±0,65) g
	Pea		1,44 (±0.10) def	-11,73 (±0,94) fg
	Rye		1,50 (±0.09) bcde	-11,84 (±0,57) g
	Vetch		1,60 (±0.03) abc	-11,36 (±1,85) fg
	Control		1,37 (±0.12) ef	-8,99 (±0,60) e
	Oats	Flowering	1,11 (±0.05) g	-7,37 (±0,88) e
	Pea		1,07 (±0.05) g	-3,59 (±1,00) bcd
	Rye		1,10 (±0.02) g	-9,53 (±1,72) ef
	Vetch		1,12 (±0.03) g	-3,88 (±0,78) cd
	Control		1,31 (±0.18) f	-1,21 (±1,08) a
One year	Oats	Vegetative	1,10 (±0.05) g	-4,27 (±0,69) d
	Pea		1,06 (±0.05) g	-3,64 (±0,75) bcd
	Rye		1,14 (±0.03) g	-2,43 (±0,66) abcd
	Vetch		1,09 (±0.04) g	-2,99 (±0,27) abcd
	Control		1,07 (±0.03) g	-2,16 (±0,57) abcd
	Oats	Flowering	1,65 (±0.02) ab	-1,60 (±0,50) ab
	Pea		1,55 (±0.08) abcd	-2,12 (±0,65) abcd
	Rye		1,67 (±0.04) a	-2,23 (±0,34) abcd
	Vetch		1,58 (±0.03) abcd	-1,84 (±0,41) abc
	Control		1,53 (±0.05) abcd	-1,12 (±0,52) a
LSD			0.15	2.21

The AI3 values were calculated with the following equation: $AI3 = 7.87 \beta\text{-glucosidase} - 8.22 \text{phosphatase} - 0.49 \text{urease}$. Each value represents the mean (n = 3) and standard error values are indicated in parenthesis. Different letters within a column indicate significant differences at P<0.05 according to Fisher's least significant difference test (LSD)

Appendix M: Chapter 3 and 4 the overall effect of termination stage (vegetative and flowering) on soil organic matter (SOM) and soil alteration index three (AI3) at different sampling times (kill and one year)

Sampling time	Termination stage	SOM (%)	Soil AI3
Kill	Vegetative	1,47 (±0.04) b	-11,19 (±0.48) d
I	Flowering	1,14 (± 0.04) c	-5,12 (± 0.72) c
One year	Vegetative	1,09 (±0.02) c	-3,10 (±0.29) b
	Flowering	1,60 (±0.02) a	-1,78 (±0.22) a
LSD		0.07	0.99

The AI3 values were calculated with the following equation: $AI3 = 7.87 \beta\text{-glucosidase} - 8.22 \text{phosphatase} - 0.49 \text{urease}$. Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

Appendix N: Chapter 3 and 4 the overall effect of termination method (slash and spray) on soil organic matter (SOM) and soil alteration index three (AI3) at one year

Termination method	SOM (%)	Soil AI3
Slash	1,37 (± 0.05) a	-2,14 (± 0.25) a
Spray	1,32 (± 0.05) b	-2,74 (± 0.30) b
LSD	0.05	0.60

The AI3 values were calculated with the following equation: $AI3 = 7.87 \beta\text{-glucosidase} - 8.22 \text{phosphatase} - 0.49 \text{urease}$. Standard error values are indicated in parenthesis. Different letters within a column indicate significant differences ($P < 0.05$) among treatments using Fisher's least significant difference test (LSD)

