THE IMPACT OF CONSERVATION TILLAGE ON SOIL QUALITY AND POTENTIAL FOR CLIMATE CHANGE MITIGATION

Shamsudheen Mangalassery, M.Sc. (Agri.)

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

2013

Abstract

Conservation tillage is generally considered as an important component of sustainable agriculture. The benefits of conservation tillage have been presented as reducing runoff, enhancing water retention and preventing soil erosion. There is also general agreement that it can be used to conserve and enhance soil organic carbon levels to some extent. However, its applicability in mitigating climate change has been extensively debated, especially when the whole profile of carbon in soil is considered along with a reported risk of enhanced N_2O emissions under conservation tillage. The suitability of conservation tillage in mitigating climate change and enhancing carbon sequestration is addressed in this research in an integrated approach combining characterisation of the soil porous architecture and other chemical and biological properties. Novel analytical tools such as X-ray Computed Tomography were used to characterise the 3-D soil pore network under conservation tillage for the first time. The study indicated zero tilled soils had a lower net emission of greenhouse gases on a CO_2 equivalent basis indicating potentially zero tillage can be used to mitigate climate change. The net global warming potential under conventional tillage was 20% higher than zero tilled soil. A model developed to predict the greenhouse gas emissions from soil found that soil pore characteristics such as porosity played a significant role in the emission of greenhouse gases such as CO_2 and CH_4 among other factors such as microbial biomass carbon, bulk density and shear strength. Soil porosity alone accounted for 39.7% of the total variation for CO_2 flux which was larger than any other parameter including microbial biomass carbon and soil carbon. Soil pore characteristics were revealed as one of the important determinant in aiding the GHG flux in soil. However N_2O emission from soil

was mainly dependent on soil moisture, microbial biomass carbon and microbial biomass nitrogen. It was also found that zero tilled soils contained 9% more soil carbon and 30% higher microbial biomass carbon than the tilled soil. It was found that tillage mediated aggregate changes could bring changes in carbon storage in soil depending on texture of soil. Increased microbial activity was evident at zero tilled soils as observed from the increased activities of hydrolysing and oxidising enzymes. The preservation of aromatic structures during residue decomposition might have contributed to enhanced sequestration of carbon under zero tilled soils as revealed by the FTIR data. The study indicates that soil management practices strongly influence other properties and by making a suitable choice of the tillage system, a comparative reduction in greenhouse gas emissions could be achieved at the same time enhancing sequestration of carbon.

Publications from this work

Peer reviewed publications included in the thesis

Mangalassery S, Sjögersten S, Sparkes DL, Sturrock CJ, Mooney SJ (2013) The effect of soil aggregate size on pore structure and its consequence on emission of greenhouse gases. Soil and Tillage Research, **132,** 39-46.

Mangalassery S, Sjögersten S, Sparkes DL, Sturrock CJ, Craigon J, Mooney SJ (2013) To what extent can zero tillage lead to a reduction in greenhouse gas emissions?

Abstract of papers presented in seminars (not included in this thesis)

Mangalassery S, Sjogersten S, Sparkes DL, Sturrock C, Mooney SJ (2012a) In 4th International Congress of European Confederation of Soil Science Societies (Eurosoil 2012)-Soil Science for the benefit of mankind and environment Bari, Italy.

Mangalassery S, Sjogersten S, Sparkes DL, Sturrock C, Mooney SJ (2012b) In British Society of Soil Science Annual Meeting 2012. University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire.

Acknowledgements

I wish to express my deep sense of gratitude and indebtedness to Prof. Sacha Mooney, Dr. Sofie Sjogersten and Dr. Debbie Sparkes for their excellent guidance and constant encouragement throughout the period of my PhD and the training they have given to develop professional academic skills. It was indeed great pleasure and privilege studying under their guidance.

I am grateful to all technical staff that helped me to complete this work in time; John Corrie and Darren Hepworth for their support for laboratory work, purchase, training and administrative help, John Alcock and William Donger for sampling help at Sutton Bonington and Julietta Marquez and Neil Saunders. I am extremely thankful to Emma Hooley for the excellent administrative support rendered. All the staff members and colleagues were always helpful and friendly to me and many thanks for that. The help I received for field sampling from Boris Lazarevic and Aveen Khalil is thankfully acknowledged.

I am overwhelmed with sincere feelings of indebtedness to my beloved, parents, brothers, sisters, brother-in-laws and sister-in-laws for their abundant love and affection showered on me, which inspired me in completing my studies.

I heartily express my special thanks to Dr. Craig Sturrock for his help and co-operation throughout my PhD study especially for training with X-ray CT, Dr. Brian Atkinson for training to use gas chromatography and Dr. Sarah Martin for helping with Biolog ecoplating.

I am grateful to the **Indian Council of Agricultural Research**, New Delhi for providing financial assistance in the form of **International Fellowship** and to the **University of Nottingham** for providing **International Research Excellence Scholarship** without which it could not have been possible to pursue my PhD at this prestigious University and also to the British Society of Soil Science for providing travel grants to attend conferences. I express my sincere gratitude to the Director, Central Arid Zone Research Institute, Jodhpur for granting me permission to pursue PhD studies.

I am extremely thankful to my lovely wife Sanu and cute daughters Munny and Minu for their love and support throughout my studies. Any omission in this brief acknowledgement does not mean lack of gratitude.

AUGUST, 2013 **(SHAMSUDHEEN M)**

CONTENTS

Abbreviations and acronyms

µ	Micro
µA	Micro ampere
AWCD	Average well colour development
CH_4	Methane
CO_2	Carbon dioxide
CT	Computed Tomography
FTIR	Fourier Transform Infrared spectroscopy
g	Gram
GC	Gas chromatography
GE	Glucose equivalents
GHG	Greenhouse gas
GWP	Global warming potential
h	Hour
IPCC	Intergovernmental Panel on Climate Change
kg	kilogram
kPa	kilo Pascal
kV	Kilo volt
L-DOPA	L-3,4-dihydroxy phenylalanine
LSD	Least significant difference
M	Molar
mg	Milligram
min	Minute
mL	Millilitre

mM	Milli molar
mM	milli molar
N_2O	Nitrous oxide
ng	Nanogram
nm	Nanometre
NMR	Nuclear Magnetic Resonance spectroscopy
PCA	Principal component analysis
PVC	Ploy Vinyl Chloride
s	Seconds
SOC	Soil organic carbon
SOM	Soil organic matter
TPF	Triphenyl formazan
VG	Volume graphics
W	watt

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; $F_{1,22}$=22.89, P <0.001.

DEDICATION

This work is dedicated to my parents (my mother Unnima and my father Saidalavi) without whose support and prayers I would not have achieved anything in my life and to my wife Shahna and daughters Liba and Mehreen for their love and patience.

1. Chapter 1: General introduction

1.1 Rationale

Globally agriculture accounts for 10-12% of total anthropogenic emissions of greenhouse gases (GHGs) which was estimated to be 5.1 to 6.1 Gt CO_2-eq/yr in 2005 (Smith et al., 2007). It has been reported that soil tillage causes a rapid loss of soil organic matter, by increasing the soil biological activity and disturbing the physical properties of soil (Gosai et al., 2009). Conservation tillage has been suggested as one of the different mitigation options to reduce GHG emission from agriculture (Six et al., 2000c). It is claimed that conservation tillage can serve as an important management strategy offering many benefits like increasing organic matter content (Kong et al., 2009), sequestration of carbon (Lal, 2009), greater aggregate stability (Six et al., 1999b) and biological activity (Chatterjee and Lal, 2009) as well as prevent soil erosion and runoff (Cássaro et al., 2011). Reduced tillage practices have been reported to reduce GHG emission directly with the reduced use of fossil fuels in field preparation in addition to increasing carbon sequestration in soil (Petersen et al., 2008). However, recently it was reported that reduced tillage could lead to stratification of soil organic carbon at the surface (Baker et al., 2007) against the more uniform distribution of carbon in conventionally tilled soils (Campbell et al., 2000). The climate change mitigation benefits such as reduced CO_2 emissions by virtue of increased sequestration of carbon and reduced CH_4 release under reduced tillage could be offset by an increased emission of N_2O, a greenhouse gas with high warming potential (Chatskikh and Olesen, 2007; Hermle et al., 2008; Six et al., 2004). Increased N_2O

emissions have been related to increased denitrification under reduced tillage due to the formation of micro-aggregates within macro-aggregates that creates anaerobic micro sites within aggregates (Hermle et al., 2008) and due to increased microbial activity leading to a higher competition for oxygen (West and Marland, 2002a) and a denser soil structure (Regina and Alakukku, 2010) due to consolidation of soil over time due to clogging of pores. Reduction of tillage can lead to increased soil densification and subsequent decrease in volume of macropores leading to soil firmess (Schjønning and Rasmussen, 2000). Soil aggregation and pore structure are important characteristics affected by tillage which impacts on the physico-chemical and hydro-thermal regime in soil and ultimately the crop yield.

Field studies concerning the effect of tillage on soil aggregation and its effect on the net balance on major greenhouse gases are sparse. Traditional methods for soil structural studies such as soil moisture retention and aggregate size distribution are destructive (Gantzer and Anderson, 2002). However advanced technologies such as X-ray Computed Tomography (CT) can be used to reveal the undisturbed structure, aggregation and pore characteristics of soils under different management practices. Gantzer et al. (2002) have demonstrated CT can be used to reveal the differences in macroporosity between conventionally and conservational managed soils.

This project aims to understand the effect of complex interactions of soil physico-chemical and biological changes under tilled and untilled conditions on the net greenhouse gas balance and carbon sequestration. This project also sought to investigate the biophysical and microbial basis of enhanced carbon sequestration in soil.

1.2 Literature review

The literature review was prepared as a review paper intended for submission to the **Journal of Agricultural Science** and is included in an unpublished paper format and this chapter illustrates the background for undertaking this research project.

Potential climate change mitigation through conservation tillage

S. MANGALASSERY, S. SJÖGERSTEN, D.L. SPARKES AND S.J. MOONEY*

School of Biosciences, Sutton Bonington Campus, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, UK

*To whom all correspondence should be addressed. Email: sacha.mooney@nottingham.ac.uk

Summary

Conservation tillage is generally considered as an important component of sustainable agriculture. The benefits of conservation tillage have been presented as reducing runoff, enhancing water retention and preventing soil erosion. There is also general agreement on the usefulness of this practice to conserve and enhance soil organic carbon levels to some extent. However, its applicability in mitigating climate change has been extensively debated, especially when the whole profile of carbon in soil is considered along with a reported risk of enhanced N_2O emissions under conservation tillage. Here we present a meta-analysis of existing literature to ascertain the climate change mitigation opportunities offered by conservation tillage. Research suggests conservation tillage is effective in sequestering carbon beyond the level of soil surface in both tropical and temperate conditions. The carbon sequestration rate in tropical soils can be about five times higher than in temperate soils. In tropical soils, carbon accumulation is generally correlated with the duration of tillage. Reduced N_2O emission under long term conservation tillage has been reported in the literature but significant variability exists in the N_2O flux

information. Long term location specific studies are urgently needed to determine the precise role of conservation tillage in driving N_2O fluxes. Considering a wide variety of crops utilised in conservation tillage studies, for example maize, barley, soybean, winter wheat; only soybean has been reported to show an increase in yield under conservation tillage (7.7% over 10 years). In most cases yield reductions have been recorded e.g. c. 1-8% over 10 years under winter wheat and barley respectively indicating that adoption of conservation tillage do not bring appreciable changes in yield. A key question that remains to be answered is, are such reductions in yield acceptable in the quest to mitigate climate change, given the importance of global food security.

Key words: Conservation tillage, carbon sequestration, net greenhouse gas emission, yield

1.2.1 Introduction

The adoption of tillage practices for crop production dates back to the invention of animal drawn implements with the benefits of tillage shown as early as the 1800s (Gebhardt et al., 1985; Lal et al., 2007). In present day conventional tillage systems, a mould board plough is typically used for primary tillage followed by the use of secondary tillage implements like power harrows for seed bed preparation. In this approach it is usual that <15% of crop residues are left on the surface (Adel, 2003) and the tillage depth is ≥ 20 cm (Jastrow et al., 2007). The environmental concerns about soil erosion, soil degradation and pollution of water brought about by tillage have resulted in development of alternative tillage systems whose popularity have varied over time (Gebhardt et al., 1985) but are currently gaining more attention. Reduction of tillage in crop cultivation was first attempted primarily as a strategy to

reduce soil erosion during the late 1950s and increased in popularity around the world especially after the discovery of the herbicides atrazine and paraquat (Hermle et al., 2008). Different forms of reduced tillage are practiced which can be collectively be grouped under the broader term 'conservation tillage'. Any tillage practice that reduces soil or water loss when compared to ploughing is considered conservation tillage. Typically, reduced tillage aims to conserve soil and water by reducing soil disturbance and leaving 30 % or more crop residues on the surface (Wang et al., 2006). Soil inversion is not permitted under conservation tillage and shallow ploughing, if done, should be less than 10 cm (Adel, 2003). In 2001, Derpsch suggested about 45 million hectares globally was under conservation tillage of which 96% was in North and South America. By 2007-08 the area under conservation tillage had more than doubled to 105 Mha spread across all continents (Table 1.1, Derpsch and Friedrich (2009)). The largest area is in South America (46.8%), followed by North America (37.8%) and the least in Africa (0.3%) and Europe (1.1%). The reported increased area under conservation tillage in United States may be due to either early introduction of such practices to prevent soil erosion problems or because US conduct regular surveys on conservation tillage and accurate data always available. Conservation tillage practices are widely documented for their benefits to protect soil against erosion and degradation of soil structure (Petersen et al., 2011), greater aggregate stability (Fernández et al., 2010; Zotarelli et al., 2007), increased soil organic matter content and sequestration of carbon (Six et al., 2000a; West and Post, 2002) and improved biological activity (Helgason et al., 2010). The reduced use of fuel in field preparation is a significant economic attraction to farmers and adds substantially to

environmental protection (Petersen et al., 2008). Further emphasis has been given in recent years to the climate change mitigation opportunities under conservation tillage systems considering the potential carbon storage in soil and reduction in emission of carbon dioxide (CO_2) in particular (Farina et al., 2011; Koga and Tsuji, 2009; Peigne et al., 2007).

Recently it was reported that reduced tillage can bring about stratification of organic carbon at the soil surface (Baker et al., 2007) compared to the more uniform distribution of carbon typically found in conventionally tilled soils (Campbell et al., 2000) questioning the effective sequestration obtainable under conservation tillage. The surface accumulated crop residues under reduced tilled soils, may decompose releasing CO_2 to the atmosphere (Petersen et al., 2008). Crucially, climate change mitigation benefits such as reduced CO_2 emission, by virtue of increased sequestration of carbon, and increased methane (CH_4) uptake under reduced tillage could be offset by an increased emission of nitrous oxide (N_2O), a GHG with high global warming potential (Chatskikh and Olesen, 2007; Six et al., 2002; Six et al., 2004). The increased N_2O emissions have been related to increased denitrification under reduced tillage due to the formation of micro aggregates within macro aggregates that create anaerobic micro sites (Hermle et al., 2008), high microbial activity leading to high competition for oxygen (West and Marland, 2002a) and a dense soil structure that could be formed due to non-disturbance (Regina and Alakukku, 2010). Soil structure and soil wetness exert a considerable role in greenhouse gas emissions from soil (Ball, 2013). Avoiding tillage in crop production can also impact on crop yields and ultimately global food security (Huang et al., 2008). A yield reduction of 21 and 15% in wheat and barley

respectively was reported over 6 years in zero tilled soil compared to conventional tillage by Machado et al. (2007). Among other factors, the yield reduction under conservation tillage was mainly attributed to increased weed growth, which makes it necessary to apply more herbicides. The potential for any mitigation by conservation tillage therefore need to be considered together with its impact on crop yields and use of agrochemicals as climate change and global food security are intrinsically linked. The objectives of this paper are to evaluate conservation tillage for the (i) mitigation of climate change by sequestration of carbon and by reducing or balancing emission of major GHGs from the soil and (ii) its effect on crop yield. In this review, the term conventional tillage will be used to represent ploughing to a soil depth of at least 20 cm and conservation tillage includes both no-till/zero till and minimum/reduced till which represent no cultivation and cultivation of surface soil (typically ≤5 cm) respectively.

1.2.2 Materials and methods

In this study we compiled data sets pertaining to carbon storage in soils, emission of greenhouse gases and crop yield under conservation tillage.

1.2.2.1 Datasets on soil organic matter

A total of 57 data sets were collected from peer reviewed research papers using the search term 'conservation tillage and carbon' in Web of Sciences. Only those papers with paired conventional tillage (CT) and no-tillage (NT) treatments were selected (Table 1.2). The C data was reported in Mg ha^{-1}. But when only C concentrations were reported, bulk density values were used to convert carbon content to C stock using the following equation.

$$\text{Mg C per ha} = \frac{\%C \times Bulk\ density\ in \frac{g}{cc} \times soil\ depth\ in\ cm \times 100}{100} \quad (1)$$

For those sites without bulk density values, the bulk density was calculated using the equation of Post and Kwon (2000) as given below.

$$\text{Bulk density } \left(\frac{g}{cc}\right) = \left(\frac{100}{\left[\left(\frac{\%Organic\ matter}{0.244}\right)+\left(\frac{100-\%organic\ matter}{1.64}\right)\right]}\right) \quad (2)$$

Where 0.244 is the bulk density of organic matter and a mineral bulk density value of 1.64 was used as suggested by Post and Kwon (2000).

1.2.2.2 Yield data sets

A review of the existing literature was made to compile a data set for comparing crop yield under conservation tillage and conventional tillage. We collected data from 59 peer reviewed research papers that made one to one comparisons with conservation tillage and conventional tillage using the search terms 'crop yield and conservation tillage' in Web of Science (Table 1.3). The relative yield was then computed as follows.

$$\text{Relative yield } (\%) = \frac{Yield\ NT\ in\ kg/ha}{Yield\ CT\ in\ kg/ha} \times 100 \text{ x} \quad (3)$$

1.2.2.3 Statistical analysis

The locations of study reported in each paper were classed into tropical and temperate based on the climatic information provided in the paper and FAO agro-ecological zoning guidelines (http://www.fao.org/nr/land/ databasesinformation-systems/ aez-agro-ecological-zoning-system/en/ (FAO). Regression equations were developed to explore the potential for carbon sequestration under conservation and conventional tillage separately under

tropical and temperate conditions and to derive conclusions regarding the effect of the duration of conservation tillage on sequestration of carbon and soil depth on net sequestration carbon rate. The yield advantage or disadvantage under conservation tillage with respect to conventional tillage was calculated from the selected published literature. Linear regressions were carried out on the yield differences against duration under conservation tillage. All the statistical analysis was carried out in Genstat (v. 14).

1.2.3 Conservation tillage and soil properties

Conservation tillage affects soil aggregation by reducing oxidation of soil organic matter which acts as a binding agent for macro aggregates. Hence water stable aggregates (>250 mm) become more stable under conservation tillage systems (Tisdall and Oades, 1980). Kasper et al. (2009) observed 18.2% of soil aggregates in the 'stable' class under conventional tillage compared with minimum tillage which contained 37.6% stable aggregates. Continuous tillage practices also make aggregates susceptible to disruption under exposure to frequent wetting and drying cycles by affecting water stability of aggregates (Six et al., 2000b).

Soil organic matter accumulates under conservation tillage practices, especially near the soil surface, when compared to conventionally tilled soils (Angers et al., 1997; Gosai et al., 2009). Under conventional tillage, crop residues are mixed with soil in the plough layer and hence nutrients are more or less evenly distributed (Wright et al., 2007), unlike conservation tillage where there might be an enhanced bio-chemical and physical environment at the surface, due to longer retention of crop residues there. Under reduced tillage practices, a reduction in soil organic matter turnover can affect net mineralisation of

nitrogen (Kong et al., 2009) and result in lower nitrogen availability for crops. Net immobilisation of nitrogen has been reported during the transition periods to conservation tillage (Jastrow et al., 2007). However, in the long term, the nitrogen concentration in the surface layer of no-till soils has been found to be higher than in conventionally tilled soils (Ussiri et al., 2009). No tilled soils have also been reported to accumulate phosphorus and potassium at the surface (Wright et al., 2007). Franzluebbers and Hons (1996) observed greater surface accumulation of P, K, Zn and Mn in no-tilled soil than in conventionally tilled soils and Bauer et al. (2002) found enhanced accumulation of Ca and Mg in the upper layers of no-tilled soils.

Tillage impacts soil macro organisms both directly and indirectly. The direct effect is by exposing them by the inversion of soil (Roger-Estrade et al., 2010) and indirectly by altering the soil microclimate, by modifying temperature and moisture conditions in soil. In the long term no-tillage practices can be beneficial for earthworm population compared with conventionally tilled soils due to enhanced availability of food resources (Eriksen-Hamel et al., 2009). An abundance of microbial biomass has been found in soils with conservation tillage, which include saprophytic fungi and arbuscular michorhyzal fungi (Roger-Estrade et al., 2010). Helgason et al. (2010) found up to 32% higher microbial biomass under long term no-till systems than conventionally tilled soils.

1.2.4 Climate change and greenhouse gases

According to the Intergovernmental Panel on Climate Change (IPCC, 2007b) the increased concentration of GHGs in the atmosphere is the major cause for global warming and associated climatic changes (Ugalde et al., 2007). The

global atmospheric CO_2 concentration increased from 280 ppm in 1750 to 392.6 ppm in 2013 which has been attributed primarily to fossil fuel use and land use change. Apart from CO_2, the atmospheric concentration of CH_4 has increased to 1874 ppb from the pre-industrial value of 700 ppb. N_2O concentration increased from 270 ppb to 324 ppb in 2013 (CDIC, 2013). Agriculture can act as both a sink and source for the GHGs of CO_2, CH_4, and N_2O based on various mitigation strategies adopted. IPCC suggested three broad mitigation options to reduce GHG emission from agriculture including reducing or avoiding emissions (IPCC, 2007a; Smith et al., 2008). Reducing soil disturbance has been advocated as a key strategy to minimise agricultural emission and also to mitigate climate change, the mitigation effect being realised by enhanced sequestration of carbon in soil (Lal, 2004a; West and Post, 2002) and reduced emission of CO_2 during decomposition of crop residues triggered by ploughing and reduced use of fossil fuel in farm operations (West and Marland, 2002a).

1.2.5 Sequestration of carbon under conservation tillage

Carbon in soil and biota forms a major component of global carbon cycle (Lal, 2004a), and increasing C sequestration in soil can mitigate increasing atmospheric CO_2 concentration (Kimble et al., 2001). A reduction in soil tillage is suggested to increases the rates of carbon sequestration by altering soil physico-chemical and biological conditions (Marland et al., 2004). Conservation tillage is regarded as an important resource management practices that help to sequester as much as 100-1000 kg C ha^{-1} per year (Lal, 2004a). The sequestration of carbon within no-till management occurs faster under humid conditions with Six et al. (2004) reporting sequestration within 5

years under such climatic conditions (194 kg C ha^{-1} yr^{-1}). Example sequestration rates obtained under various conservation tillage studies are presented in Table 1.4. West and Marland (2002a) obtained a mean carbon sequestration rate of 340 kg ha^{-1} per year from 76 long term experiments for extending soil depth of up to 30 cm over 20 years. Similarly a comparable sequestration of carbon was noticed by Six et al. (2002) in both tropical and temperate soils. The carbon sequestration capabilities increased considerably with an increase in duration under conservation tillage, with the increment more evident under tropical conditions (Fig. 1.1, $P < 0.05$ for tropical and NS in case of temperate). Our meta-analysis suggests the carbon sequestration rate under conservation tillage of the top 25 cm soil was 735 kg ha^{-1} per year in tropical regions against 165 kg ha^{-1} per year in temperate soils (Fig. 1.2, $P < 0.05$ for tropical and $P < 0.001$ for temperate). The changes in carbon sequestration is also dependent on many other variables such as crop rotation, soil type (Gaiser et al., 2009) and soil drainage (Duiker and Lal, 1999). Mc Conkey et al. (2003) noticed a linear relationship with clay content and increase in carbon stock under no-till which was further confirmed by Grace et al. (2012) who recorded more than double the sequestration rate in clay soils compared to sandy soils in India. The ability to sequester carbon also depends on the initial carbon content at the initiation of conservation tillage practices as there is an upper limit of maximum carbon that could be sequestered. Therefore, it is crucial to consider these parameters when evaluating the benefits of any conservation tillage programme.

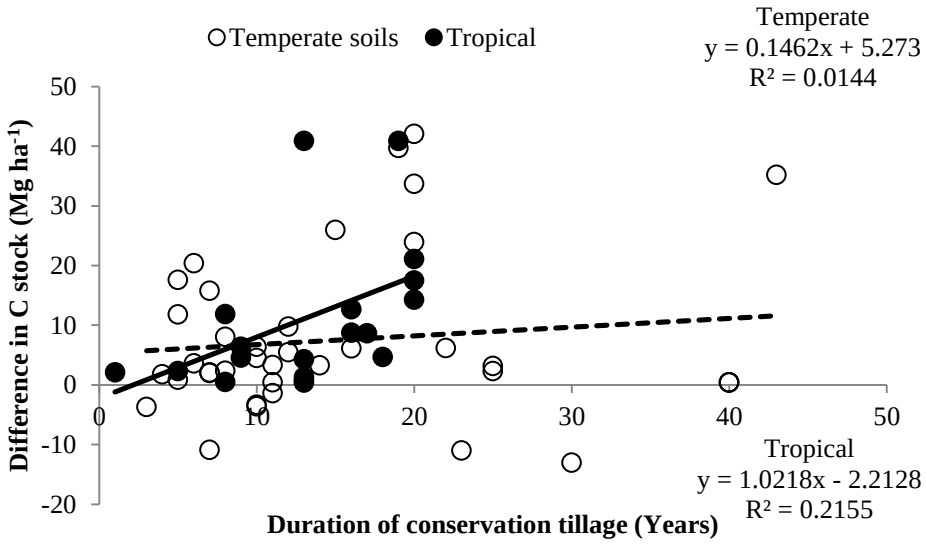

Fig. 1.1. Net sequestration of carbon (Mg ha^{-1}) under conservation tillage in comparison to conventional tillage as affected by duration under conservation tillage in tropical and temperate soils. ($F_{1,55}$ = 1.42, NS overall, $F_{1,16}$ = 4.40, P <0.05 tropical, $F_{1,37}$ = 0.54, NS temperate; for the data sets used please refer to Table 1.2)

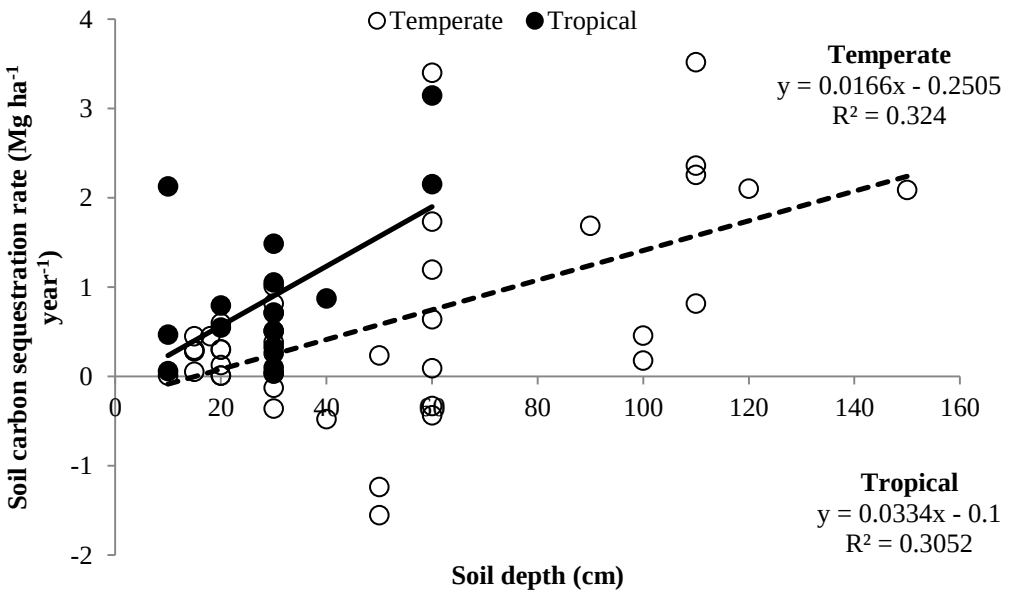

Fig. 1.2. Carbon sequestration rate in tropical and temperate soils ($F_{1,55}$ = 16.57, P <0.001 overall, $F_{1,16}$ = 7.03, P <0.05 tropical, $F_{1,37}$ = 17.73, P <0.001 temperate; Please refer to table 1.2 for the sources of data used in this figure).

1.2.5.1 Longevity of sequestered carbon under conservation tillage

Lal (2004b) suggested carbon sequestration by conservation tillage might be viewed as a short-term strategy only. An initial decline of soil carbon has been reported under conservation tillage compared to conventional tillage due to the absence of the incorporation of residues, and organic inputs into deeper layers of soil (Kong et al., 2009). After five years de Rouw et al. (2010) reported a net loss of carbon (1.33 Mg ha^{-1}) under no-till plots in comparison to tilled plots in Laos. The initial delayed response to sequestration of carbon after conversion from conventional tillage was also reported by West and Post (2002) who observed little or no increase during 2-5 years and a large increase between 5-10 years. The time required to reach steady state in carbon sequestration varies with respect to climate, soil type and the management practices and can range

from 5 to 30 years according to the studies listed in Table 1.4. The initial soil carbon content in relation to the equilibrium level that a particular soil can achieve is important in deciding the effectiveness of conservation tillage with respect to the sequestration (de Rouw et al., 2010). Angers and Eriksen-Hamel (2008), found a weak but significant correlation for soil organic carbon (R^2 = 0.15, P = 0.05) with the duration of no-tillage and hypothesised the positive effect of no-tillage would increase with time. In our analysis, carbon under conservation tillage in tropical regions were significantly correlated with the duration of the system (R^2 = 0.22, P = <0.001) but this was not significant for temperate regions. This is in agreement with the reports that in temperate soils, the time period to attain sink saturation is around 100 years, with much lower values for tropical soils (Alvaro-Fuentes and Paustian, 2011; Smith, 2004).

1.2.5.2 Physical aspects of carbon sequestration under conservation tillage

1.2.5.2.1 Aggregation

Tillage generally reduces soil aggregation and consequently particulate organic matter content (Wright and Hons, 2005). Under tillage, macro aggregates are both physically broken up due to shearing forces and by exposure to wet-dry and freeze-thaw cycles (Conant et al., 2007). Conservation tillage is known to increase sequestration of soil carbon especially in the surface layer and the major mechanisms underlying such sequestration is an increase in micro-aggregation (Lal and Kimble, 1997) and decrease in decomposition of soil organic matter (Chatterjee and Lal, 2009). Six et al. (1999b) found proportions of crop-derived C in macro aggregates (250‑2000 μm) were similar under no-till and conventional tillage, but proportions of crop derived C were three times greater in micro aggregates (<53 μm) from no-tillage than micro

aggregates from conventional tillage. Although the crop derived carbon in macro aggregates was similar in both conventional tillage and no-till, the no-till system showed 28% more total organic carbon in all aggregate size classes compared to conventional tillage (Madari et al., 2005). Six et al. (2000a) developed a conceptual model to explain the C sequestration under conservation tillage which hypothesised that tillage enhances macro aggregate turnover and decreases the formation of new micro aggregates. The improvement of soil aggregation and organic carbon by no-tillage has been demonstrated by other workers including Wright and Hons (2005) and Mrabet et al. (2001b). Six et al. (1999a) attributed the decrease of C sequestration by tillage to increased macro-aggregate turnover. Under conservation tillage the turnover of macro aggregates are decreased and formation of stable micro aggregates occur within macro aggregates (Denef et al., 2007) which serve as long term carbon stabilisation sites. The increased macro aggregation and its decreased turnover under conservation tillage can cause a 1.5 times slower carbon turnover, due to carbon stabilisation within micro aggregates (Six et al., 2002).

1.2.5.2.2 Soil Compaction

A number of studies have indicated that continuous conservation tillage practices over the long term reduce bulk density of soil (Dam et al., 2005a; Li et al., 2011). Lal et al. (1994) found that after 28 years of maize and soybean, the lowest bulk density soil was in no-till soils. In another study a continuous no-till system for 43 years significantly decreased bulk density at the surface (0-15 cm) of a silt loam soil in Ohio with little effect on the subsurface layer (15-30 cm) (Ussiri et al., 2009); the surface decrease being explained by the

changes in soil pore structure, carbon content and biological activity with greater impact mainly at the surface. The reduction in soil compaction under reduced tillage is mainly due to less traffic, additional crop residues at the surface (Jastrow et al., 2007) and increased biological activity provided by soil macro and micro fauna (Simmons and Coleman, 2008). The lower bulk density under conservation tillage may be beneficial for easier root penetration into deeper layers and thereby increasing the crop derived carbon input to the soil. This is specifically important in the case of deep rooted plants, since photosynthates, which are translocated into the below ground portions are added to soil through rhizodeposition (Baker et al., 2007). The decreased soil bulk density can aid in the downward movement of surface accumulated carbon (Luo et al., 2010b), by preferential accumulation of plant residues moving in the soluble fraction (Angers and Eriksen-Hamel, 2008). Blanco-Canqui et al. (2011) also found a moderate negative correlation between bulk density and soil organic carbon throughout a 1 m soil depth under no-till. However, there are reports stating continuous conservation tillage might also lead to increased soil strength and soil density (Hernanz et al., 2009; Schjønning and Rasmussen, 2000). Hill (1990) noticed increased bulk density and soil strength in the no-till treatments over a 11-12 year no-tillage experiment under continuous maize cultivation in Maryland, USA. Lopez-Fando and Pardo (2011) found significantly higher surface bulk density under no-till soil than conventionally tilled soil over 20 years of experimentation in central Spain with a crop sequence of Cheap pea (Cicer arietinum L)/ barley (Hordeum vulgare L.). The reasons attributed to increased bulk density under conservation tillage systems are increased settling of soil due to lack of

cultivation (Hermle et al., 2008) which can lead to soil consolidation (Peigne et al., 2007). However, the enhanced bulk density might not prevent the growth of roots if pore continuity is enhanced by creation of more biological macropores (Peigne et al., 2007).

1.2.5.2.3 Soil structure and porosity

Soil structure is an important factor in determining the sequestration or decomposition of organic matter as it governs the physical space for microorganisms aiding their actions in terms of aeration and moisture supply (Strong et al., 2004). A soil's porous network and organic matter are inseparable entities and the relative dynamic changes between the two entities vary in space and time. Kay and VandenBygaart (2002) reported reduced tillage might cause a decline in total porosity with an increased porosity in the uppermost layer of soil near to the crop residues. Direct drilling or reduced tillage practices initially lead to a reduction in macro pore volume in soil which ultimately reduces diffusion of air into soil in comparison to conventional tillage (Schjønning and Rasmussen, 2000). However, with the adoption of conservation tillage macro porosity increases gradually, especially in the soil surface (Zhang et al., 2007) due to retention of stubble (Bronick and Lal, 2005) and formation of macro pores by the activities of soil organisms and plant roots (Kay and Vanden Bygaart 2002). Arshad et al. (1999) observed more micro pores under conservation tillage than conventional tillage. The smaller aggregates have a higher capacity for protection of organic matter than larger aggregates due to their smaller pore sizes (Bachmann et al., 2008). In undisturbed conditions, as in the case of conservation tillage, the organic

matter lying between aggregates or inside larger aggregates are less prone to microbial attack.

1.2.5.2.4 X-ray Computed Tomography- Advanced techniques to measure soil pore characteristics

1.2.5.2.4.1 *Introduction*

The development of X-ray Computed Tomography has been attributed foremost to Godfrey Hounsfield (Hounsfield, 1973). Initially the first uses were in the medical field with its first use in soil science by Petrovic et al. (1982). The basic principle of CT is the attenuation of an electromagnetic beam from an object of interest. When the X-ray passes through the sample, attenuation of the X-ray beam occurs, which is then recorded on a detector (Heeraman et al., 1997) (Fig. 1.3). For homogenous samples, the attenuation of monochromatic beam like X-ray can be described by Beer`s law as:

$I/Io = \exp(-\mu h)$

Where, μ is the linear attenuation coefficient (L^{-1}), Io the intensity of incident X-ray beam and I that of attenuated and h is the sample thickness. The image obtained from CT scanning represents the linear attenuation coefficient of an object which is related to density of material. Although, the attenuation of an X-ray beam caused by highly heterogeneous soil cannot be accurately described by this law. Different workers have modified the equation to suit an heterogeneous systems like soil by summing up the length of the path corresponding to each component of soil (Ferraz and Mansell, 1979).

X-ray CT is now widely used in the study of soil physical properties following the initial work of Petrovic et al. (1982), who studied soil bulk density. The

technique provides a good tool for assessing soil structural changes induced by cultivation practices (Papadopoulos et al., 2009; Pires et al., 2002; Taina et al., 2008).

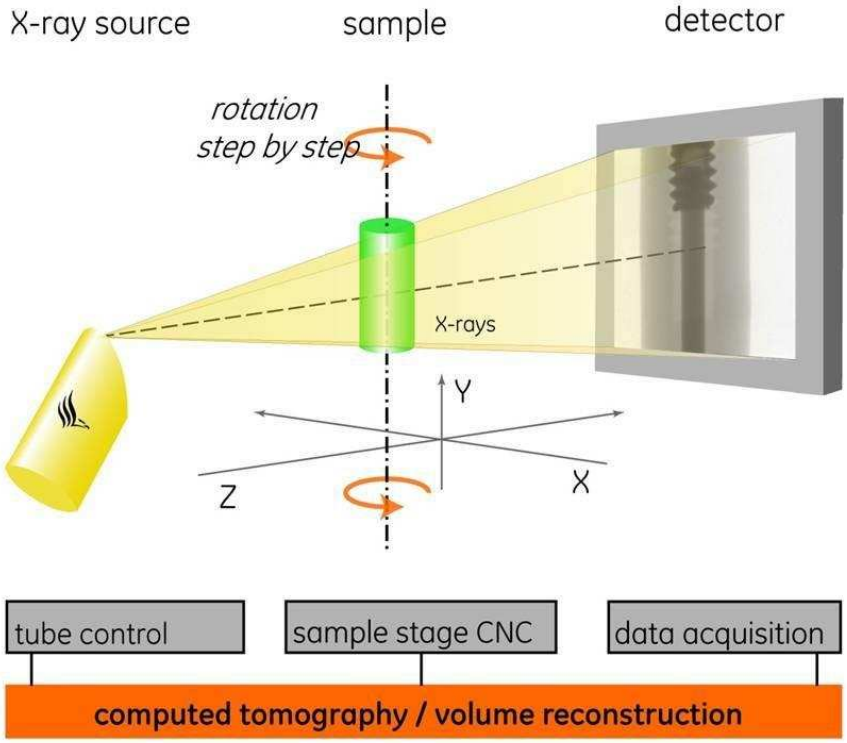

Fig. 1.3. Schematic representation of working principle of X-ray CT (http://www.ge-mcs.com)

1.2.5.2.4.2 *Study of tillage systems by X-ray CT*

Olsen and Borresen (1997) used X-ray CT to compare different tillage practices and found a compacted layer with reduced macropores at depths below the plough layer whilst soils with reduced tillage exhibited a more uniform profile uniform in bulk density. Atkinson et al. (2009) used micro-CT to study the impact of cultivation on soil structure and the establishment of winter wheat. The technique was used for study of soil surface sealing (Pires et al., 2002) and impact of falling water on soil (Macedo et al., 1998).

1.2.5.2.4.3 *Studies on pore size and distribution in soil by X-ray CT*

Soil pores can be classified as macro-pores (75-100 µm), meso pores (30-75 µm), micro pores (5-30 µm), ultra-micro pores (0.1-5 µm) and crypto pores (0.01-0.1 µm) (Lugato et al., 2009). Soil macropores constitute an important pathway in aiding the flow and transport of water and air in soil (Perret et al., 1999). Different characteristics of soil pores like pore size, pore shape, pore continuity and tourtosity affect the liquid and air transport (Luo et al., 2010a). These pore characteristics are greatly influenced by textural properties of soil (Mooney and Morris, 2008) and land use practices (Zhou et al., 2008). The use of X-ray CT for study of pore characteristics at a finer resolution have been used by different workers. Udawatta and Anderson (2008) found land use like trees and grass possessed deeper and longer pores when compared to cultivated fields. Mooney and Morris (2008) and Lugato et al. (2009) used X- ray CT to study water flow mechanisms in texturally different soils and found mean pore size decreased with decreasing particle size. Luo et al. (2010a) illustrated that soil type and land use significantly impacted the soil pore characteristics.

1.2.5.3 Chemical aspects of carbon sequestration under conservation tillage

Soil organic matter consists of different fractions with varying physico-chemical properties, each of which differs in turnover time (Del Galdo et al., 2003). Tillage alters aggregate dynamics and prevents the formation of stabilised carbon fractions such as intra particulate organic carbon (Six et al., 1999a). The turnover of soil organic matter is dependent upon the type of organic matter in soil with the labile fraction requiring only 0.4 to 1.2 years for decomposition whereas many years (400-2200) are required to decompose passive pools comprising of humic fractions for cold temperate soil (Lal and

Kimble, 1997). Microbially transformed substances are converted into humic forms through the intermediaries of quinones and amino compounds, the reaction being mediated by biological and inorganic catalysts (Stevenson, 1994). The main determinant in this phenol oxidation is oxygen availability which is directly related to cultivation practices in soil and soil porosity (Jastrow et al., 2007). Thus conservation tillage, by directly affecting the physical characteristics, governs the chemistry of soil carbon dynamics.

1.2.5.4 Biological aspects of carbon sequestration under conservation tillage

The number and diversity of soil organisms has been reported to increase with a reduction in tillage (Roger-Estrade et al., 2010). Soil microorganisms improve soil aggregation and thus indirectly influence carbon cycling by helping the physical protection of soil organic matter (Noguez et al., 2008). Peigne et al. (2007) found conservation tillage systems contained more fungi than bacteria in the surface layers. Fungi have the capacity to efficiently sequester carbon in aerobic conditions in agricultural systems. Fungi are reported to have greater carbon utilisation efficiency than bacteria. Fungi attach more frequently on lignitic materials, producing monomers which are important constituents of humic materials and the residues of fungal death cells are resistant to microbial degradation (Jastrow et al., 2007). Mycorrhizal fungi are effective in increasing soil organic carbon through their effect on soil aggregation and also are efficient in securing carbon from the plant and thus add extra carbon to soil organic matter (Manns et al., 2007). Tillage incorporates crop residues and places them close to decomposers while under conservation tillage they are initially kept away from decomposers (de Rouw et al., 2010). Under conservation tillage systems, with less disturbance, fungal

hyphae grow and form bridge structures between soil and surface residues and form a major component of the soil fabric (Jastrow et al., 2007). These hyphal masses, upon decomposition, add to the soil carbon pool by way of the recalcitrant by-products of decomposition.

1.2.5.5 Impact of soil depth on carbon sequestration under conservation tillage

Previous work to estimate carbon sequestration benefits under conservation tillage has been criticised, as the depth of sampling has mostly been limited to the surface 20 cm or less (Baker et al., 2007). In our meta-analysis it was found that carbon sequestration under conservation tillage takes place irrespective of soil depth (Fig. 1.2). Significantly higher carbon was sequestered under conservation tillage compared to conventional tillage, under both tropical (R^2 = 0.31, $P < 0.05$) and temperate conditions (R^2 = 0.32, $P < 0.001$) to a depth of upto 160 cm. Multiple linear regression of carbon sequestration with depth and duration of tillage also indicated significant carbon increases under tropical ($P < 0.01$) and temperate conditions ($P < 0.001$). Angers and Eriksen-Hamel (2008) also found significantly greater soil organic carbon under no-tillage compared to full inversion tillage at depths up to 30 cm, by comparing 23 studies with duration of zero-till of more than 5 years sampled to more than 30 cm depths. The greater soil carbon under subsurface depths in full inversion tillage was not sufficient to offset the surface gain under no-tillage. Similarly Six et al. (2002) also found a net sequestration of carbon to a depth of 50 cm after 20 years of no-tillage. In a long term tillage experiment ofr 17 years by Lopez-Fando and Pardo (2011) a significant effect of conservation tillage on carbon sequestration

in the top 30 cm depth was found. This indicates that a net carbon sequestration is possible under conservation tillage when the whole soil profile is considered.

1.2.6 Greenhouse gas emission under conservation tillage

1.2.6.1 Carbon dioxide emissions under conservation tillage

Decomposition of plant residues and organic matter by the action of soil microbes and respiration of microbes and plant roots are the major sources of emission of CO_2 in soil (Oorts et al., 2007). Immediately after tillage, the emission of CO_2 is rises. Chatskikh et al. (2007) reported a 34 % increase in emissions under tilled soil compared to reduced tilled soil in Denmark. Ellert and Janzen (1999) showed enhanced release of CO_2 immediately after tillage which was associated with the release of CO_2 stored in soil pores and from stimulated biological production. The CO_2 flux soon after soil disturbance has been related to depth and the degree of soil disturbance (Álvaro-Fuentes et al., 2007). In the initial periods after tillage the soil CO_2 emission might be governed by soil structural changes associated with pore structure and soil organic carbon substrate might not be the limiting factor controlling production (Al-Kaisi and Yin, 2005). Over an intermediate to long term period (10-100 days) enhanced biological production of CO_2 is the major driver of the increased emissions. Reduced turnover of soil organic matter under conservation tillage leads to decreased emission of CO_2 under long term conservation tillage. In south-western Saskatchewan, Canada, there was a 20-25% reduction in CO_2 flux under soils that had been zero tilled for 13 years compared to conventional tillage attributed to slower decomposition of surface left crop residues under zero tilled soil (Curtin et al., 2000). In a long term tillage experiment maintained for 25 years, Bauer et al. (2006) found that

irrespective of season, the CO_2 flux from conventional tillage was higher compared to conservation tillage. Zero tillage is reported to reduce the CO_2 emission rate by 0.6 Mg C ha^{-1} yr^{-1} compared to conventional tillage in long term experiment under maize (43 years) in the USA (Ussiri and Lal, 2009). In contrast, a long term study by Oorts et al. (2007) found on more than half of the sampling days, no-tillage exhibited larger CO_2 emissions and they attributed this to the achievement of equilibrium under long periods (32 years) of no-tillage. The authors attributed this larger CO_2 emission under no-tillage due to the decomposition of old weathered residues.

1.2.6.2 Nitrous oxide emissions under conservation tillage

Many workers have reported increased N_2O emission under no-tillage compared to conventional tillage (Ball et al., 1999; Chatskikh and Olesen, 2007; Oorts et al., 2007). This has been attributed to decreased water filled pore space , mineral nitrogen concentration (Oorts et al., 2007), reduced gas diffusivity and air-filled porosity (Chatskikh and Olesen, 2007), increased water content (Blevins et al., 1971) and a denser soil structure (Beare et al., 2009; Schjønning and Rasmussen, 2000). Increased N_2O fluxes under conservation tilled soils might be attributed to the increased anaerobic conditions provided by the increased bulk density and decreased soil porosity due to soil consolidation (Ball et al., 1999). The physical characteristics of the soil in different layers, as modified by different tillage practices, affect the flux of N_2O. If N_2O is produced at surface layers, which are more permeable, the gas is likely to be emitted, but if the point of production is in lower layers, overlaid by compact layers, the N_2O produced may be consumed within the profile. Although N_2O emission is quantitatively less in comparison to CO_2

emission, it assumes significance due to its larger global warming potential (approximately 300 times that of CO_2) (IPCC, 2001). Indeed, increased N_2O emissions have the potential to offset 75-310% of the climate change mitigation obtainable from the sequestration of carbon in soil (Regina and Alakukku, 2010). The adoption of conservation tillage over a long term (20 years) was reported to nullify this adverse effect of N_2O emissions with lower N_2O emissions under no-tillage than under tilled soil in humid climates and similar emissions under both tillage types in dry climates (Six et al., 2004). Similar reports were also made by Kessavalou et al. (1998) and Chatskikh et al. (2008) attributable to increased N_2O consumption in soil (Luo et al., 2010b). However the uncertainty associated with estimation of N_2O remains high in most experiments due to significant spatial and temporal variability (Chatskikh et al., 2008; Ussiri et al., 2009). It seems that further long term location-specific studies combining different greenhouse gases and carbon sequestration are urgently needed to investigate the impact of conservation tillage on N_2O flux.

1.2.6.3 Methane emissions under conservation tillage

Most studies indicate an increased absorption of CH_4 in soils under no tillage due to reduced surface disruption (Kessavalou et al., 1998; Regina and Alakukku, 2010), and due to greater pore continuity with the presence of more micro sites for methanotrophic bacteria (Hütsch, 1998). This increased soil bulk density under conservation tillage might prevent the efflux of CH_4 leading to its oxidation within soil (Li et al., 2011). Long term studies by Ussiri et al. (2009) indicated a net CH_4 uptake in no-till silt loam soils under maize in the USA. They found an uptake of 0.32 kg CH_4-C ha^{-1} $year^{-1}$ against an emission

of 2.76 kg CH_4-C ha^{-1} $year^{-1}$ in conventional till. Continuous ecological disturbance under tillage can be detrimental to methane oxidisers. Most previous studies indicate conservation tilled soils act as a net sink for methane. However, both increased and decreased CH_4 consumption has been reported in no-till soils (Hütsch, 1998; Venterea et al., 2005). If a conservation tillage system creates anaerobic micro sites or makes conditions favourable for enhanced water logging conditions, then it is likely CH_4 production and therefore emissions will increase.

1.2.6.4 Net emission of greenhouse gases

To obtain a realistic assessment on the potential of conservation tillage reducing GHG, the combined emission of all major GHGs need to be considered. There are very few studies that have considered the global warming potential of different gases under conservation tillage systems. Some long term studies have indicated a stabilisation of N_2O emissions under reduced tillage over 20 years especially in humid climates (Six et al., 2004). Ussiri et al. (2009) observed a lower total emission of N_2O under 43 years of no-till in comparison to conventional tillage. In their study the global warming potential under no-till systems were 51 to 58% less than under conventional tillage. A complete life cycle analysis of a no-till system and conventional till system was carried out by West and Marland (2002b) based on comparison of 76 long-term experiments up to a soil depth of 30 cm. After accounting for the CO_2 emissions from different inputs and production activities for maize, wheat and soybean in the US and comparing carbon sequestered under no-till, they calculated a net carbon sequestration of 368 kg C ha^{-1} $year^{-1}$ (In this study C emissions from machinery and agricultural inputs were also included in

calculations) However a global data analysis of no-till versus conventional tillage covering tropical and temperate soils found that, after accounting for the carbon sequestered and CH_4 taken up in soil, net sequestration was negative with an overall negative greenhouse balance of 214 kg CO_2- equivalents ha^{-1} $year^{-1}$ (Six et al., 2002). This analysis only compared systems with tillage or no-tillage elements excluding experiments with the potential for additional carbon sequestration such as cover crops and crops in rotation. Robertson et al. (2000) found, during eight years of experimentation, a low net global warming potential under no-till (14 g CO_2- equivalents m^{-2} $year^{-1}$) compared to conventional till (114 g CO_2- equivalents m^{-2} $year^{-1}$). The slightly higher or comparable N_2O emission under no-till was compensated for by enhanced carbon storage. Reduced tillage can decrease net GHG release by 0.56 Mg CO_2- equivalents ha^{-1} $year^{-1}$ compared to conventionally tilled soil as shown by Chatskikh et al. (2008) under a 30 years simulation experiment while field studies for 43 years by Ussiri et al. (2009) found a decrease of 1.03 Mg CO_2-equivalents ha^{-1} $year^{-1}$ under conservation tillage compared to conventional tillage (52% reduction).

Overall the literature suggests zero tillage reduces GHG emissions in the long term, but crucially some uncertainty exists as to how long these effects can be observed. To date most studies indicate a reduction in the overall release of radiatively active trace gases suggesting no-tillage may have significant potential for reducing the impact of climate warming. However, large uncertainties remain and further work is needed both to define the underlying mechanisms and understand the variation between agricultural systems. At present a quantitative meta-analysis of the greenhouse gas data from soil was

not possible due the large variation in the data sets with regard to methodology adopted.

1.2.7 Soil quality and yield responses under conservation tillage

Our analysis of previous research suggests little consistent effect of zero till on yield with 53% of publications in this area reporting an increase in crop yield under conservation tillage, whereas only 46% reported higher yield under conventional management (n=63). The most negative effects have been recorded in maize with an average of 36.4% reduction in maize yield under conservation tillage over 10 years reported in 15 publications (Fig. 1.4). The data on winter wheat generally suggest little effect on yield following the adoption of conservation tillage over conventional tillage (0.94% reduction) (Fig. 1.5), though an 8% reduction in barley yield was noted over 10 years. However, the research is conflicting with Machado et al. (2007) reporting a yield reduction of 21 and 15% in wheat and barley respectively over six years, with zero tilled soils compared with conventionally tilled soils. Reduced cereal yields under short term conservation tillage practices have been also reported by Kankanen et al. (2011). A meta-analysis of 47 European studies by Van den Putte et al. (2010) compared the crop yields under conservation tillage with conventional tillage and reported a yield reduction ranging from 0 to 30% depending on crop type, tillage depth, and texture of soil as well as crop rotation, with an average yield reduction of 4.5%. The major constraint for realising good yields under conservation tillage is the infestation of weeds (Vakali et al., 2011). Weeds compete with the seedlings for the important resources necessary for growth such as light, water, nutrients and space, that

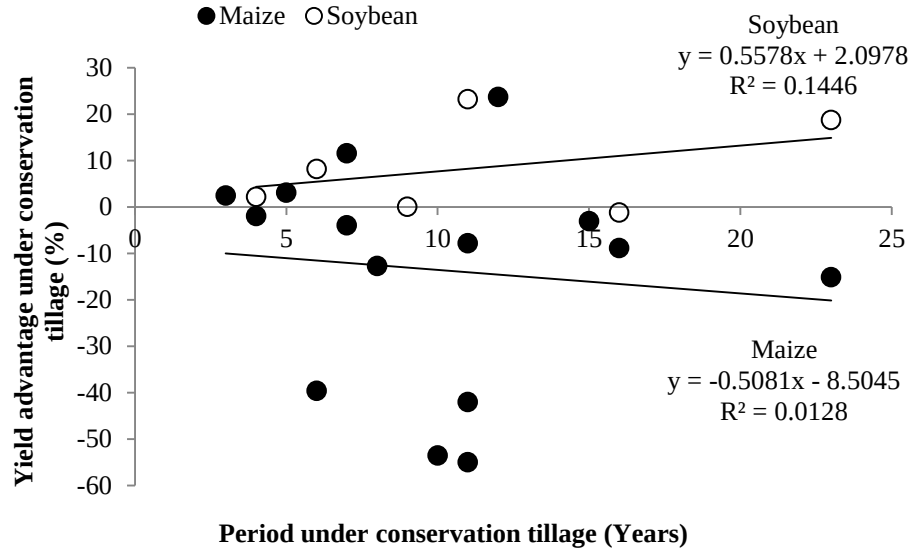

Fig. 1.4. Yield advantage over years under conservation tillage over conventional tillage in maize and soybean (Statistically non-significant, this figure is based on references cited in table 1.3).

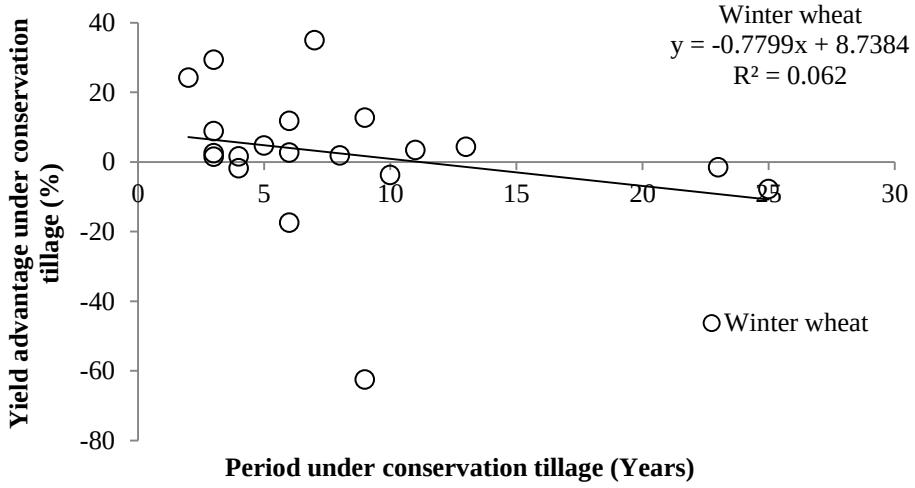

Fig. 1.5. Yield advantage over years under conservation tillage over conventional tillage in winter wheat (Statistically non-significant, this figure is based on references cited in table 1.3).

may lead to poor germination, establishment and crop growth (Gruber et al., 2012). The surface retention of crop residues may adversely affect the crop yield. Increased accumulation of crop residues, especially straw residues in poorly drained soils, can increase water logging and reduce crop yield by affecting germination (Wang et al., 2006). Surface residues may also impact on crop yields by affecting initial seedling establishment by acting as a favourable niche for pests and disease causing organisms (Wuest et al., 2000) and reducing the efficiency of applied fertilisers and pesticides as well as affecting the drying and wetting regimes of the soil (Carter, 1994; Känkänen et al., 2011). Residues left on the surface may also affect nutrient availability to the crops, especially, with nitrogen due to immobilisation.

Through the stabilisation of the soil physico-chemical and biological environment, the negative effects of conservation tillage on yield can be offset in the long term thereby supporting enhanced crop yields in the future. Wang et al. (2006) found an increased yield under soybeans of 7.7% with conservation tillage over 10 years compared to conventional tillage (Fig 1.3). The increased yields under conservation tillage were mainly ascribed to non-disturbance and retention of crop residues at the surface. The positive aspects of surface retention of crop residues are a reduction in evaporation losses from soil, reduction in crust formation and potential of soil erosion (Guérif et al., 2001). In dry regions such as north west China, crop residues left at the surface can be helpful for storing water (Huang et al., 2008) and in temperate regions it can prevent frost damage. Long term tillage experiments in Switzerland over 15 years found comparable yields of wheat under reduced and conventional tillage systems (Anken et al., 2004), as also reported for maize yield under 11 years of

experimentation in Canada (Dam et al., 2005b). When combining conservation tillage with retention of stubbles, Huang et al. (2008) obtained 12.5% more field pea yield and 14% more spring wheat yield under conventional tillage over four years of experiments. They observed that the yield advantage of conservation tilled plots with respect to conventional plots, disappeared when the stubbles were removed, indicating the necessity of combining both no-tillage and residue retention to maximise productivity.

From these studies it can be concluded that there is potential for crop yields to be increased or maintained under conservation tillage by carefully addressing the yield limiting factors such as weed growth, slow initial growth, nutrient deficiency, pest pressure and hardened subsurface etc. (Lyon et al., 1998; Machado et al., 2007). It is worth noting that when considering the benefits of conservation tillage over conventional tillage, there are other considerations than yield, as often a slight reduction in yield can be overcome by reduction in cultivation costs.

The adoption of conservation tillage as a part of a change in management system in combination with other sustainable land use management options such as diversified crop rotation involving non-cereals (Van den Putte et al., 2010) and controlled traffic farming (Hamza and Anderson, 2005) can harness even better results. Infrequent tillage has been suggested as an alternate strategy to address the problem of compaction and weed growth. Conant et al. (2007) observed such practices can sequester nearly as much as carbon as continuous no-till systems based on a modelling study. Indeed, field studies on periodic tillage by Yang et al. (2008) found tilling of a long term no-till experiment (13 years) destroyed the surface stratification of soil carbon in the

0-5 cm layer, which was offset by soil carbon gains in the 10-20 cm depth. Similar results were reported by Kettler et al. (2000) and Pierce et al. (1994). However, such studies need to be conducted for each agro-ecological region to determine the fine balance between offsetting greenhouse gas emissions and maintaining good yields. The yield perspective is also important from a global change stand point. As C sequestration is also affected by biomass which in turn is correlated with higher crop yield (de Rouw et al., 2010) and hence maintaining crop yield at satisfactory levels is important both for food security and climate change mitigation.

Conservation tillage can be beneficial in sequestering carbon not only at the surface, but also in deeper layers, in both tropical and temperate climatic conditions. The greatest concern regarding the ability to contribute to mitigating climate change through conservation tillage relates to the reported enhanced emission of N_2O. However reduced N_2O emissions under conservation tillage over longer timescales (e.g. 20 years) have been reported recently (Chatskikh et al., 2008; Six et al., 2004). Adopting appropriate agronomic management including weed control, crop rotation and cover crops and controlled traffic systems to control N_2O emissions may be beneficial in addressing the problem of yield reduction along with the environmental benefits. The location specific yield reduction under conservation tillage can potentially be overcome by careful attention to yield limiting factors such as weed growth, pest outbreak and nutrient deficiency.

Summary

The previous studies on the effect of conservation tillage on mitigating climate change were mainly carried out in isolation looking into the effect individually.

Also physical factors governing emission of greenhouse gases and carbon sequestration in soils under conservation tillage is not given due attention in previous studies. No previous studies have considered the effect of the soil porous architecture created by tillage on net balance of greenhouse gas emissions. Traditional methods for inferring soil structure such as soil moisture retention curves are limited as they are destructive and do not provide the soil pore size distribution in three dimensions(Gantzer and Anderson, 2002). However, imaging technologies such as X-ray Computed Tomography (CT) can be used to reveal the undisturbed structure, aggregation and pore characteristics of soils at high resolutions (e.g. microscale). To address these, following research aims and objectives were formulated.

1.3 Research aim and objectives

The overall aim of this project was to investigate the effect of soil physical properties, especially soil pore characteristics, as affected by different cultivation practices on microbial activity, carbon sequestration and greenhouse gas (GHG) release from soils. X-ray Computed Tomography (X-ray CT) was used to study the soil pore characteristics. Chemical and microbial analysis and observations on gaseous release was used to understand how the emission of GHG depends upon different soil management systems and the physical and microbial basis of carbon sequestration in soil. The overarching hypothesis is:

"Conservation tillage can be used in the mitigation of climate change through reduction of greenhouse gas loss from soil and sequestration of carbon in soil, and is both microbially and physically mediated*"*

To address this, the following sub aims have been developed.

1. To investigate the effect of different soil tillage practices on soil pore characteristics.

   – This is addressed in Chapter 2 and 4

2. To understand the effect of different aggregate size classes as derived by different soil management systems on the physical characteristics of soil and emission of GHGs such as CO_2, CH_4 and N_2O.

   – This is addressed in Chapter 3

3. To study the climate mitigation capabilities of conservation tillage practices in comparison to conventional tillage systems based on its physico-chemical and biological properties.

   -This is addressed in both Chapters 4 and 5.

4. To investigate the biophysical and microbial mechanisms of carbon sequestration in soil.

   – This is addressed in Chapter 5.

1.4 Thesis structure

This thesis is presented in a research paper format. **Chapter 1** has provided an overview of the problem to be addressed in this thesis, the rationale of the study, some background information in the form of a literature review along with the research aims and objectives. **Chapter 2** provides the results from a preliminary pilot experiment conducted on soils both texturally different and under contrasting management regimes. **Chapter 3** assesses the effect of aggregates of different sizes in sandy loam and clay loam soil on soil pore

characteristics, hydraulic properties and greenhouse gas emission from soil. This work has been published in Soil and Tillage Research. **Chapter 4** assesses impact of conservation and conventional tillage practices on soil pore characteristics, carbon sequestration and greenhouse gas emissions and net effect on total global warming potential, based on soil sampling on conservation and conventional tilled farms across East Midlands, UK. **Chapter 5** provides the microbial and biological basis of carbon sequestration in soils under conservation and conventionally tilled soils. **Chapter 6** provides a general discussion of key results and findings presented in each chapter and highlight the major conclusions from the research along with possible future lines of work. A detailed description of most of the laboratory techniques and procedures are given in appendix.

Table 1.1. Area under conservation tillage in different countries (Adopted from (Derpsch and Friedrich, 2009)

Country	Area under conservation tillage (Mha) as of 2007-2008
USA	26593
Brazil	25502
Argentina	19719
Canada	13481
Australia	12000
Paraguay	2400
China	1330
Kazakhstan	1200
Bolivia	706
Uruguay	672
Spain	650
South Africa	368
Venezuela	300
France	200
Finland	200
Chile	180
New Zealand	162
Colombia	100
Ukraine	100
Others	1000
Total	**105863**

Table 1.2. Carbon stock reported under conventional and zero-tillage around the globe.

Sl No	Author	Study area	Soil texture	Years under no-tillage	Crops	Depth to which C reported	Carbon-Conventional (Mg ha^{-1})	Carbon - under ZT (Mg ha^{-1})	Climate
1	Sombrero et al. (2010)	Burgos, Spain	Loamy sand in surface	10	Cereal – fallow, Cereal legume	30	4.6	17.80	Temperate
2	Deen et al. (2003)	Ontario, Canada	Silt loam	25	Maize, Soybean	60	36.7	39.0	Temperate
3	Lopez-Fando et al. (2011)	Toledo, Central Spain	Loamy sand	16	Chick pea, barley	30	26.5	32.6	Temperate
4	Chatterjee et al. (2009)	Michigan, US	Clay loam	10	Maize-soybean	60	97.6	104.0	Temperate
5	Chatterjee et al. (2009)	Ohio, US	Clay loam, silty clay loam	10	Maize-soybean	60	82.3	79.0	Temperate
6	Chatterjee et al. (2009)	Ohio, US	Loam	15	Maize-soybean	60	117.0	143.0	Temperate
7	Chatterjee et al. (2009)	Ohio, US	Silt loam	6	Maize-soybean	60	46.3	66.7	Temperate
8	Chatterjee et al. (2009)	Pennsylvania, US	Loam	30	Maize-alfalfa	60	96.4	83.4	Temperate
9	Puget et al. (2005)	Ohio, US	Silty clay loam	8	Maize	20	88.5	90.9	Temperate
10	Dolan et al. (2006)	Minnesota, US	Silt loam	23	Soybean, maize	40	117.0	106.0	Temperate
11	Kahlon et al. (2013)	Ohio, US	Silt loam	22	-	15	21.4	27.6	Temperate

12	Yang et al. (2008)	Ontario, Canada	Clay loam	8	Maize, maize-soybean rotation	30	104.8	112.9	Temperate
13	Yang et al. (1999)	Urbana, US	Silt loam	8	Soybean	30	46.6	58.5	Tropical
14	Lou et al. (2012)	Jianping county, China	Sandy loam	12	Maize	100	87.6	93.1	Temperate
15	Lou et al. (2012)	Changtu county, China	Loam	5	Maize	100	95.4	96.3	Temperate
16	Jemai et al. (2012)	Mateur, Tunisia	Clay loam	3	Wheat/faba bean rotation	50	83.9	80.2	Temperate
17	Jemai et al. (2012)	Mateur, Tunisia	Clay loam	7	Wheat/sulla rotation	50	83.9	73.1	Temperate
18	Lal (1997)	Ibadan, Nigeria	Sandy	8	Maize	10	2.0	2.4	Tropical
19	Larney et al. (1997)	Alberta, Canada	Sandy clay loam to clay loam	7	Spring wheat - fallow	15	27.1	29.2	Temperate
20	Larney et al. (1997)	Alberta, Canada	Sandy clay loam to clay loam	7	Continuous spring wheat	15	31.0	33.0	Temperate
21	Sisti et al. (2004)	Passo Fundo, Brazil	Clay	13	Wheat-soybean rotation	30	60.7	65.0	Tropical
22	Metay et al. (2007)	Cerrados, Brazil	Clay	5	Leguminous cover crops	10	19.9	22.3	Tropical
23	Dendooven et al. (2012)	Central Mexico	Clay	19	Wheat and maize	60	76.8	117.7	Tropical
24	Varvel et al. (2011)	Lincoln, US	Silty clay loam	20	Maize, soybean	60	90.5	114.4	Temperate
25	Varvel et al. (2011)	Lincoln, US	Silty clay loam	20	Maize, soybean	90	104.8	138.6	Temperate
26	Varvel et al. (2011)	Lincoln, US	Silty clay loam	20	Maize, soybean	120	123.3	165.4	Temperate
27	Plaza et al. (2012)	Santa Olalla, Toledo, Spain	Sandy loam	25	Barley	20	21.5	24.6	Temperate

28	Dalal et al. (2011)	Queensland, Australia	Clay	40	Wheat, barley	10	19.8	20.2	Temperate
29	He et al. (2011)	Hebei province, China	Silt loam	11	Summer maize, winter wheat	30	6.1	6.6	Temperate
30	Ussiri et al. (2009)	Ohio, US	Silt loam	43	Maize	30	44.8	80.0	Temperate
31	Hao et al. (2001b)	Alberta, Canada	Clay loam	4	Spring wheat–sugar beet–spring wheat–annual legume	15	28.3	30.1	Temperate
32	Jacobs et al. (2009)	Göttingen, Germany	Silt loam	40	Forage maize, winter wheat/mustard, pea, winter wheat and winter wheat.	20	2.7	3.2	Temperate
33	Jacobs et al. (2009)	Göttingen, Germany	Silt loam	40	Forage maize, winter wheat/mustard, pea, winter wheat and winter wheat.	20	3.0	3.4	Temperate
34	Jantalia et al. (2007)	Planaltina, Distrito Federal, Cerrado, Brazil	Clay	20	Soybean based rotations	30	64.8	85.9	Tropical
35	Bayer et al. (2000)	Rio Grande do Sul State, Brazil	Sandy clay loam	9	Oat /maize	30	44.6	49.2	Tropical
36	Bayer et al. (2000)	Rio Grande do Sul State, Brazil	Sandy clay loam	9	Oat+common vetch /maize +cowpea	30	50.2	56.6	Tropical
37	Fuentes et al. (2010)	Central Mexico	Clay	16	Maize	20	27.5	36.2	Tropical

38	Fuentes et al. (2010)	Central Mexico	Clay	16	Wheat	20	27.3	40.0	Tropical
39	Clapp et al. (2000)	Minnesota, US	Silt loam	13	Maize, soybean, oats	15	49.7	50.4	Temperate
40	Jantalia et al. (2007)	Planaltina, Distrito Federal, Brazil	Clay	20	Rice, soybean, maize	30	71.6	85.9	Tropical
41	Varvel et al. (2011)	Lincoln, US	Silty clay loam	19	Continuous maize and soybean	150	131.6	171.3	Temperate
42	He et al. (2011)	Gaocheng North China	Silt loam	11	Summer maize and winter wheat	30	19.6	18.2	Temperate
43	Ernst et al. (2009)	Southern Eifel, Germany	Silt loam	10	Rape, winter wheat, winter barley, and spring barley	30	57.8	54.2	Temperate
44	De M. Sa et al. (2001)	Tibagi, Ponta Grossa- Brazil	Clay	20	Rice, Soybean, Wheat	40	97.9	115.4	Tropical
45	Sainju et al. (2002)	Georgia, USA	Sandy loam	6	Tomato or silage maize	20	20.8	24.4	Temperate
46	Kushwaha et al. (2001)	Banaras, India	Sandy loam	1	Barley	10	9.9	12.0	Tropical
47	Freixo et al. (2002)	Passo Fundo Brazil	Clayey	13	Wheat- Soybean	30	68.1	68.5	Tropical
48	Freixo et al. (2002)	Passo Fundo Brazil	Clayey	13	Wheat- Soybean, Veltch-Maize	30	65.4	66.7	Tropical
49	Castellanos-Navarrette et al. (2012)	Central Mexico	Clay loam	17	Maize–wheat rotation	30	35.4	44.1	Tropical
50	Jarecki et al. (2005)	Ohio	Silt loam	14	Continuous maize	50	51.4	54.7	Temperate

51	Ernst et al. (2009)	Paysandú, Uruguay	Clay loam	10	Wheat, barley, and oat for winter crops and maize, sunflower, sorghum, and soybean for summer crops	18	47.3	51.8	Temperate
52	Mrabet et al. (2001a)	Sidi El Aydi, Morocco	Clay	11	Wheat- maize, lentils fallow	20	33.9	37.3	Temperate
53	Abreu et al. (2011)	Oklahoma, US	Silt loam	5	Soybean–maize–wheat–soybean–maize	110	101.6	119.2	Temperate
54	Abreu et al. (2011)	Oklahoma, US	Silt loam	7	Wheat–soybean–maize	110	111.6	127.4	Temperate
55	Abreu et al. (2011)	Oklahoma, US	Silt loam	5	Maize–wheat	110	104.5	116.3	Temperate
56	Abreu et al. (2011)	Oklahoma, US	Silt loam	12	Wheat/soybean/grain sorghum	110	72.1	81.9	Temperate
57	Zanatta et al. (2007)	Rio Grande do Sul State, Brazil.	Sandy clay loam	18	Oat/maize	30	41.8	46.5	Tropical

Table 1.3. Reported yields under various crops in zero till and conventional tillage systems.

Sl no.	Reference	Study area	Soil texture	Annual Rainfall	Years under zero till	Crops	Yield Zero till (kg ha^{-1})	Yield-Conventional till (kg ha^{-1})
Studies reporting increased yields under zero till								
1	Chen et al. (2011)	Northeast China	Clay loam	530	6	Soybean	2659	2441
2	Su et al. (2007)	Henan Province, China	Loam	614	6	Winter wheat	4679	4125
3	Hemmat and Eskandari (2006)	East Azerbaijan Province, Iran	Clay loam	375	3	Winter wheat	1435	1014
4	Vogeler et al. (2009)	Braunschweig, Germany	Silty loam	620	8	Winter wheat	5790	5680
5	Vogeler et al. (2009)	Braunschweig, Germany	Silty loam	620	8	Field beans	2910	2520
6	He et al. (2011)	Gaocheng in Hebei, China	Silt loam	494	11	Winter wheat	6154	5945
7	Hao et al. (2001a)	Lethbridge, Canada	Clay loam	283	4	Spring wheat	5591	5547
8	Morell et al. (2011)	Agramunt , Spain	Sandy silt loam	435	10	Winter barley	1590	1148
9	Ekeberg and Riley (1997)	Southeast Norway	Loam	415	9	Spring barley	4310	4020
10	Ekeberg and Riley (1997)	Southeast Norway	Loam	415	9	Spring wheat	3760	3280
11	Cantero-Martinez et al. (2003)	Guissona, Spain	Clay loam	<350	3	Barley	4163	3803
12	Cantero-Martinez et al. (2003)	Agramunt, Spain	Sandy silt loam	<350	3	Barley	3770	3230
13	Buschiazzo et al. (1998)	Córdoba, Argentina	Silt loam	760	11	Soybean	3230	2480
14	Buschiazzo et al. (1998)	Córdoba, Argentina	Silt loam	760	11	Sorghum	5720	4780
15	Buschiazzo et al. (1998)	Buenos Aire, Argentina	Sandy loam	660	7	Wheat	1600	1040
16	Mrabet (2000)	Casablanca, Morocco	Clay	296	3	Maize	2470	2410
17	Wang et al. (2012)	Luoyang, Henan, China	Sandy loam	570	6	Winter wheat	4534	4413
18	Franchini et al. (2012)	Paraná, southern Brazil	Clay	1651	23	Soybean	3071	2496
19	Kutcher and Malhi (2010)	Saskatchewan, Canada	Sandy loam	-	5	Barley	3069	2796
20	Kutcher and Malhi (2010)	Saskatchewan, Canada	Clay loam	-	5	Barley	3133	2760

21	Arshad et al. (1994)	Alta, Canada	Clay	449	3	Wheat	1570	1530
22	Filipovic et al. (2006)	north-west Slavonia, Croatia	Silt loam	817	4	Winter wheat	5680	5590
23	Wang et al. (2011)	Shanxi province, China	Sandy loam	520	5	Maize	5347	5185
24	Karunatilake et al. (2000)	Willsboro, New York	Clay loam	-	7	Maize	7260	6420
25	Sanchez-Giron et al. (2004)	Madrid, Spain	Loam	430	13	Winter wheat	3169	3032
26	Kumar et al. (2013)	western Uttar Pradesh, India	Sandy loam	800	3	Winter wheat	4490	4090
27	Lafond et al. (1992)	Saskatchewan, Canada	Clay	534	3	Winter wheat	2070	2039
28	Hemmat and Eskandari (2004)	Maragheh, Iran	Clay	476	2	Winter wheat	1717	1301
29	Halvorson et al. (2000)	North Dakota, US	Silt loam	422	12	Spring wheat	1881	1830
30	Aulakh et al. (2012)	Ludhiana, India	Loamy sand	563-995	4	Soybean	2226	2178
31	Verhulst et al. (2011)	El Batán, Mexico	Clay	625	12	Maize	5650	4310
32	Halvorson et al. (2002)	Akron, US	Silt loam	419	5	Winter wheat	3122	2975
33	Lampurlanes et al. (2001)	Catalonia, Spain	Loamy	440	4	Barley	3608	3371
	Studies reporting increased yields under conventional tillage							
34	Chen et al. (2011)	Northeast China	Clay loam	530	6	Maize	4860	6787
35	Gruber et al. (2012)	Hohenheim, Germany	Loam	715	10	Winter wheat	8100	8400
36	Gruber et al. (2012)	Hohenheim, Germany	Loam	715	10	Oil seed rape	4000	4100
37	Gruber et al. (2012)	Hohenheim, Germany	Loam	715	10	Oats	3800	4700
38								
39	Vogeler et al. (2009)	Braunschweig, Germany	Silty loam	620	8	Maize	4780	5390
40	He et al. (2011)	Gaocheng in Hebei, China	Silt loam	494	11	Summer maize	9945	10727
41	Carter (2005)	Prince Edward Island, Canada	Loam	403	8	Barley	2730	2790
42	Nyborg et al. (1995)	North central Alberta	Loam	547	11	Maize	2090	3240
43	Nyborg et al. (1995)	North central Alberta	Silty clay loam	452	11	Maize	2640	3750
44	Buschiazzo et al. (1998)	Buenos Aire, Argentina	Sandy loam	660	7	Maize	5000	5200
45	Buschiazzo et al. (1998)	La Pampa, Argentina	Sandy loam	639	9	Sorghum	3960	4070
46	Buschiazzo et al. (1998)	La Pampa, Argentina	Sandy loam	639	9	Wheat	1440	2340

47	Buschiazzo et al. (1998)	San Luis, Argentina	Loamy sand	591	10	Maize	1400	2150
48	Patil (2013)	Bellary, India	Clay	507	2	Sorghum	1905	2151
49	Wang et al. (2012)	Shouyang, Shanxi, China	Sandy loam	520	15	Spring maize	4683	4827
50								
51	Franchini et al. (2012)	Paraná, southern Brazil	Clay	1651	23	Maize	5751	6623
52	Franchini et al. (2012)	Paraná, southern Brazil	Clay	1651	23	Wheat	2253	2287
53	Filipovic et al. (2006)	north-west Slavonia, Croatia	Silt loam	817	4	Maize	7540	7690
54	Sanchez-Giron et al. (2004)	Madrid, Spain	Loam	430	16	Winter barley	3024	3046
55	Machado et al. (2007)	Oregon, US	Silty	398	6	Winter wheat	2180	2560
56	Machado et al. (2007)	Oregon, US	Silty	398	6	Spring wheat	1640	2200
57	Machado et al. (2007)	Oregon, US	Silty	398	6	Spring barley	1700	3360
58	Lafond et al. (1992)	Saskatchewan, Canada	Clay	534	3	Spring wheat	2548	2553
59	Lyon et al. (1998)	Sidney, US	Silty	440	25	Winter wheat	2430	2620
60	Aulakh et al. (2012)	Ludhiana, India	Loamy sand	563-995	4	Winter wheat	3226	3283
61	Wilhelm and Wortmann (2004)	Nebraska, US	Silty clay loam	708	16	Maize	6200	6750
62	Wilhelm and Wortmann (2004)	Nebraska, US	Silty clay loam	708	16	Soybean	2450	2480
Studies reporting little difference in yields under both tillage systems								
63	Carter (2005)	Prince Edward Island, Canada	Sandy loam	403	9	Soybean	1540	1540

Table 1.4. Soil carbon sequestration rates under conservation tillage

Region	Carbon sequestration rate achievable by conservation tillage (g C/m^2 per year)	Time period to attain the sequestration rate	Depth of soil (cm)	Reference
Global soils	57	15 years	Top 22 cm	West and Post (2002)
US Great plains	30-60	-	-	Follet (2001)
US Croplands	10-50	In 5-10 years	Top 20 cm	Lal et al. (1998)
US Croplands	34	20 years	Top 30 cm	West and Marland (2002b)
Global soils	33	30 years	Top 30 cm	Hermle et al. (2008)
Tropical-humid	3-20	30 years	Top 100 cm	Farina et al. (2011)
Sub tropical humid	2.67	10 years	60 cm	Sainju et al. (2008)
Sub tropical humid	0.7	7 years	40 cm	Al-Kaisi et al. (2005)
Semi arid	0.55	20 years	20 cm	Hernanz et al. (2009)
Semi arid	0.5	17 years	60 cm	Lopez-Fando and Pardo (2011)
Semi arid	2.46	16 years	30 cm	Álvaro-Fuentes et al. (2009)
Arid areas in India	2.69	20 years	30 cm	Grace et al, (2012)

2. Chapter 2: The effect of soil texture and management on soil biophysical and chemical behaviour

This chapter contains the results from a small experiment conducted by sampling tilled soils and a grass stewardship site at the University of Nottingham campus at Sutton Bonington. This experiment helped to devise sampling strategy, X-ray computed tomography (CT) and greenhouse sampling procedures that would be used in more detail in subsequent studies. In this chapter the first sub aims of the project was addressed. i.e. the effect of tilled and untilled soils on soil pore characteristics and its impact on CO_2 emission.

2.1 Introduction

It has been suggested that soil tillage can cause deterioration of soil structure and a rapid loss of soil organic matter, by changing biological activity in soil and disturbing the physical properties of soil, along with reduction in crop yields over a long term (Gosai et al., 2009). Reduced tillage has become an important management strategy offering many benefits like increased organic matter content (Kong et al., 2009), sequestration of carbon (Lal, 2009), mitigation of greenhouse gas emissions (Kong et al., 2009), greater aggregate stability (Six et al., 1999),biological activity (Chatterjee et al., 2009) and prevention of soil erosion and runoff (Cássaro et al., 2011). However it is also reported that reduced tillage practices increase soil compaction and reduce porosity (Petersen et al., 2008), decrease air and water movement (Hubert et al., 2007) and increase emission of greenhouse gases due to the decomposition

of crop residues retained at the surface. Advanced technologies such as X-ray CT can be used to reveal the structure, aggregation and pore characteristics of soil under different management practices, and supported by studies of microbial properties in soil can yield new knowledge on involvement of biota in the sequestration of carbon. The aim of this experiment was to study the effect of different tillage practices on soil structure, pore dynamics and release of CO_2 in soils under two contrasting soils and under different levels of soil management.

2.2 Materials and methods

2.2.1 Field site and sampling

The sites used were at the University of Nottingham experimental farm, Sutton Bonington Campus, Leicestershire, UK (52.5^{o}N, 1.3^{o}W) and at the Controlled Traffic Farming demonstration farm, (CTF Europe Ltd), Bedfordshire UK. At Sutton Bonington treatments consisted of two soil types/textures (sandy loam of the Dunnington Heath series (FAO: Stagno Gleyic Luvisol) and clay loam of the Fladbury series (FAO: Pelo-alluvial gley soil) and two soil management regimes (tilled crop land and grass stewardship). The tilled sites were under constant cultivation for long periods of time (>20 years) in a rotation of winter wheat and winter oats whereas the stewardships (grassed strips between cropped fields) were seeded in September 2001 prior to previously being used as arable crops. The tillage operations included a single pass of a heavy disc cultivator, followed by power-harrowing, drilling with crop seeds followed by rolling. At the CTF farm, the sites were under no-tillage since 2004 with use of tractor for sowing and harvest only. The soil textures at Bedford are clay and clay loam. Sampling was done at three sites subjected to three different soils

trafficking regimes; namely random traffic, no-traffic and intermediate traffic. In the case of random traffic sites, the tractor pass was at random in the field year after year; whereas in no-traffic sites the wheeled traction is restricted to permanent tram lines. The intermediate traffic treatments had wheeled traction in between both. The sampling was done from the middle of the sites, making sure to avoid the tram lines.

Intact soil cores were collected from the topsoil using polyvinyl chloride pipes of 150 mm length and 50 mm internal diameter. Firstly the soil surface was cleared of vegetation and the PVC pipes were pushed in to a depth of 100 mm. The cylinders were pushed into the soil by a hammer with a flange on top to ensure vertical penetration. The core samples were trimmed, labelled and placed in plastic bags. Field moist bulk samples were also collected to measure particle size, organic matter content and microbial biomass carbon. The bulk samples were stored at 4^{o}C and soil cores in a constant temperature room at 15^{o}C until analysed.

2.2.2 X-ray Computed Tomography

For X-ray CT scanning, intact soil cores collected in the field using polythene pipes were used. The X-ray CT scanner was Nanotom, Phoenix X-ray system made by GE Sensing and Inspection Technologies GmbH, Germany (Fig. 2.1). This Micro CT system is characterised by the presence of high resolution detectors with the detectability of 1 μm. The X-ray tube is characterised by nanofocus <800 nm spot size with maximum voltage of 180 kV and a maximum output of 15W.

Fig. 2.1. Phoenix nanotom X-ray Computed Tomography scanner (http://www.ge-mcs.com)

2.2.2.1 Sample preparation and acquisition of CT data

The samples were scanned over a range of angular orientations using the X-ray beam generated by passing high energy current (expressed in μA) over a tungsten target. The energy levels of X-ray beam generated is described as the peak X-ray energy in kV. The soil core was placed on the sample stage and the position was adjusted to ensure that the sample was within the field of view and was fitted firmly to avoid sample movement during the scan. After placing the soil core in the sample stage, the energy levels and current were adjusted to obtain good quality images in a reasonable time period (detector time). This was done by looking into the histogram to get a grey scale value of ≥20% of

the dynamic range of detector, in the densest part of the soil core (centre). Use of a copper filter to control the incident X-ray beam aided getting good quality images. The possibilities of changing detector position from the X-ray gun were also tested to get best possible resolution. Once the scanning parameters such as energy current, detector timings, binning, spin and resolutions were decided, the sample was removed from the sample stage to calibrate the X-ray signals. Two calibrations were undertaken namely offset and gain. During offset calibration the X-ray was switched off while X-ray was switched on during the scanning for "gain" calibration. These calibrations are done to standardise the detector with respect to X-ray signals being generated and it serves as the baseline from which all sample scan data are subtracted. Then the core sample is introduced back to the sample stage and CT scanning was performed. Scanning was done at energy levels of 130 kV and a current of 110 μA. The soil cores were scanned in a vertical upright position. A total of 2000 images of resolution 27.5 μm were recorded over 60 minutes for each core.

2.2.2.2 Image reconstruction

Image reconstruction is a mathematical process to generate images from projection data obtained by CT scanning. The reconstruction of images was performed by datos|x software (GE Sensing and Inspection Technologies GmbH, Germany) and then using VG Studiomax (volume graphics); an image processing software. In the datos|x software the raw projection intensity data are converted to CT numbers in a range of grey scales (12 bit) which in turn correspond to the X-ray attenuation coefficient which is a function of density, atomic number and X-ray energy (Ketcham, 2005). A total of 2000 images were acquired for each scan..

2.2.2.3 Artefacts

Using the scan optimiser option in datos|x the difference between first and last image was computed and the value was accepted. This step eliminates artefacts caused by movement, if any, during scanning.

2.2.2.4 Beam hardening

Since the size of sample used in all the experiments was large, hardening of X-ray beam was expected. Beam hardening makes the edges of sample brighter than the centre parts. It is caused by an increase in mean energy of X-ray as it passes through the sample to be scanned; since lower energy X-rays in a polychromatic beam get attenuated more readily. To reduce this artefact a copper filter of thickness 0.1 mm was used in front of X-ray tube to pre-harden the X-ray beam, before beginning of scanning.

2.2.2.5 Image resolution

By scanning the soil cores through a 360^{o} rotation, image data is recorded in the form of stack of slices. To account for the thickness element of each slice, which provides the three-dimensional capabilities for CT images, the pixels in CT images are referred to as voxels. The resolution of CT images is given as voxel size in μm which indicate the size of a 3D pixel that can be identified as an independent entity. The image resolution varied with each experiment and is given separately in each chapter.

2.2.2.6 Image visualisation and saving for analysis

The reconstructed data of each scan was opened in VG Studio Max software and saved as image stack for further analysis (Fig. 2.2)

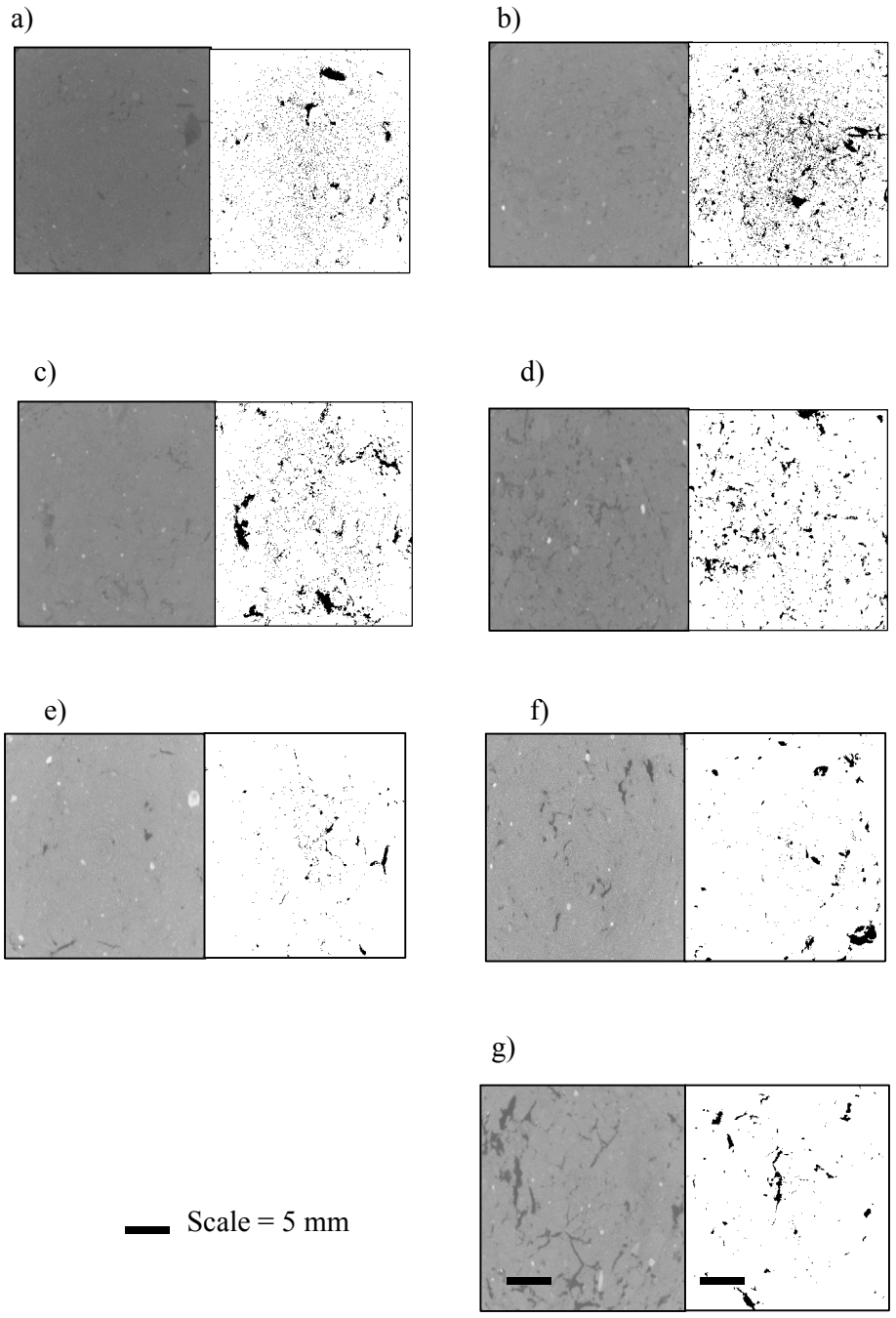

Fig. 2.2. Selected original and gray scale images of (a) clay loam tilled (b) clay loam grass strip (c) sandy loam tilled (d) sandy loam grass strip from Sutton Bonington and (e) Random traffic (f) Intermediate traffic and (g) No-traffic soils of Bedford (Pore space is shown in black).

2.2.2.7 Image analysis

Images analysis was carried out using *ImageJ* software (Rasband, 2002) to study the soil pore characteristics. *ImageJ* is an open source software written in Java. A rectangular region of interest (33 x 33 mm^2) was selected in the reconstructed CT images to exclude pores adjacent to the sample edges. A total of 1800 images were used in the analysis excluding 100 images from the start and the end which are more prone to cone beam artefacts. A suitable image routine was developed after trying different filters and image enhancement techniques. The contrast of all images was enhanced, normalised and equalised. The function 'sharpen' increases the contrast and accentuates details in the image. A median filter was used reduce noise. The differentiation of pores from solids was made by thresholding with a suitable automated algorithm and the image was converted to an 8-bit gray scale image. Thresholding is used to convert a gray scale image into binary by defining a segmentation point on a histogram. This step facilitated classifying the image into features of interest (pores) and background (solids). The thresholding algorithms used were 'minimum' 'MinError' and 'MaxEntropy'. The noise in the subsequent binary image was then removed by the 'remove outlier' option which replaces a pixel by the median of the pixels in the surrounding if it deviates from the median by more than the value assigned for threshold (ImageJ, 2012).The statistics on pore characteristics of each individual pore were generated using the 'analyse particles' option in *ImageJ*. The information on number of pores, average pore size, porosity, pore size distribution, surface area and circularity of pores were obtained. A coefficient of uniformity was calculated to statistically compare the pore size distribution. This was

determined as the ratio of size of pores at 10% and 60% of total macro pore distribution (Atkinson et al., 2009). The image routine followed in each experiment varied slightly and is given in respective chapters. A general flow chart showing the image analysis is depicted in Fig. 2.3.

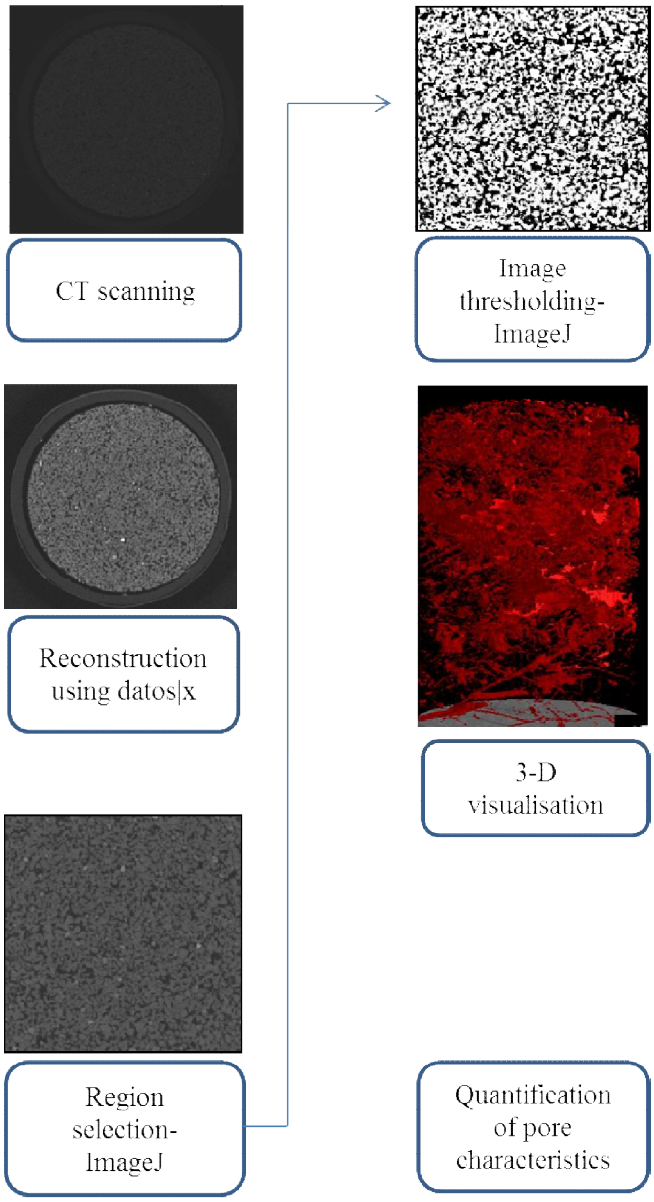

Fig. 2.3. Flow chart showing the procedures used in image acquisition and analysis.

2.2.3 Carbon dioxide emissions from soil

For the procedure adopted for greenhouse gas estimation please see Appendix.

2.2.4 Soil organic matter and microbial biomass carbon

For a detailed description of procedure adopted please refer to Appendix.

2.2.5 Soil physical properties

Soil shear strength data were recorded using procedures described in Appendix. The shear strength data were not recorded in Bedford due to more wetter and clay texture of soil that made recording shear strength difficult. The laboratory estimation of physical characteristics such as time to ponding, saturated hydraulic conductivity, water content and bulk densitywere also estimated. The detailed procedures are provided in Appendix.

2.2.6 Statistical analysis

The statistical software package Genstat (v. 14) was used for the analysis of variance (Šimon et al. 2009) to test the significance of differences. The two fields at Sutton Bonington were analysed separately. A two-way analysis of variance was applied to results obtained from laboratory, field measurements and image analysis in the samples from Sutton Bonington with soil texture and soil management as two factors. A one-way analysis of variance was used to test the significance of differences in different trafficking in Bedford. The treatment means were compared at the $P < 0.05$ level using the LSD. Standard errors of means were calculated and provided as required. Multiple regression analysis was performed with average CO_2 emissions as the dependent variable and other physical, chemical and biological properties studied in this

experiment as explaining variables to find out the relative effect of these parameters on CO_2 emission and to predict the best model describing the fluxes of GHGs from soil. By using stepwise backwards elimination process, only the variables that contributed significantly to the model and reduced the residual sum of squares were retained in the model. For illustrative purposes single linear regression was also carried out between the parameters that contributed to the multiple regression models.

2.3 Results

2.3.1 Soil pH

The soil pH did not exhibit any significant difference between contrasting soil management. The textural difference was also not significant.

2.3.2 Soil organic matter

Both texture and soil management affected the organic matter content in soil. There was significantly more soil organic matter content (9.02% SOM) in the clay loam under grass stewardship (P <0.001, Fig. 2.4). Sandy loam soil when tilled had the lowest soil carbon content of 4.2% (P <0.001). The SOM content in the grass stewardship soil was higher by 4.6% in sandy loam soil compared to 40.3% in clay loam soil indicating a less prominent effect of soil management on net carbon changes under coarse textured soil. Among the controlled traffic farming treatments, no-traffic treatments recorded the highest SOM content (9.3%) and least in randomly trafficked sites (7.9%) with a P value of <0.05.

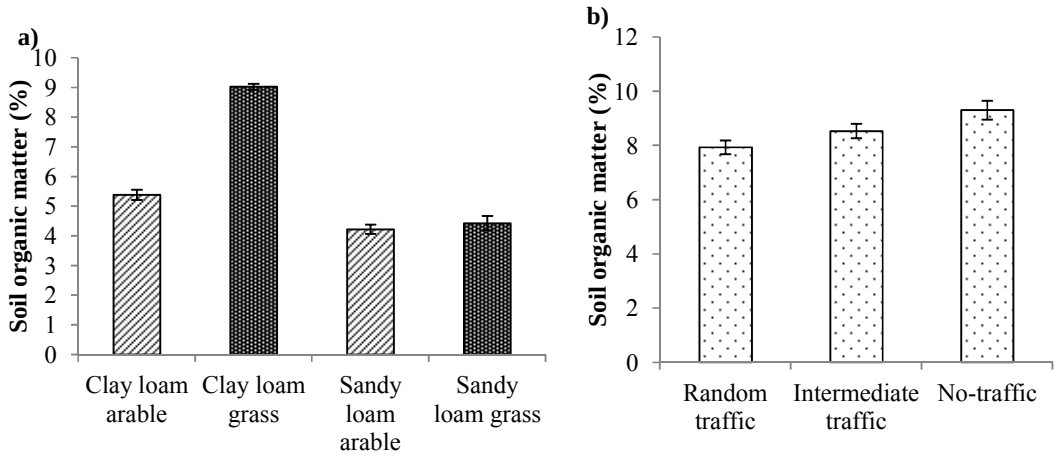

Fig. 2.4. Soil organic matter (SOM) content in different soils (a) Sutton Bonnington (Texture: $F_{1,12}$ = 268.01, P <0.001; Soil management: $F_{1,12}$ = 119.46, P <0.001;Texture x Soil management: $F_{1,12}$ = 95.57, P <0.001) (b) Bedford ($F_{2,9}$ = 5.43, P <0.05). Bars indicate ±SEM.

2.3.3 Soil organic carbon and carbon stock

Soil management practices had a significant effect on soil organic carbon content in both clay loam and sandy loam soils (P <0.001, Table 2.1). The clay loam in grass stewardship soil recorded almost double the soil organic carbon (3.1%) than the tilled arable fields (1.6%). The soil organic carbon follows the pattern same as of humus in these soils. Both soil texture and soil management significantly influenced the soil organic carbon stock (P <0.001). There was significantly more soil organic carbon stock under clay loam soil than sandy loam soil, similarly under grass stewardship soil than tilled arable soil. The SOC stocks under grassland were 66.4% higher than under arable clay loam soil and 9.2% higher in the sandy loam under grassland than under arable sandy loam soil. The soil organic carbon stock in differently trafficked soil did not exhibit significant difference.

Table 2.1. Soil organic carbon stock (Mg ha^{-1}) in different soils in Sutton Bonington and Bedford

Treatments	Soil organic carbon stock (Mg ha^{-1})*
Clay loam arable	18.62±0.59
Clay loam grass	42.07±1.11
Sandy loam arable	19.41±0.59
Sandy loam grass	21.19±0.72
Random traffic	22.82±0.88
Intermediate traffic	23.59±0.88
No-traffic	25.78±0.90

*Mean ± standard error of mean

2.3.4 Microbial biomass carbon and nitrogen

Both texture and soil management affected the microbial biomass carbon in soil (Fig. 2.5a). Clay loam soils had significantly greater microbial biomass carbon than sandy loam soils (552 mg kg^{-1} soil) (P <0.01). Soil management significantly (P <0.05) reduced the microbial biomass in both clay loam and sandy loam soil by 40.4% and 28.3% respectively than in the grassland soils. However the interaction of soil texture and soil management was not significant. In the controlled traffic soils, the soils with intermediate traffic recorded significantly higher (1329 mg kg^{-1} soil) (P <0.01) microbial biomass carbon with not much difference between random traffic and no-traffic soils (Fig. 2.5b). The microbial biomass nitrogen followed similar trend as that of

microbial biomass carbon (Fig. 2.6). The interaction of texture and soil management was significant in the case of microbial biomass nitrogen. In the clay loam and sandy loam soils the grassland soils contained higher microbial biomass nitrogen (181 and 68 mg kg^{-1} soil respectively) than arable managed soil (82 and 55 mg kg^{-1} soil respectively).

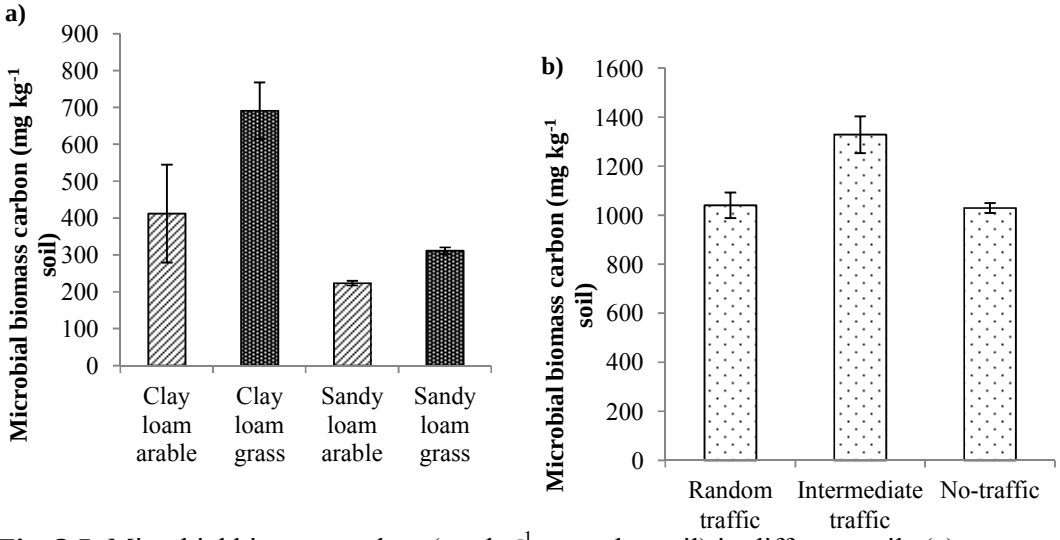

Fig. 2.5. Microbial biomass carbon (mg kg^{-1} oven dry soil) in different soils (a) Sutton Bonington (Texture: $F_{1,12}$ = 13.67, P <0.01; Soil management: $F_{1,12}$ = 5.71, P <0.05;Texture x Soil management: $F_{1,12}$ = 1.55, P NS) (b) Bedford ($F_{2,9}$ = 9.87, P <0.01). Bars indicate ±SEm.

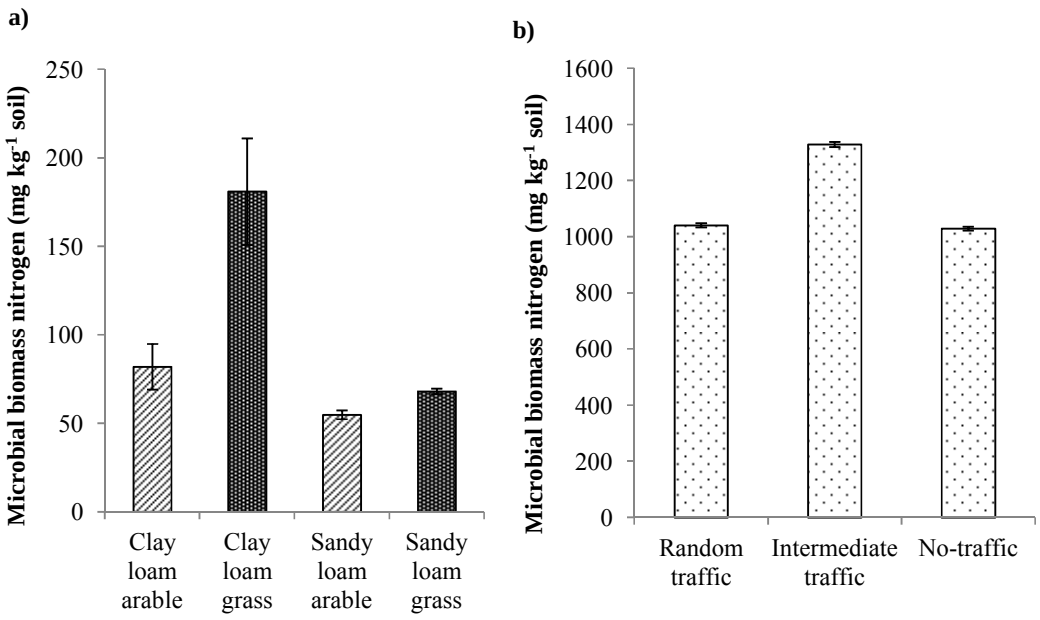

Fig. 2.6. Microbial biomass nitrogen (mg kg^{-1} oven dry soil) at (c) Sutton Bonington (Texture: $F_{1,12} = 18.15$, $P < 0.001$; Soil management: $F_{1,12} = 11.66$, $P < 0.01$;Texture x Soil management: $F_{1,12} = 6.82$, $P < 0.01$) (d) Bedford ($F_{2,9} = 6.82$, $P < 0.05$). Bars indicate ±SEm.

2.3.5 Hydraulic conductivity, shear strength and ponding limit

Soil texture and soil management significantly affected the saturated hydraulic conductivity of soil ($P < 0.01$ and < 0.001, Fig. 2.7a). The highest saturated hydraulic conductivity of 0.498 cm s^{-1} was recorded under sandy loam with grassland stewardship soil which was significantly higher than under arable soil ($P < 0.05$). Similarly in clay loam soil the arable (0.05 cm s^{-1}) and grassland stewardship treatments (0.22 cm s^{-1}) varied significantly in hydraulic conductivity. In case of shear strength, the textural differences on soil strength were not statistically significant. Soil management had a significant effect on soil strength ($P < 0.001$), with lower soil strength under arable soil (Fig. 2.7b).

The shear strength under arable clay loam soil was 16.6 kPa against 22.8 kPa under grassland soil, whereas in sandy loam soil, the arable field had 12.9 kPa strength against 23.5 in grassland soil.

The ponding limit gives indirect estimates of soil porosity and soil hydraulic conductivity. Clay loam soils under arable ponded earlier than any other treatments (Fig. 2.8, *P* <0.001) while the sandy loam soil under grassland stewardship was by far the slowest to pond.

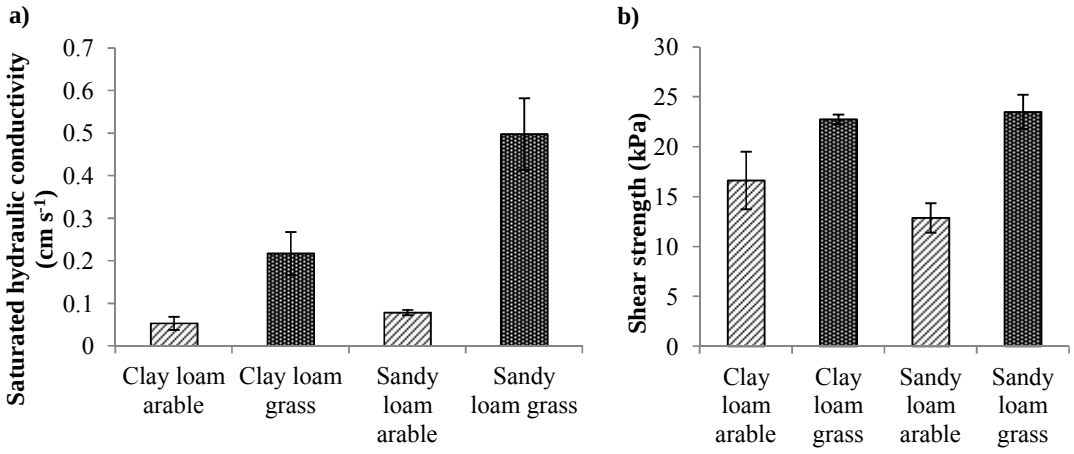

Fig. 2.7. (a) Saturated hydraulic conductivity (Texture: $F_{1,12}$ = 9.41, P <0.01; Soil management: $F_{1,12}$ = 34.24, P <0.001;Texture x Soil management: $F_{1,12}$ = 6.56, P <0.05) (b) and Shear strength (Texture: $F_{1,12}$ = 0.66, P NS; Soil management: $F_{1,12}$ = 20.58, P <0.001;Texture x Soil management: $F_{1,12}$ = 1.49, P NS) in different soils at Sutton Bonington. Bars indicate ±SEm.

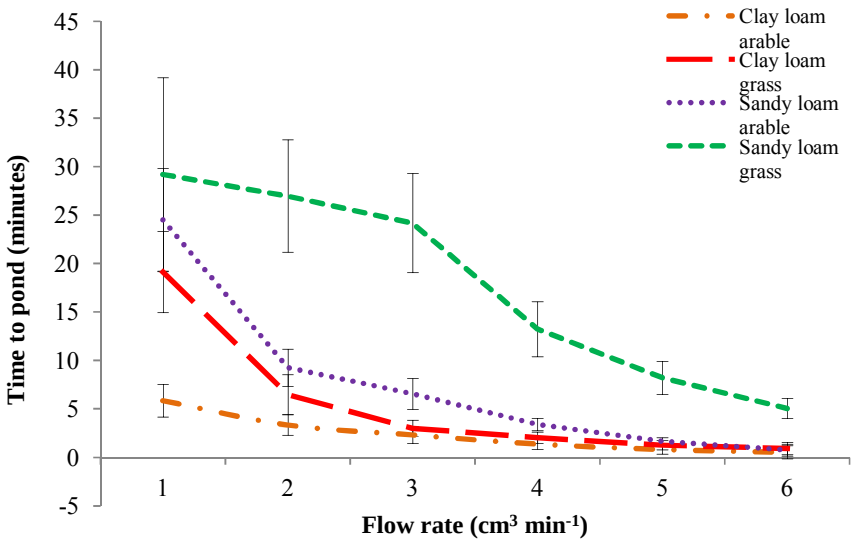

Fig. 2.8. Time to pond (minutes) by soils under different category. Bars indicate ±SEm, 15 d.f.

2.3.6 Bulk density

Sandy loam textured soil generally had higher bulk density (26.9%) than the clay loam soils (P <0.05, Fig. 2.9). The soil management effect on bulk density was not significant. Bulk density did not differ significantly in differently trafficked soils.

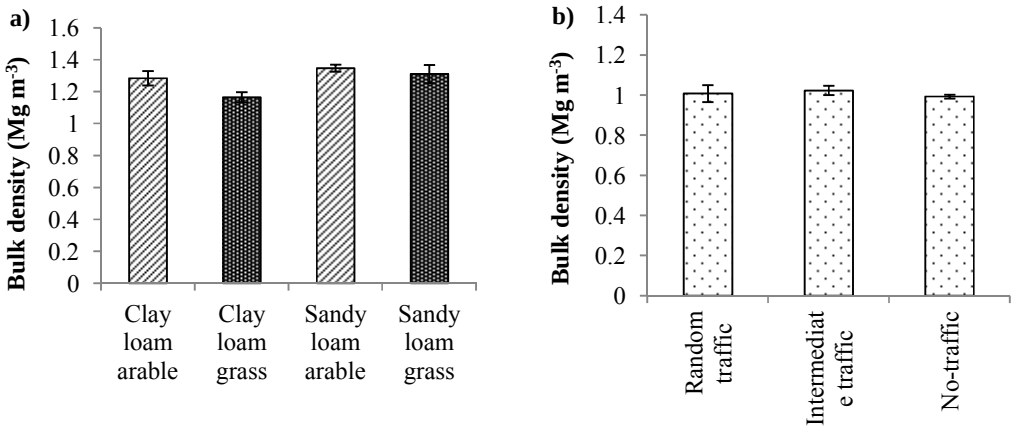

Fig. 2.9. Bulk density (Mg m^{-3} oven dry soil) in different soils (a) Sutton Bonnington (Texture: $F_{1,12}$ = 6.34, P <0.05; Soil management: $F_{1,12}$ = 3.57, P

NS;Texture x Soil management: $F_{1,12} = 0.98$, P NS) (b) Bedford ($F_{2,9} = 0.3$, P NS). Bars indicate ±SEm.

2.3.7 Carbon dioxide emissions

Different soil textural classes did not influence the emission of carbon dioxide from the soil incubated at constant temperatures. Soil management practices significantly influenced the CO_2 fluxes with greater emission from soils under grassland stewardship (368 mg m^{-2} h^{-1}) compared to arable soil (283 mg m^{-2} h^{-1}) (P <0.05, Fig. 2.10). There were no significant differences among differently trafficked soils.

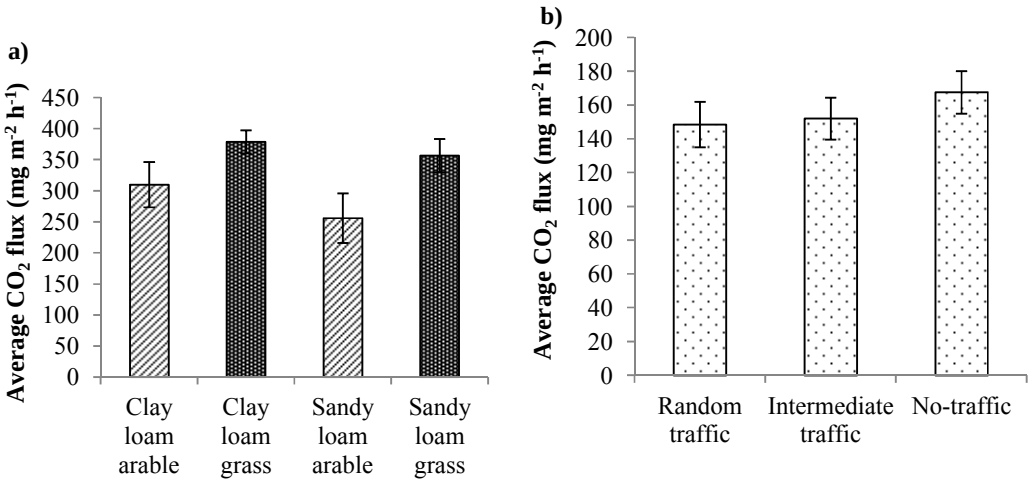

Fig. 2.10. Soil CO_2 flux in different soils (a) Sutton Bonington (Texture: $F_{1,12} = 6.08$, P NS; Soil management: $F_{1,12} = 3.24$, P <0.05;Texture x Soil management: $F_{1,12} = 0.06$, P NS) (b) Bedford ($F_{2,9} = 0.63$, P NS). Bars indicate ±SEm.

2.3.8 Soil structural analysis

2.3.8.1 Porosity, pore size and pore size distributions

Increased macro porosity was noticed in sandy loam and clay loam soils under grassland stewardship than their arable counterparts, although not significant (Table 2.2). Randomly trafficked soils recorded the highest porosity among the differently trafficked soil, but was also not significant. Grassland stewardship soils recorded maximum average pore size with clay soils having mean value of 0.25 mm^2 and sand soil 0.22 mm^2. Average pore size was also not significantly different between treatments (Table 3.2). The average pore size was greater under untilled condition in both clay and sand.

Table 2.2. CT measured average soil porosity (%), and average pore size (mm^2) under different treatments*

Treatments	Soil porosity (%)	Pore size (mm^2)
Clay loam arable	10.98±1.57	0.18±0.04
Clay loam grass	11.87±2.17	0.20±0.06
Sandy loam arable	10.04±1.47	0.13±0.04
Sandy loam grass	10.76±1.99	0.22±0.07
Random traffic	7.04±1.18	0.18±0.05
Intermediate traffic	6.52±1.20	0.19±0.03
No-traffic	4.90±1.16	0.22±0.05

*Mean±Standard Error of Mean

The pore size distribution varied with respect to soil texture and soil management practices adopted (Fig. 2.11). Tillage increased total pore area in both clay loam and sandy loam soils, but was statistically not significant. Similarly, soil trafficking also increased the pore size distribution. The arable sandy loam soil and intermediate traffic soil had comparatively more uniformly distributed pore size classes. Random traffic and no-traffic appeared to increase the number of larger pores.

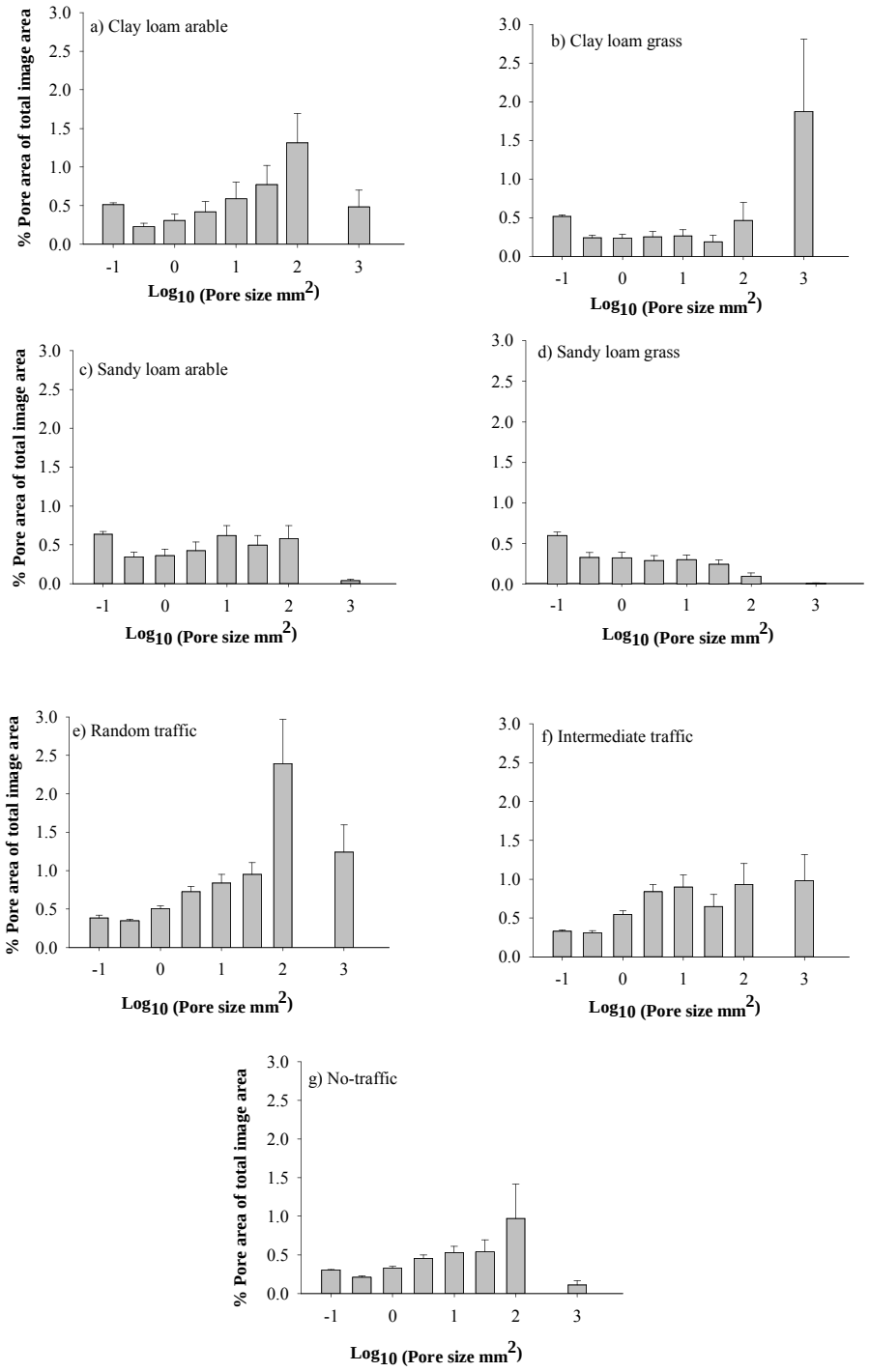

Fig. 2.11. Pore size distributions of a) clay loam arable, b) clay loam grassland, c) sandy loam arable d) sandy loam grassland of Sutton Bonington and e) Random traffic, f) Intermediate traffic and g) no- traffic soils in Bedford, by image analysis on X-ray CT images. Error bars represents s.e.d.

2.3.9 Relationship between different soil properties

CO_2 fluxes was predicted by a multiple regression model ($P < 0.001$) including soil organic carbon and soil porosity (P) which accounted for 69.4% of the variation. The optimal model for CO_2 flux is provided in equation 1. The variation contributed by porosity was 43.3% compared to 26.1% for SOC.

$$CO_2 \text{ flux (mg } g^{-1} h^{-1}) = 0.000225 + 0.000131P + 0.000383SOC \quad (1)$$

The linear regression studies indicated that soil structural properties were related to other chemical properties. As porosity increased, soil microbial biomass carbon (P <0.01, R^2 = 0.31, Fig. 2.12a) and soil organic matter content (P 0.08, R^2 = 0.12, Fig. 2.12b) decreased. The increased aeration associated with soil pore space might have triggered microbial activity which brings about faster decomposition of soil organic matter.

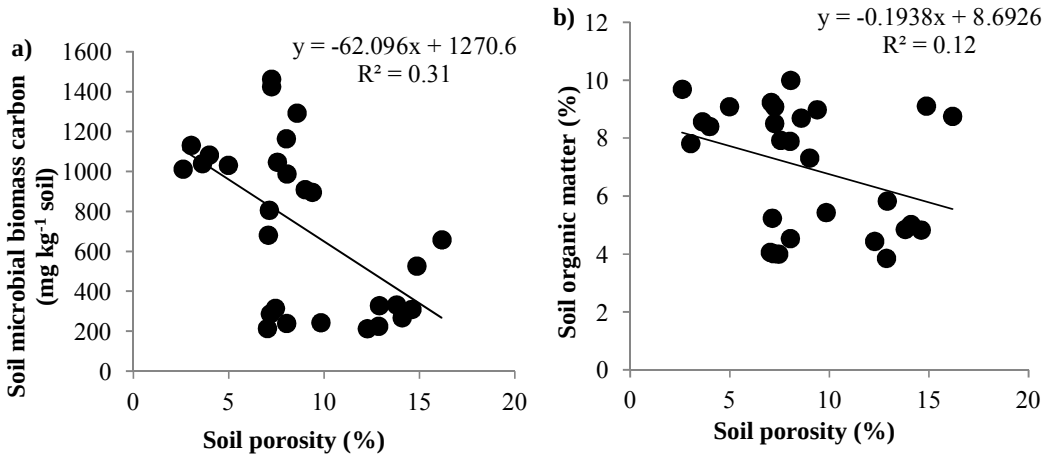

Fig. 2.12. Relationship between (a) porosity and soil microbial biomass carbon ($F_{1,26}$ = 11.88, P <0.01) and (b) porosity and soil organic matter ($F_{1,26}$ = 3.45, P NS).

The emission of CO_2 from soil was negatively related to the microbial biomass carbon (P <0.01, R^2 = 0.29, Fig. 2.13a). Soil physical properties had a strong

impact on the CO_2 fluxes. As the bulk density decreased the CO_2 flux from soil increased ($P < 0.01$, $R^2 = 0.55$). In parallel, soil porosity had a significant positive relationship with the CO_2 emission from the soil ($P < 0.001$, $R^2 = 0.65$, Fig. 2.13b).

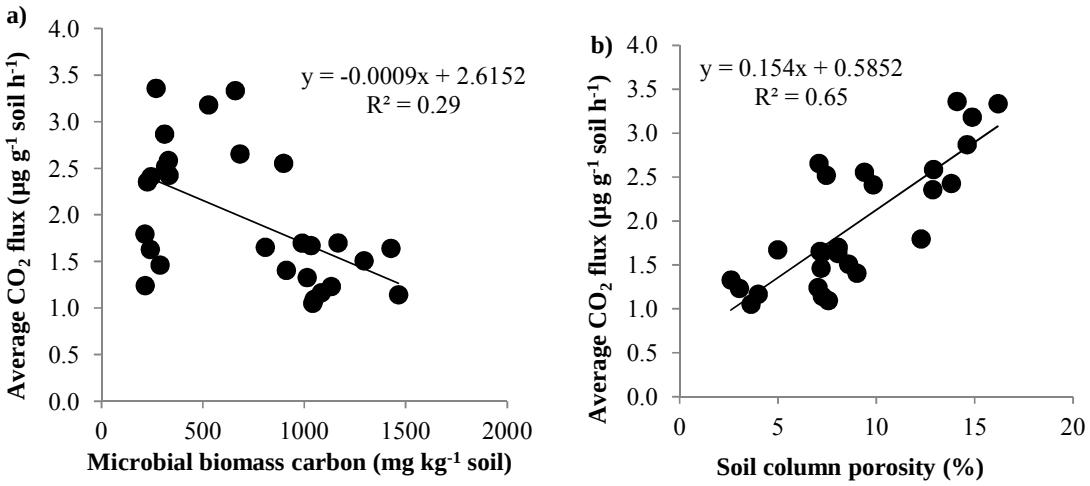

Fig. 2.13. Relationship between CO_2 flux with (a) soil microbial biomass carbon ($F_{1,26} = 10.48$, $P < 0.01$) (b) porosity ($F_{1,26} = 48.55$, $P < 0.001$).

2.4 Discussions

Soil texture and physical disturbance by soil management affected the physico-chemical and biological properties of soil. Surprisingly bulk density was not affected by different tillage systems (tilled crop land and untilled grass steward). The slightly lower bulk density, although not significant, under untilled soil may be due to increased growth of grass roots and resultant increase in soil pores and also absence of tillage and traffic (Udawatta et al., 2008). The adoption of grass strips decreased soil macroporosity compared to arable soils. Evrendilek et al. (2004) reported that the conversion of grass land to arable led to a 9.1% reduction in soil porosity over 12 years in the 0-20 cm layer of Typic Haploxeroll in central Taurus mountains of Turkey. The

presence of extensive fibrous root system and constant supply of organic materials in soils under grassland stewardship might have improved the soil physical properties compared to the arable soils. However the distribution of various pore size classes indicated the occurance of larger pore classes under tilled soil condition due to the cultivation practices. The soil porous architecture has a significant effect on soil hydraulic properties. The increased macroporosity under grassland soils resulted in increased saturated hydraulic conductivity (Fig. 2.7a) which minimises the ponding risk (Fig. 2.8) in the event of excessive rainfall and minimise runoff losses.

The highest carbon content, soil organic matter and SOC stock under grass stewardship was attributed to increased biomass addition in line with findings in an improved fallow compared to continuous maize in Zimbabwe by Nyamadzawo et al. (2009). This is supported by the positive correlation of soil organic carbon with microbial biomass carbon also reported by Hassink (1995). Disturbance of soil is also believed to enhance macroaggregate tunover leading to decomposition of protected soil organic matter (Six et al., 1999). This indicates that reducing soil disturbance as in the case of grassland strips could lead to increased sequestration of carbon (Beare et al., 1994). The C sequestration capacity of clay loam soil is greater than in sandy soil due to the possibility of absorption of organic carbon to clay surfaces, entrappment of carbon on pores of aggregates or encapsulation of organic carbon by clay particles (Nyamadzawo et al., 2009). Balota et al. (2003) attributed the increase in microbial biomass carbon under no-tillage to factors such as higher moisture content, greater soil aggregation and higher C content. In a land use practice such as grass stewardship, steady organic sources, both labile and non labile

are avaialble for sustained microbial activity. Tufekcioglu et al. (2003) reported detritus by grasses might stimulate the heterotrophic microbes in soils, later contributing to soil organic matter by way of microbial products. Increased microbial activities are favorable for the formation of stable soil aggregates, which help with further physical protection of carbon (Lal, 2004). Although both microbial biomass carbon and nitrogen showed significant differences with respect to both texture and tillage, C/N ratio did not differ significantly as also observed by Helgason et al. (2010).

Avoiding soil disturbance leads to accumulation of soil organic carbon (Bayer et al., 2000; Bayer et al., 2006) especially in the surface soil horizons (Baker et al., 2007; Chatterjee et al., 2009; Lal, 2009). The greatest average emission of CO_2 in the present study was obtained under the grassland stewardship soils that can be attributed in part to increased biological activity triggered by favourable soil physico-chemical conditions and increased availability of labile carbon sources from the grass roots. Medeiros et al. (2011) found more C-CO_2 emissions under no-till management system due to retention of residues in no-tillage in contrast to residue removal in conventional tillage. The positive corelation between porosity and CO_2 flux is indicative of the release of CO_2 physically entrapped in the soil pores (Álvaro-Fuentes et al., 2007). Such a physical effect can be related to, the preservation of soil structure (Tormena et al., 1999), creation of ideal pore sizes, greater macropore volume and better pore connectivity (Medeiros et al., 2011) under reduced tillage. The greater number of pores under untilled condition permits increased water and gas storage and transmission (Kim et al., 2010). Different traffiking at Bedford did

not provide statistically significant results interms of pore characteristics and CO2 emission and hence is not discussed.

2.5 Conclusions

This study indicated that non disturbance of soil by grassland stewardship management increased soil microbial biomass carbon, soil organic carbon and SOC stock in both clay loam and sandy loam soil, indicating the potential capability of reduced tillage system to sequester more carbon compared with a tilled soil. Soils under grass stewardship were also found to be better at water conducting than arable soils and helped to prevent water ponding on the soil surface. However significantly increased CO_2 fluxes was recorded under grassland stewardship compared to arable soils which was independent of soil texture. Multiple regression analysis showed CO_2 fluxes were mainly affected by soil organic carbon and porosity indicating the importance of soil structural properties in the release of biogenic gases from soil. This study suggests that soil management practices that affect soil structural properies might have significant influence on greenhouse gas emission. However further work is required to probe these complex relationships further. This study indicated that tilling or not tilling have a significant effect on soil physical charcateristics which in turn linked to emission of CO_2. Since tillage brings about changes in soil physical properties especially soil aggregates and structure, this will be addressed in the next chapter.

3. Chapter 3: The effect of soil aggregate size on pore structure and its consequence on emission of greenhouse gases

The experiment in previous chapter (Chapter 2) showed that CO_2 fluxes in soil were affected by soil organic carbon and soil porosity which in turn was modified by tillage. It was shown by other workers that tillage brings about significant changes in soil aggregate characteristics. In this chapter the effect of aggregates of different sizes in sandy loam and clay loam soil on soil pore characteristics, hydraulic properties and greenhouse gas emission from soil was assessed. This chapter has been published as research article in **Soil & Tillage Research** (Mangalassery et al. 2013. 132, 39-46) and is presented in published paper format.

Summary

This chapter addresses the second sub aim set out for this thesis; to understand the effect of different aggregate size classes as derived by different soil management systems on the physical characteristics of soil and emission of GHGs such as CO_2, CH_4 and N_2O. Manually re-packed soil aggregates were used to generate desired soil aggregate classes. Soil aggregation is an important physical property that influences the physico-chemical and biological properties of soil. Soil disturbances such as tillage can have a significant effect on soil aggregation. Columns of aggregates in the size ranges of 2-4 mm, 1-2 mm, 0.5-1 mm and <0.5 mm were tested along with a field structured soil (i.e. aggregates <4 mm). Soil pore characteristics were quantified using X-ray Computed Tomography (CT). The average porosity in the soil columns ranged

from 38.7 to 50.7%. Aggregate size influenced the total soil organic matter content with average values ranging from 7.5 to 8.6% in the clay loam soil and 2.8 to 5.2% in the sandy loam soil. CO_2 and CH_4 flux was significantly affected by size of aggregates. Clay loam soils emitted the most CO_2 from the small sized aggregates, whereas in sandy loam soils the larger aggregates produced the maximum CO_2 flux. Smaller aggregates produced higher CH_4 flux in both soil textures. No significant difference between aggregate sizes and soil textures were found for N_2O fluxes. Soil pore characteristics such as porosity and pore size significantly affected fluxes of GHGs such as CO_2 and CH_4. These results indicate that management practices such as tillage that heavily influence soil aggregation and pore characteristic development can have a direct impact on emission of greenhouse gases and subsequently have implications for global warming. Having established that soil aggregate changes could greatly influence soil physical characteristics including pore structure with resultant effect on greenhouse gas emissions, the field level effect of zero tillage and tillage on soil physico-chemical and biological properties and the complex interactions among these properties are covered in next chapters.

Soil & Tillage Research 132 (2013) 39–46

Contents lists available at SciVerse ScienceDirect

Soil & Tillage Research

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The effect of soil aggregate size on pore structure and its consequence on emission of greenhouse gases

S. Mangalassery, S. Sjögersten, D.L. Sparkes, C.J. Sturrock, S.J. Mooney *

School of Biosciences, Sutton Bonington Campus, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire LE12 5RD, UK

ARTICLE INFO

Article history:
Received 17 January 2013
Received in revised form 10 May 2013
Accepted 13 May 2013

Keywords:
X-ray CT
Saturated hydraulic conductivity
Soil aggregates
Pore characteristics
Greenhouse gases

ABSTRACT

Soil aggregation is an important physical property that influences the physico-chemical and biological properties of soil. Soil disturbances such as tillage can have a significant effect on soil aggregation. This study sought to examine the effect of soil aggregate size on soil pore characteristics and the subsequent effect on emission of greenhouse gases (GHGs) for both sandy loam and clay loam soils. Columns of aggregates in the size ranges of 2–4 mm, 1–2 mm, 0.5–1 mm and <0.5 mm were tested along with a field structured soil (i.e. aggregates <4 mm). Soil pore characteristics were quantified using X-ray Computed Tomography (CT). The average porosity in the soil columns ranged from 38.7 to 50.7%. Aggregate size influenced the total soil organic matter content with average values ranging from 7.5 to 8.6% in the clay loam soil and 2.8 to 5.2% in the sandy loam soil. CO_2 and CH_4 flux was significantly affected by size of aggregates. Clay loam soils emitted the most CO_2 from the small sized aggregates, whereas in sandy loam soils the larger aggregates produced the maximum CO_2 flux. Smaller aggregates produced higher CH_4 flux in both soil textures. No significant difference between aggregate sizes and soil textures was found for N_2O fluxes. Soil pore characteristics such as porosity and pore size significantly affected fluxes of GHGs such as CO_2 and CH_4. These results indicate that management practices such as tillage that heavily influence soil aggregation and pore characteristic development can have a direct impact on emission of greenhouse gases and subsequently have implications for global warming.

1. Introduction

Greenhouse gas (GHG) emissions from agricultural soils are a substantial contributor to climate change (Smith et al., 2008) and developing agricultural practices that bring mitigation of GHG emissions from agricultural soils is important. Currently, several different soil management strategies have been considered with regard to their potential to reduce the release of GHG from agriculture e.g. no-till practices (Ugalde et al., 2007; Uri, 2000), cover crops (Tubiello and Ewert, 2002) and agroforestry (Calfapietra et al., 2010; Pandey, 2002). Studies investigating the impact of such changes in practice on GHG emissions and soil C storage have illustrated wide-ranging results, possibly due to differences between studies in climatic zones, soil types, length of management practice and cropping systems. This highlights the importance of developing a mechanistic understanding of how soil management directly impacts on GHG release and C storage through changes in soil biophysical properties in particular.

It is well known that crop management activities, such as tillage, exert significant influence on soil physical properties. For example, tillage brings about the disruption of soil aggregates especially at the zone of disturbance and potentially the creation of hard pans at lower depths (Zotarelli et al., 2007). Soil micro aggregates are typically formed by binding microbial polysaccharides with smaller soil particles such as silt and clay whereas macro aggregates are typically formed around plant roots and coarse organic fragments (Ian, 2011). Also, stable micro aggregates (<250 μm) can reorient themselves into macro aggregates with the help of newly formed particulate organic matter (Jastrow et al., 1996). The protection of soil aggregates depends on their stability on contact with water and responses to mechanical stresses like tillage. Tisdall and Oades (1980) showed conventional tillage leads to oxidation of soil organic matter, which act as binding agents for macro aggregates, and hence water stable aggregates (>250 mm) become less stable under intensive tillage systems. Kasper et al. (2009) observed reduced stability in aggregates under conventional tillage (18.2%, compared with 37.6% under minimum tillage). Different sized aggregates exert varying contributions on the soil porous system and this in turn governs water and gas movement in soil (Perret et al., 1999).

* Corresponding author. Tel.: +44 115 9516257.
E-mail addresses: sacha.mooney@nottingham.ac.uk, shamsudheenm@gmail.com (S.J. Mooney).

0167-1987/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.still.2013.05.003

Both soil GHG release and soil C storage are linked to soil pore and aggregate structure. Aggregation is directly related to storage of soil organic carbon and affects movement of gases and water in soil (Marland et al., 2004) by influencing both biological processes in soil (Beare et al., 2009) and pore characteristics which regulate the flow of water and gases. The decomposition of soil organic matter can vary in soils between different aggregate size classes (Zhang et al., 2012). In addition, soil aggregates of different sizes also behave differently with regard to accumulation of carbon, with the greatest amount of carbon typically found in intermediately sized aggregates (1–4 mm) (Fernández et al., 2010; Helgason et al., 2010).

Soil aggregate composition determines intra and inter aggregate porosity and largely controls gaseous composition and transport in soil (Blagodatsky and Smith, 2012). Aggregates play a vital role in deciding the aerobic or anaerobic status of soil (Lipiec et al., 2007) which impacts on the production and release of GHGs from soil. Increased CO_2 emissions have been reported from micro aggregates (<0.25 mm) compared with macro aggregate (>0.25 mm) fractions (Sey et al., 2008). In contrast, Strong et al. (2004) found soil with relatively large pore volumes (15–60 μm) led to faster decomposition of carbon in soil with decomposition rates less in both air-filled macro and micro pores. In a clay loam soil Drury et al. (2004) found decreased CO_2 production with increasing aggregate size, and increased N_2O production with increasing aggregate size. Kimura et al. (2012) found increased uptake of CH_4 in smaller aggregates and higher emission from aggregates >2 mm.

To date there have been few studies that have linked the impact of different aggregate sizes on the soil pore characteristics and their effect on the emission of GHGs. Different pore characteristics such as size, continuity and shape affect fluid transport (Udawatta and Anderson, 2008) and gas transport and hence aeration of the soil (Luo et al., 2010) which is an important determinant of microbial activity in soil. These pore characteristics are greatly influenced by textural properties of soil (Mooney and Morris, 2008) and land use practices (Zhou et al., 2008). X-ray Computed Tomography (CT) has been successfully used to study pore characteristics at a finer resolution (<1 mm) (Lugato et al., 2009; Mooney and Morris, 2008; Udawatta and Anderson, 2008). The objectives of this research were to ascertain the effect of different aggregate size classes, prepared in soil columns, on soil physical properties and the emission of CO_2, CH_4 and N_2O.

2. Materials and methods

2.1. Sample preparation

Samples were collected from two soil types; a sandy loam from the Dunnington Heath series (FAO: Stagno Gleyic Luvisol) and a clay loam from the Fladbury series (FAO: Pelo-alluvial gley soil) from University of Nottingham Farm, Sutton Bonington, Nottinghamshire, UK (52.52° N, 1.07° W). Bulk samples were collected from a depth of 10 cm and air dried before being separated into the different aggregate size fractions of 2–4 mm, 1–2 mm, 0.5–1 mm and <0.5 mm by manual disaggregation and sieving (Fernández et al., 2010). In addition to this a core repacked with <4 mm sized aggregates referred to as field structured soil was included in the experiment. These soil aggregate fractions were packed into polyvinyl chloride (PVC) columns (5 cm internal diameter, 10 cm long) with eight replicates of each treatment, to a bulk density of 1.2 Mg m^{-3}. The bottom portion of each column was covered with a fine nylon mesh (0.1 mm) to retain the soil in columns. Half of the replicates of each treatment, i.e. 4 samples/replicates per aggregate size class, were saturated and then drained for 48 h to attain a notional field capacity. The cores were then placed in an incubation chamber at 15 °C and maintained at field capacity. These cores were then examined using X-ray CT. GHG release was measured on these samples at monthly intervals for a period of five months. The other four replicates per treatment were used to derive the saturated hydraulic conductivity using the falling head method (Klute and Dirksen, 1986).

2.2. Greenhouse gas emission and soil carbon

GHG measurements were conducted by placing the soil cores in glass jars of 1.5 dm^3 volume. The glass jars were fitted with rubber septa in the lid for head space gas sampling with a syringe. Gas sampling was performed after ensuring mixing of the air within the jar using a magnetic stirrer for 30 s. The gas sampling was repeated at defined time intervals in 1 h (namely 0, 15, 30 and 60 min). The gas samples were analysed with a gas chromatograph for GHGs such as CO_2, CH_4 and N_2O (GC-2014, Shimadzu). The gas sampling was repeated at monthly intervals on all soil cores which were maintained at the same moisture level in a constant temperature room. The linear response obtained from the time series data was used for calculating the emission rate of GHG. The gas data was converted to mass per volume and mass per weight basis using the ideal gas equation and the molecular mass of each gas (Denef et al., 2007).

$$n = \frac{PV}{RT} \quad (1)$$

where n is the number of moles of CO_2, N_2O or CH_4, P is atmospheric pressure (≈1 atm), V is the volume of head space (dm^3), R is the ideal gas constant (0.08205746 L atm K^{-1} mol^{-1}) and T is the temperature of sampling (273.15 + room temperature in °C). From this it was possible to calculate the gas flux.

$$E = \frac{nm}{at} \times 1000 \quad (2)$$

where E is the flux of each gas in mg m^{-2} h^{-1}, n is the number of moles of CO_2, N_2O or CH_4, m is the molar weight of CO_2 (44.01), N_2O (44.01) or CH_4 (16.04), a is an area of the soil core used and t is the time in hours. In the paper the data is presented as ng g^{-1} h^{-1} of oven dried soil. The gas sampling and analysis was carried out in the months of October, November, December, January and February of the year 2011–2012 and average values over the five sampling times are reported.

The GHG flux data was also calculated on a per organic matter basis (ng g^{-1} h^{-1} of soil organic matter) to determine the effect of soil organic matter on soil pore characteristics and are presented separately in the paper.

Finally, total soil organic matter (SOM) content in soil was determined following the loss on ignition method, by igniting oven dried soil at 550 °C in a muffle furnace.

2.3. X-ray Computed Tomography (CT)

Four soil cores per treatment were subjected to X-ray CT scanning using a high resolution micro CT scanner (Nanotom, Phoenix X-ray, GE Sensing and Inspection Technologies GmbH, Germany). Scanning was performed at energy levels of 140 kV and 140 μA. All soil cores were scanned in a vertical upright position. A total of 2000 projection images at a resolution of 28.75 μm were collected over a 33 min scanning time for each core. Images were reconstructed using Phoenix X-ray software and visualised using VG StudioMax (Volume Graphics). Fig. 1 illustrates a single 2-D binary image example of each aggregate size class in sandy loam and clay loam soils. Image analysis was carried out using *ImageJ* software (Rasband, 2002) to study the soil pore characteristics. A rectangular region of interest (27.92 mm × 27.92 mm) was select-

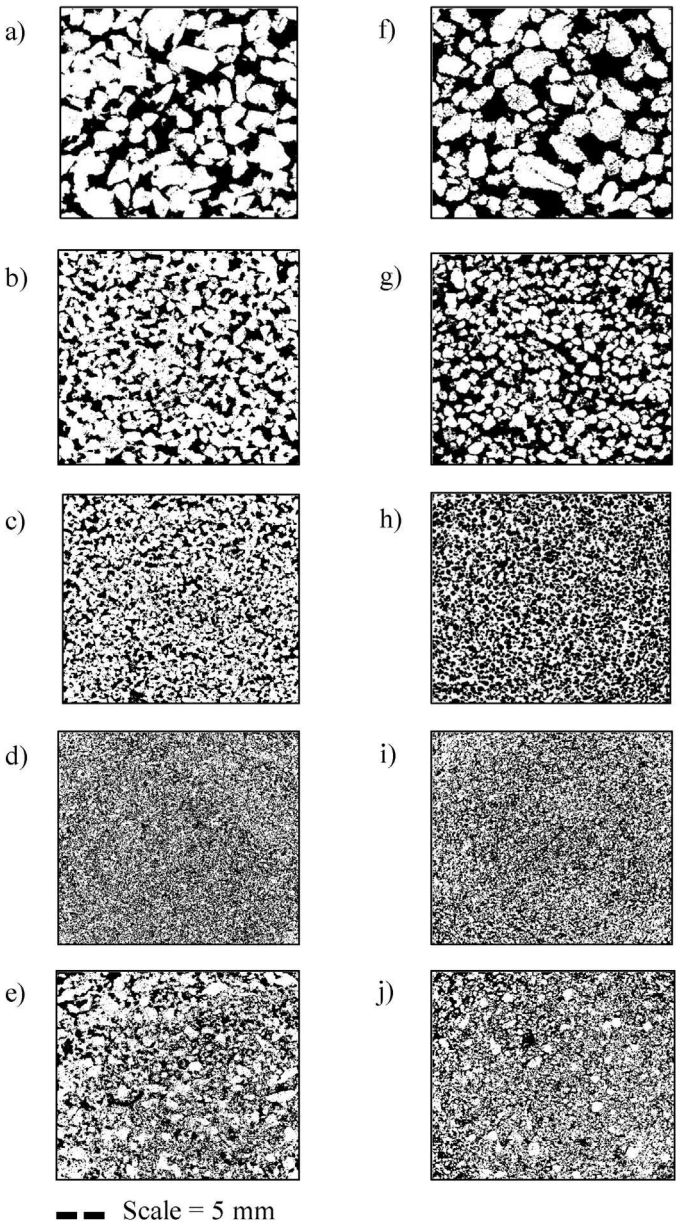

Fig. 1. Selected 2-D binary image for the different aggregate size classes. Images a to e on the left are for clay loam soils (a) 2–4 mm, (b) 1–2 mm, (c) 0.5–1 mm, (d) <0.5 mm and (e) field structured. The images from f to j on the right are for sandy loam soils (f) 2–4 mm, (g) 1–2 mm, (h) 0.5–1 mm, (i) <0.5 mm and (j) field structured.

ed to exclude those pores adjacent to the core edges. A total of 1800 images were used in the analysis excluding 100 images from the start and the end. A suitable image routine was developed after testing several different filters and image enhancement techniques. The contrast of all images was enhanced, normalised and equalised. A median filter was then applied prior to image thresholding/segmentation. The differentiation of pores from solids was made by using the MinError algorithm and the images were subsequently converted to 8-bit grey scale images. Any pixel that deviated more than the median of the surrounding pixels was removed with a threshold value of 1 to reduce the image noise. Information on the number of pores, average pore size (area), total porosity, pore size distribution and pore surface area were obtained. A coefficient of uniformity was calculated to quantify the pore size distribution. This was determined as the ratio of size of pores at 10% and 60% of total pore size distribution (Atkinson et al., 2009).

2.4. Statistical analysis

The statistical software package Genstat (v. 14) was used for all data analysis. A two-way analysis of variance (ANOVA) was applied to results obtained from laboratory measurements and image analysis in the samples with soil texture and aggregate size as two factors. The treatment means were compared at the $P < 0.05$ level using the LSD. Standard errors of means were calculated and provided as required. Simple linear regressions were carried out to examine the relationship between different parameters.

3. Results

3.1. Saturated hydraulic conductivity

Both texture and aggregate size significantly affected the saturated hydraulic conductivity ($P < 0.001$). As expected, the sandy loam soil was more permeable than clay loam soil with an average saturated hydraulic conductivity of 1.2 cm s^{-1} compared to 0.38 cm s^{-1} in the clay loam soil. There was a linear relationship between hydraulic conductivity and aggregate sizes with the larger aggregates permitting the water flow more readily than small sized aggregates (Fig. 2). The field structured soil behaved most similarly to the <0.5 mm aggregate columns.

3.2. Soil organic matter

Soil texture and aggregate size had a significant effect on total soil organic matter content ($P < 0.001$). Clay loam soil contained more organic matter (8.2%) than sandy loam soil (3.7%). The smallest aggregates (<0.5 mm) in clay loam soil had the highest organic matter (8.7%) with the lowest in the field structured soil (7.5%). Whereas in sandy loam soil the larger aggregates (1–2 mm and 2–4 mm) contained more organic matter (5.2 and 3.5%, respectively) than small aggregates (<0.5 mm) (2.8%) (Fig. 3).

3.3. Greenhouse gas release

CO_2 emission decreased from soil over time; however, there was an initial increase in CO_2 flux immediately after incubation (data not shown). CH_4 flux showed a definite decrease during incubation whereas the N_2O flux pattern did not follow any clear trend.

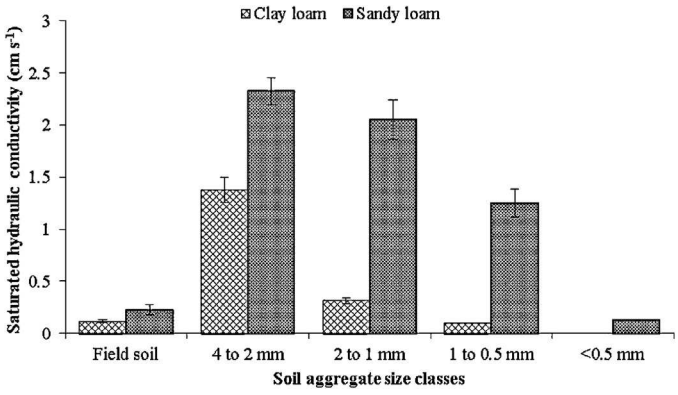

Fig. 2. Saturated hydraulic conductivity (cm s^{-1}) for different aggregate size fractions in clay loam and sandy loam soils (texture: $F_{1,30} = 188.32$, $P < 0.001$; aggregates: $F_{1,30} = 125.12$, $P < 0.001$; texture × aggregates: $F_{1,30} = 27.82$, $P < 0.001$). Mean values are shown, error bars indicate standard error of mean, $n = 4$.

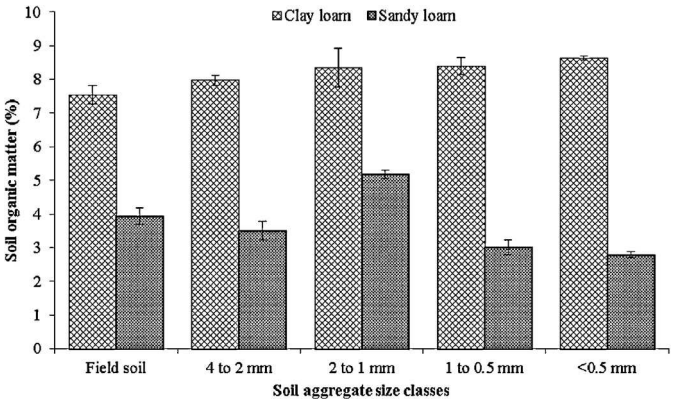

Fig. 3. Variations in soil organic matter content as derived by loss on ignition for different aggregate size fractions in clay loam and sandy loam soils (texture: $F_{1,30} = 718.83$, $P < 0.001$; aggregates: $F_{1,30} = 6.15$, $P < 0.001$; texture × aggregates: $F_{1,30} = 9.16$, $P < 0.001$). Mean values are shown, error bars indicate standard error of mean, $n = 4$.

Both texture and aggregate size significantly influenced CO_2 emission ($P < 0.001$). The average CO_2 emission was greater from clay loam textured soils (704 ng g^{-1} h^{-1}) compared to sandy loam soil (624 ng g^{-1} h^{-1}). In the clay loam, the maximum CO_2 flux was recorded in the field structured soil (704 ng g^{-1} h^{-1}) followed by the <0.5 mm aggregate fraction (612 ng g^{-1} h^{-1}) with lowest value in 2–4 mm aggregates (387 ng g^{-1} h^{-1}). In contrast, in the sandy loam soil, larger aggregates (2–4 mm) recorded the maximum CO_2 flux (719 ng g^{-1} h^{-1}), followed by field structured soil (624 ng g^{-1} h^{-1}) and 1–2 mm aggregates (618 ng g^{-1} h^{-1}) (Fig. 4a).

When the comparison was made between field structured soils, the higher CH_4 flux was recorded from sandy loam soil (0.35 ng g^{-1} h^{-1}) than clay loam soils (0.24 ng g^{-1} h^{-1}). But individually among different aggregate classes the CH_4 flux was higher in clay loam aggregates than aggregates from sandy loam. Increased CH_4 emission was recorded as aggregate size decreased in both soils ($P < 0.05$, Fig. 4b). Among different sized aggregates, the highest CH_4 flux was from <0.5 mm aggregates in both clay loam (0.57 ng g^{-1} h^{-1}) and sandy loam soils (0.47 ng g^{-1} h^{-1}). Similarly the lowest CH_4 flux was from 2 to 4 mm sized aggregates in both clay loam (0.29 ng g^{-1} h^{-1}) and sandy loam soils (0.22 ng g^{-1} h^{-1}).

N_2O fluxes significantly varied with the soil texture ($P < 0.05$) with maximum values in the clay loam soil (1.7 ng g^{-1} h^{-1}) compared to sandy loam soil (1.2 ng g^{-1} h^{-1}). Although the effect of aggregate size on N_2O flux was not significant, the interaction of soil texture with aggregates was significant. In the clay loam soil, the highest N_2O emission was recorded in the field structured soil (1.9 ng g^{-1} h^{-1}) followed by 1–2 mm sized aggregates (1.8 ng g^{-1} h^{-1}) and lowest in 0.5–1 mm size class (1.4 ng g^{-1} h^{-1}). −1). In the sandy loam soil the highest emission was from 2 to 4 mm (1.8 ng g^{-1} h^{-1}) followed by <0.5 mm (1.6 ng g^{-1} h^{-1}) and least from 1 to 2 mm size class (1.2 ng g^{-1} h^{-1}).

When the GHG flux data was expressed in terms of organic matter, the CO_2 flux pattern was similar to the per soil basis in the clay loam soil (Fig. 5). However in the sandy loam soil the lowest CO_2 emission was from 1 to 2 mm sized aggregates. CH_4 flux on per gram of organic matter basis exhibited a trend similar to when expressed on per gram soil basis. N_2O flux per gram of organic matter was significantly affected by both texture and aggregates with the maximum flux from sandy loam soil ($P < 0.001$). N_2O flux was highest in the field structured soil followed by 1–2 mm aggregates and the lowest flux was recorded with 0.5–1 mm and <0.5 mm aggregates in clay loam soil. In sandy loam soils the N_2O flux per gram of organic matter was highest in the <0.5 mm aggregates and lowest in the 1–2 mm aggregates.

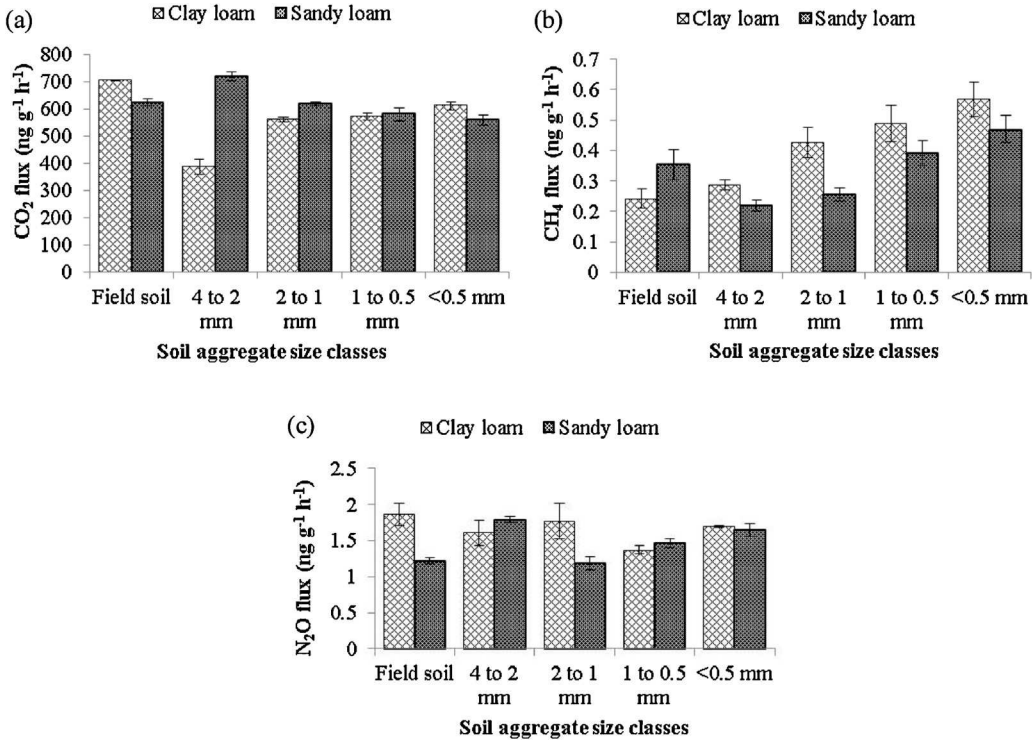

Fig. 4. Differences in average fluxes of CO_2, CH_4 and N_2O (ng g^{-1} oven dried soil h^{-1}) in different aggregate size fractions in clay loam and sandy loam soils (a) CO_2 flux: texture: $F_{1,30} = 26.59$, $P < 0.001$; aggregates: $F_{1,30} = 13.30$, $P < 0.001$; texture × aggregates: $F_{1,30} = 51.43$, $P < 0.001$; (b) CH_4 flux: texture: $F_{1,30} = 5.93$, $P < 0.05$; aggregates: $F_{1,30} = 13.39$, $P < 0.001$; texture × aggregates: $F_{1,30} = 3.21$, $P < 0.05$; (c) N_2O flux: texture: $F_{1,30} = 6.97$, $P < 0.05$; aggregates: $F_{1,30} = 2.03$, NS; texture × aggregates: $F_{1,30} = 5.18$, $P < 0.01$; Mean values are shown, error bars indicate standard error of mean, $n = 4$.

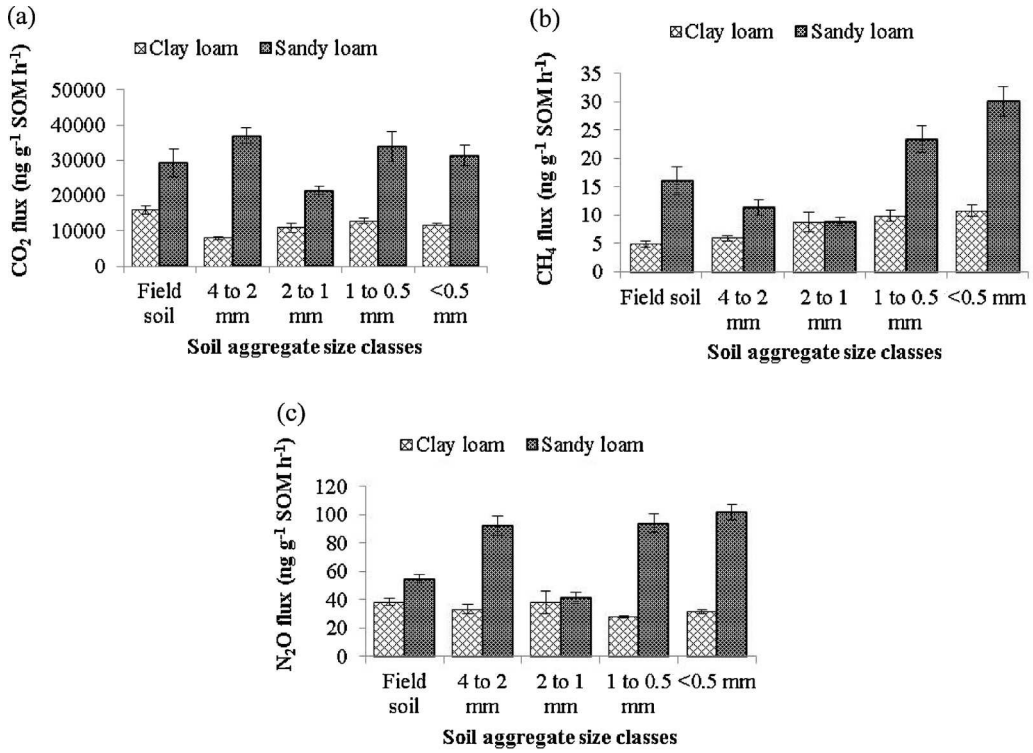

Fig. 5. Variations in average fluxes of CO_2, CH_4 and N_2O (ng g^{-1} organic matter h^{-1}) in different aggregate size fractions in clay loam and sandy loam soils (a) CO_2 flux: texture: $F_{1,30} = 171.33$, $P < 0.001$; aggregates: $F_{1,30} = 3.28$, $P < 0.05$; texture × aggregates: $F_{1,30} = 5.07$, $P < 0.01$; (b) CH_4 flux: texture: $F_{1,30} = 92.03$, $P < 0.001$; aggregates: $F_{1,30} = 21.05$, $P < 0.001$; texture × aggregates: $F_{1,30} = 10.35$, $P < 0.001$; (c) N_2O flux: texture: $F_{1,30} = 198.97$, $P < 0.001$; aggregates: $F_{1,30} = 11.51$, $P < 0.001$; texture x aggregates: $F_{1,30} = 20.57$, $P < 0.001$; Mean values are shown, error bars indicate standard error of mean, $n = 4$.

3.4. Soil pore characteristics

Both soil texture and size of aggregates significantly affected the number of pores measured per sample using CT. The fine textured soil had more pores than the coarse textured soil ($P < 0.001$). As the aggregate size reduced, the number of pores increased ($P < 0.001$) in both textural classes (Fig. 6). In the clay loam soil the smaller aggregates (0.5 mm) contained 88% more pores than larger aggregates (2–4 mm) and in sandy loam soil it was 92% more. The pore size significantly varied with soil texture and aggregate size ($P < 0.001$, Fig. 7). Larger aggregates in soil columns facilitated the creation of large sized pores, with pore size greater in the sandy loam soil in all the aggregate size classes compared to clay loam soil.

Among all the aggregate size classes and field structured soil, sandy loam soil had a significantly higher average porosity than clay loam textured soil except in the <0.5 mm sized aggregates ($P < 0.001$, Fig. 8). In clay loam soil the porosity increased as the aggregate size decreased and the converse was the case with the sandy loam soil ($P < 0.001$). The surface area of the soil pores varied significantly with soil texture and aggregate size ($P < 0.001$, Fig. 9). The total surface area of pores was greater in the sandy loam soil. As the size of aggregates increased, the surface area of pores generally increased in both textural classes, with the highest value for the sandy loam in the 2–4 mm class compared to the 1–2 mm class for the clay loam. It is worth noting the soil pore characteristics were limited by the image resolution of 28.75 μm, hence pores smaller than these were not measured. The coefficient of uniformity of the pore size distribution was

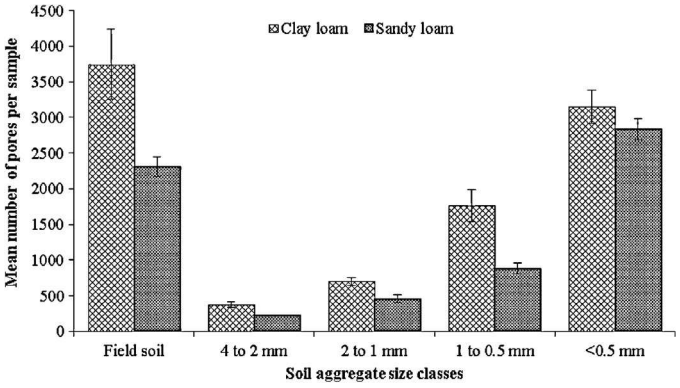

Fig. 6. Mean number of pores per sample for the different aggregate size classes in clay loam and sandy loam soils as measured by X-ray Computed Tomography (texture: $F_{1,30} = 22.63$, $P < 0.001$; aggregates: $F_{1,30} = 84.77$, $P < 0.001$; texture × aggregates: $F_{1,30} = 3.71$, $P < 0.05$). Mean values are shown, error bars indicate standard error of mean, $n = 4$.

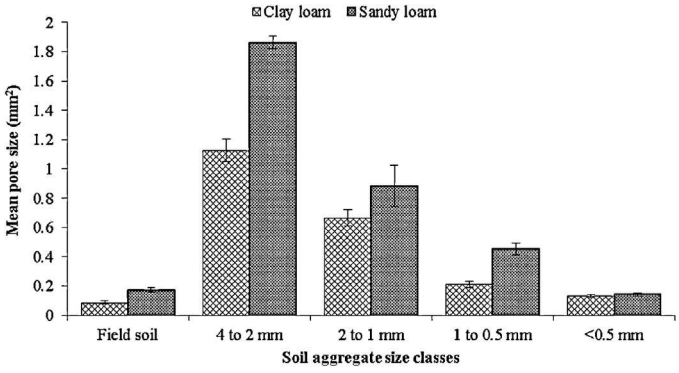

Fig. 7. Mean pore size (mm^2) for the different aggregate size classes in clay loam and sandy loam soils as measured by X-ray CT (texture: $F_{1,30} = 49.45$, $P < 0.001$; aggregates: $F_{1,30} = 196.07$, $P < 0.001$; texture x aggregates: $F_{1,30} = 11.76$, $P < 0.001$). Mean values are shown, error bars indicate standard error of mean, $n = 4$.

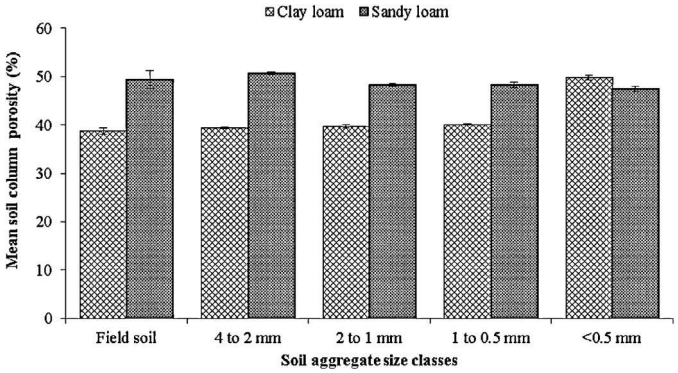

Fig. 8. Mean soil column porosity (%) for the different aggregate size classes in clay loam and sandy loam soils as measured by X-ray CT (texture: $F_{1,30} = 266.05$, $P < 0.001$; aggregates: $F_{1,30} = 15.54$, $P < 0.001$; texture × aggregates: $F_{1,30} = 30.21$, $P < 0.001$). Mean values are shown, error bars indicate standard error of mean, $n = 4$.

significantly different for both texture ($P < 0.05$) and aggregates ($P < 0.001$, data not shown).

3.5. Relationship between fluxes of greenhouse gases and soil physical properties

Total soil carbon was statistically not correlated with the average CO_2, CH_4, N_2O gas flux ($P > 0.05$). However, some aspects of the soil pore structure were related to GHG emissions. Soil porosity significantly affected CO_2 flux ($P < 0.05$, $R^2 = 0.13$, Fig. 10) but not CH_4 and N_2O fluxes. Only CH_4 was related to average pore sizes among different aggregate size classes with a negative relationship ($P < 0.001$, $R^2 = 0.21$, Fig. 11).

4. Discussion

Many complex physico-chemical and biological processes govern the turnover and protection of carbon in soil (Lugato et al., 2009). These results showed variable soil aggregate sizes from contrasting soils store carbon differently. In the sandy loam soil the intermediate aggregate fraction (1–2 mm) recorded significantly higher carbon, similar to Fernández et al. (2010) who also found highest carbon contents in intermediate aggregate size fractions. In contrast the clay loam soil had greater total carbon contents in the smallest aggregate size fraction. Smaller aggregates possess greater ability to protect organic matter and hence retain more carbon content and for longer (Papadopoulos et al., 2009). Six et al. (2002) reported slower turnover of carbon in

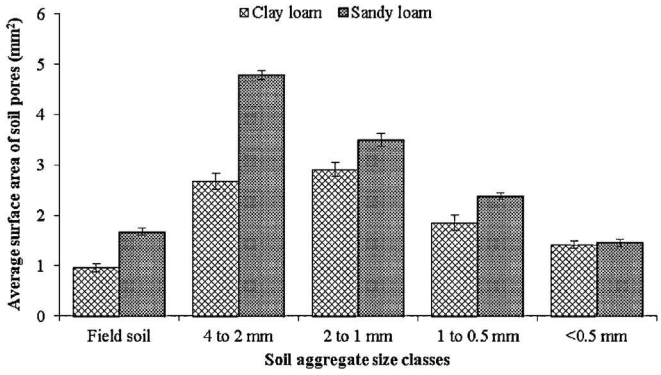

Fig. 9. Average surface area of soil pores (mm^2) for the different aggregate size classes in clay loam and sandy loam soils as measured by X-ray CT (texture: $F_{1,30} = 133.13$, $P < 0.001$; aggregates: $F_{1,30} = 192.43$, $P < 0.001$; texture × aggregates: $F_{1,30} = 25.45$, $P < 0.001$). Mean values are shown, error bars indicate standard error of mean, $n = 4$.

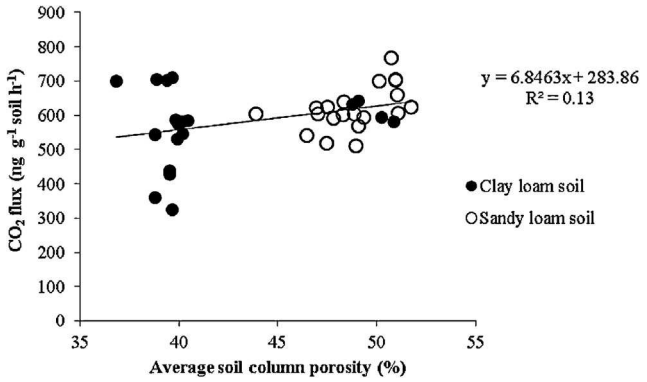

Fig. 10. The relationship between average soil column porosity and CO_2 flux ($F_{1,38} = 5.92$, $P \leq 0.05$, $R^2 = 0.13$).

micro aggregates compared to macro aggregates. These changes in soil organic matter may have implications on tillage associated impacts on soil aggregation and carbon sequestration. Nyamadzawo et al. (2009) observed 18% higher macro aggregation under no tillage (NT) compared to continuous maize cropping and increased macro aggregate protected carbon under NT. Soil aggregation is reported to be enhanced under no-tillage systems (Six et al., 2000b) which might increase soil organic carbon content by protecting humic substances within aggregates (Six et al., 2000a).

Both texture and aggregate sizes affected the fluxes of various GHGs. These results clearly demonstrate the importance of soil aggregates and then subsequent pore characteristics on emission of CO_2 and CH_4. The arrangement of soil aggregates determined the soil porous characteristics which directly mediated the emission of GHGs such as CO_2 and CH_4 from soil. Soil CO_2 fluxes were affected by soil porosity in both textures indicating the soil pore network plays a major role in driving CO_2 produced by microbial respiration to the soil surface. On the other hand the increased soil porosity might also favour the aeration of soil making more oxygen available to microbes to act on organic matter and crop residues. CO_2 release was greatest in the sandy loam soil for the largest aggregate size class which suggests the impact tillage may have on gas release; although in the clay the largest release was in the smaller aggregate class which shows the relationship in soil is texture dependent. Although the effect of total organic matter on CO_2 flux was not statistically significant in this study, its effect might be through microbial action which warrants more studies in this direction. This is in agreement with Al-Kaisi and Yin (2005) who also reported non-significant relationship between soil CO_2 and different forms of carbon and indicated that CO_2 emissions in such case was not limited by soil organic C substrate, instead might be governed by soil pore characteristics. Regardless of soil texture,

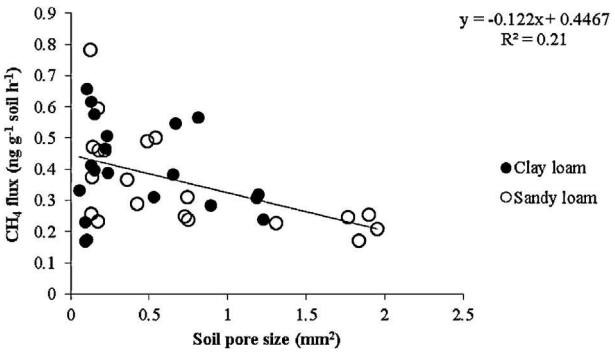

Fig. 11. The relationship between average CT measured soil pore size and CH_4 flux ($F_{1,38} = 9.93$, $P < 0.001$, $R^2 = 0.21$).

smaller aggregates produced more CH_4 flux, indicating that the repacked micro aggregates provided sufficiently anaerobic conditions for methane production. This is further supported by the negative relationship between average pore size and CH_4 flux (Fig. 11) as a more anaerobic environment may be provided by the decreased size of soil pores. The different niches occupied by methanogenic vs. aerobic microorganisms may be linked to the level of aggregation in the soil (Sey et al., 2008). The aeration in packed aggregates increases with increasing aggregate sizes due to larger inter aggregate pore space (Uchida et al., 2008). The innermost micro aggregates provided the anaerobic conditions which might have triggered the activity of methanogens which predominantly require anaerobic environments for their activity. These results hence suggest emissions of CH_4 can occur in drained soils due to anaerobic microsites found in the smaller aggregates in the soil.

N_2O emission was not related to the soil pore characteristics. The N_2O flux in soil is related to many factors such as inorganic nitrogen supply in soil, availability of carbon compounds for the activity of microbes, soil pH, aeration status, moisture and temperature (Chapuis-Lardy et al., 2007). The factors reported to cause negative N_2O fluxes such as moisture and temperature was controlled in this experiment. N_2O flux in soil is highly variable and dynamic and N_2O produced in top soil might soon dissipate to atmosphere without significant residence time in soil (Yoh et al., 1997). In some aggregate fractions the net N_2O produced within the soil core may have been consumed within soil and hence not reached the top surface (Arah et al., 1991). When the effect of soil organic matter on flux was eliminated by expressing the flux in terms of per organic matter basis, increased gas flux was noticed in the sandy loam soil indicating the effect of soil pore structure in controlling the emission of gases to the atmosphere.

The sandy loam soil typically contained more and larger pore sizes in the different aggregate treatments than the clay loam soil. The total average porosity also was higher for sandy loam soil compared to clay loam soil for all the aggregate classes studied except <0.5 mm size fraction for the given resolution used here (pores >28.75 μm). Soil hydraulic properties are largely governed by soil structure (Zhou et al., 2008). Under saturated conditions the hydraulic conductivity was positively affected by soil pore characteristics such as the size of pores ($R^2 = 0.69$, $P < 0.001$) and average porosity ($R^2 = 0.10$, $P \leq 0.05$). Among different CT-measured soil pore characteristics, pore size accounted for 69% variation in hydraulic conductivity. Udawatta and Anderson (2008) attributed 76% variation in saturated hydraulic conductivity to CT measured fractal dimension of pores and 54% to number of pores, similar to as found in our study. However Kim et al. (2010) found CT measured macro porosity as the parameter most correlated with saturated hydraulic conductivity ($R^2 = 0.95$).

Rapid changes in soil aggregation and pore characteristics are created by soil management practices such as tillage. Tillage influences aggregate size and shape directly by physical disruption of macro aggregates and indirectly by modifying the biological environment (Zhang et al., 2012). Several studies indicate decreased macro aggregate stability under tillage (Malhi et al., 2006). This is especially important since 92% of the world's cropped area are currently tilled (FAO, 2010a,b). We have shown that the production, consumption and transport of GHGs can be directly linked to soil structural properties. Tillage induced changes in soil aggregation govern GHG emission by modifying the physico-chemical and biological regimes. The disruption of aggregates releases the physically protected soil organic matter which increases microbial turnover of soil organic matter and GHG release (Six et al., 2002). Soil pore characteristics created by aggregates highly influence the storage and emission of GHG produced by microbial activities.

5. Conclusions

Our study demonstrated large differences in macropore characteristics between two soil types and different aggregate size classes. Aggregate size had a significant influence on macroporosity, number of pores and pore size. Soil texture and aggregate sizes play an important role in building a soil's porous architecture, which has implications on release of GHGs from soil. Soil pore characteristics such as total porosity and pore size significantly influenced the release of different GHGs such as CO_2 and CH_4 from soil, but not N_2O. Small sized aggregates produced the highest CO_2 flux in clay loam textured soil whereas it was in the largest and the intermediate sized aggregates in sandy loam textured soil. CH_4 flux was highest with small sized aggregates in both the textures. The study suggest that soil management practices such as tillage which have profound implications on soil structure and pore characteristics, may influence the GHG release and water transport through the soil. Soil management strategies that seek to reduce the emissions of GHG from soil need to carefully consider the role of soil aggregate size whilst appreciating that its impact is highly variable between different soil textures.

Acknowledgements

Funding support to this work was provided by the International Fellowship programme of the Indian Council of Agricultural Research, India and a Research Excellence Scholarship by the University of Nottingham, UK.

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4. Chapter 4: To what extent can zero tillage lead to a reduction in greenhouse gas emissions?

It was shown in Chapters 2 that tillage influences soil physical properties significantly and emission of greenhouse gases such as CO_2 was influenced by soil pore characteristics apart from soil carbon content. Also the tillage mediated changes in soil aggregation could play an important role in modifying soil porous architecture that will decide the fate of greenhouse gas flux in soil as shown in Chapter 3. In this chapter the impact of conservation and conventional tillage practices on soil pore characteristics, carbon sequestration and greenhouse gas emissions and net effect on total global warming potential was assessed, based on soil sampling from zero tilled and tilled farms across the East Midlands, UK. The purpose of this experiment was to assess the climate mitigation capabilities of conservation tillage and to find out the factors governing greenhouse gas emissions under changed condition. This chapter address the sub aims 1 and 3, This has been prepared in the paper format.

To what extent can zero tillage lead to a reduction in greenhouse gas emissions?

Running heading: ZERO TILL REDUCE GREENHOUSE GAS EMISSIONS FROM SOIL

S. MANGALASSERY[1], S. SJÖGERSTEN[1*], D.L. SPARKES[2], C.J. STURROCK[1], J. CRAIGON[2] and S.J. MOONEY[1]

[1] Division of Agricultural and Environmental Sciences, School of Biosciences, Sutton Bonington Campus, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, UK

[2] Division of Plant and Crop Sciences, School of Biosciences, Sutton Bonington Campus, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, UK

*Correspondence: Sofie Sjögersten, Division of Agricultural and Environmental Sciences, School of Biosciences, Sutton Bonington Campus, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, UK. tel. +44 115 9516257, fax +44 115 9516261, e-mail: sofie.sjogersten@nottingham.ac.uk

Key words: greenhouse gases, microbial biomass, soil organic carbon, soil pore characteristics, X-ray Computed Tomography, zero tillage.

Type of paper: Primary Research Article

Abstract

Soil tillage practices have a profound influence on soil physical properties and the greenhouse gas balance. Conservation tillage practices such as zero tillage have been proposed to reduce greenhouse gas emissions, however there have been very few integrated studies on emission of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) along with soil physico-chemical characteristics under different soil management systems. We conducted a study to evaluate the impact of zero tillage and conventional tillage in the United Kingdom that assessed soil physical properties such as 3-D pore characteristics, soil biochemical characteristics and emission of CO_2, CH_4 and N_2O. The soils considered in the zero tillage treatment had been untilled for between 5-10 years. Soil macro porosity was greater under conventional tillage whereas zero tilled soils retained more moisture and had a higher carbon and microbial biomass carbon than tilled soils. Significantly higher fluxes of CO_2 (21.2% higher) and CH_4 (57.6% higher) were observed under tilled soils than zero tilled soils. Although increased N_2O flux (43.5% higher) was observed under zero tilled soils, the net global warming potential was significantly higher for conventional tillage systems (20% higher than zero tillage systems). In the case of CO_2 flux, the soil pore characteristics modified by tillage played a significant role, whereas for CH_4 flux soil strength was found to be the dominant factor in multiple regression analysis. The variation in N_2O flux was explained mainly by microbial biomass carbon followed by soil moisture and to a lesser extent by soil pore size. The physical environment created by not tilling a soil plays a major role in modifying the production and release of greenhouse gases. The study indicates that reducing tillage practices could play

a significant role in minimising emissions of GHGs from soils and contribute to efforts to mitigate against climate change.

4.1 Introduction

Globally agriculture accounts for 10-12% of total anthropogenic emissions of greenhouse gases (GHGs), estimated to be 5.1 to 6.1 Gt CO_2-eq yr^{-1} in 2005 (Smith et al., 2007). Conservation tillage is one among many different mitigation options suggested to reduce GHG emission from agriculture. Conservation tillage practices such as reduced/minimum/zero tillage, direct drilling and strip cropping are also widely recommended to protect soil against erosion and degradation of soil structure (Petersen et al., 2011), create greater aggregate stability (Zotarelli et al., 2007; Fernández et al., 2010), increase soil organic matter content and enhance sequestration of carbon (Six et al., 2000; West et al., 2002b), mitigate GHG emissions (Kong et al., 2009) and improve biological activity (Helgason et al., 2010). Derpsch (2009) estimated that approximately 45 million hectares of land was under conservation tillage management worldwide in the year 2001, by 2007-08 this area had more than doubled. Minimum tillage practices have been previously reported to reduce GHG emissions from soil directly with the reduced use of fossil fuels in field preparation, in addition to increasing carbon sequestration in soil (Petersen et al., 2008). However, recently it was reported that reduced tillage could lead to a stratification of soil organic carbon at the surface (Baker et al., 2007) against the more uniform distribution of carbon in conventionally tilled soils (Campbell et al., 2000). Although Hermle et al. (2008) found a net carbon sequestration to a depth of 50 cm after 20 years of no tillage. The surface accumulated crop residues under reduced tilled conditions may result in carbon

being lost to the atmosphere upon decomposition (Petersen et al., 2008). Furthermore, climate change mitigation benefits such as reduced CO_2 emissions by virtue of increased sequestration of carbon and increased CH_4 uptake under reduced tillage could be offset by an increased emission of N_2O, a greenhouse gas with higher warming potential (Six et al., 2004; Chatskikh et al., 2007; Hermle et al., 2008). Increased N_2O emissions have been related to increased denitrification under reduced tillage due to the formation of micro-aggregates within macro-aggregates that create anaerobic micro sites (Hermle et al., 2008) with increased microbial activity leading to a higher competition for oxygen (West et al., 2002a). Reduction of tillage can also create increased soil densification and a subsequent decrease in the volume of macropores (Schjønning et al., 2000) leading to soil compaction and reduced gaseous exchange. Soil aggregation and the resultant geometry of the pore structure are vitally important characteristics affected by tillage practices which impacts on the physico-chemical and hydro-thermal regime in soil and ultimately crop yield. Additionally the effect of tillage on the environment varies across farms geographically since the impacts of tillage on soil organic matter and net greenhouse balance depends on soil type, climatic variables and management (Chatskikh et al., 2007).

There are no previous studies that have considered the effect of tillage on net balance of greenhouse gas emissions and the combined role of soil porous architecture. Traditional methods for measuring soil structure such as soil moisture retention curves and aggregate size distribution are limited as they are destructive and do not provide the soil pore size distribution in three dimensions (Gantzer et al., 2002). However, imaging technologies such as X-

ray Computed Tomography (CT) can be used to reveal the undisturbed structure, aggregation and pore characteristics of soils at high resolutions (e.g. microscale). Gantzer et al. (2002) previously already demonstrated CT can be used to reveal the differences in macroporosity between conventionally and conservatively managed soils. Here we sought to evaluate the impact of zero tillage and conventional tillage practices on soil pore characteristics, carbon sequestration and greenhouse gas emissions. We hypothesised that zero tillage improves C sequestration and reduces greenhouse gas emissions compared to conventional tillage, through the nature of the porous network that is developed.

4.2 Materials and methods

4.2.1 Site selection and sample collection

A selection of 22 farms in Leicestershire, Nottinghamshire and Lincolnshire in the East Midlands of the UK where zero tillage is practised were chosen for analysis (Fig. 4.1). All sampling sites comprised pairs of intensely tilled farms and farms where zero tillage practices were practised. Each paired field was located directly adjacent to each other and the distances between paired fields never exceeded 10 metres (Fig. 4.2 to 4.5). The zero tilled soils had been managed in this way for a minimum of 5 years to a maximum of 10 years whereas the tilled soils were subjected to ploughing every year to a depth of 10 cm. Selected site characteristics are presented in Table 4.1. In fields under zero tillage, stubble was left at the surface after the harvest of previous crop. Weeding was achieved by spraying glyphosate before drilling. Seed drilling was carried out between the root stocks of previous crop using a range of min-till seed drills. Wheat, oil seed rape and oats were cultivated under zero tilled

fields. The tilled soil sites were annually ploughed to depths of 20-25 cm and contained the same crops as the zero tilled fields.

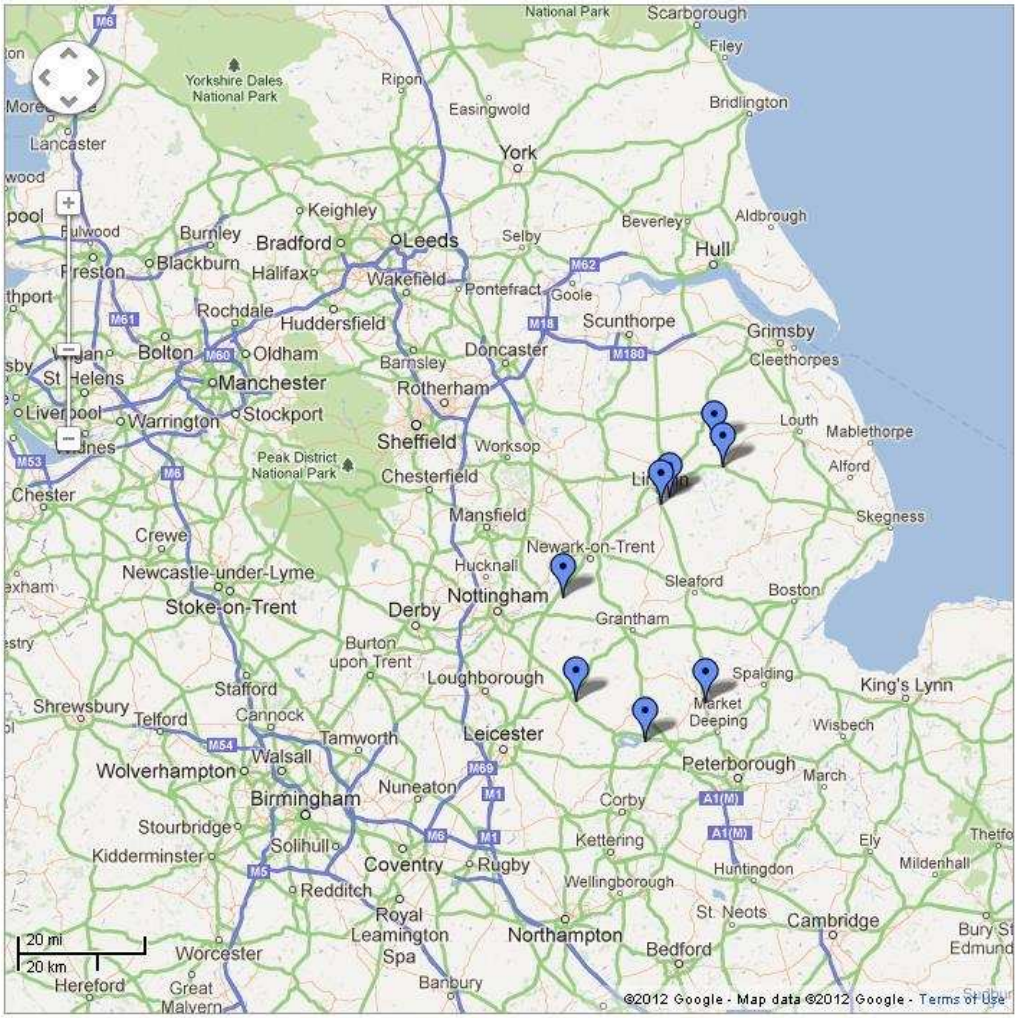

Fig. 4.1. A map showing the location of sampling sites for experiment 3

Intact soil cores were collected using a manual core sampler, following harvest of the previous crop, between November-December 2011. The core sampling was performed to a depth of 20 cm with a diameter of 5 cm cores. The sampling was replicated in random locations three times at each site. These

core samples were labelled and sealed in plastic bags before being transported to the laboratory. Samples were stored at 4^{o}C until analysed. Bulk soil samples of about 1 kilogram were also collected from two depth ranges (0 to 10 cm and 10 to 20 cm) and were also stored at 4^{o}C until measurement. Smaller soil cores were collected in the field using stainless steel cylinders (radius 3.4 cm, height 4 cm) for the measurement of bulk density (Page et al., 1982).

Fig. 4.2. Some of the min-till devices used by farmers in East Midlands

Fig. 4.3. Sampling sites at Thurlby A (Zero tilled, left and tilled, right)

Fig. 4.4. Sampling sites at Oahkam B (Zero tilled, left and tilled, right)

Fig. 4.5. Sampling sites at Canwick (Zero tilled, left and tilled, right)

4.2.2 Soil physical properties

Soil physical properties such as shear strength,volumetric water content, And particle size analysis were estimated by standard procedures (Appendix).

4.2.3 X-ray Computed Tomography (CT)

Prior to the study of GHGs, the soil core samples were subjected to morphological analysis using an X-ray CT scanner (Nanotom, Phoenix X-ray, GE Sensing and Inspection Technologies GmbH, Germany) to visualise and measure the internal soil structure. The cores were scanned at a voltage of 140 kV and a current of 100 mA. A copper filter of thickness 0.25 mm was used to minimise artefacts such as beam hardening. The image resolution was 64 µm per voxel. The soil core was positioned vertically onto the scanner platform. Each scan lasted 100 minutes per core, scanning both top and bottom 10 cm portions in a split scan. Whilst it is possible to achieve much faster scan times than this, a larger scan time was used to achieve the highest possible image quality. For each scan 1000 images were collected.

The images obtained were visualised using the software, VG StudioMax (Volume Graphics). The images were converted to the .tiff format and analysed using ImageJ (Rasband, 2002) to study the pore characteristics. A rectangular region of interest (27.94 x 27.94 mm^2) was selected to avoid the edges of the soil cores. In addition the first 100 images each from the beginning and end of the scan were discarded due to cone beam artefacts. The images were sharpened to highlight the image features and then smoothed by a median filter before being converted to binary scale using the minimum threshold algorithm in ImageJ (Fig. 4.6). Both dark and bright outliers were removed and

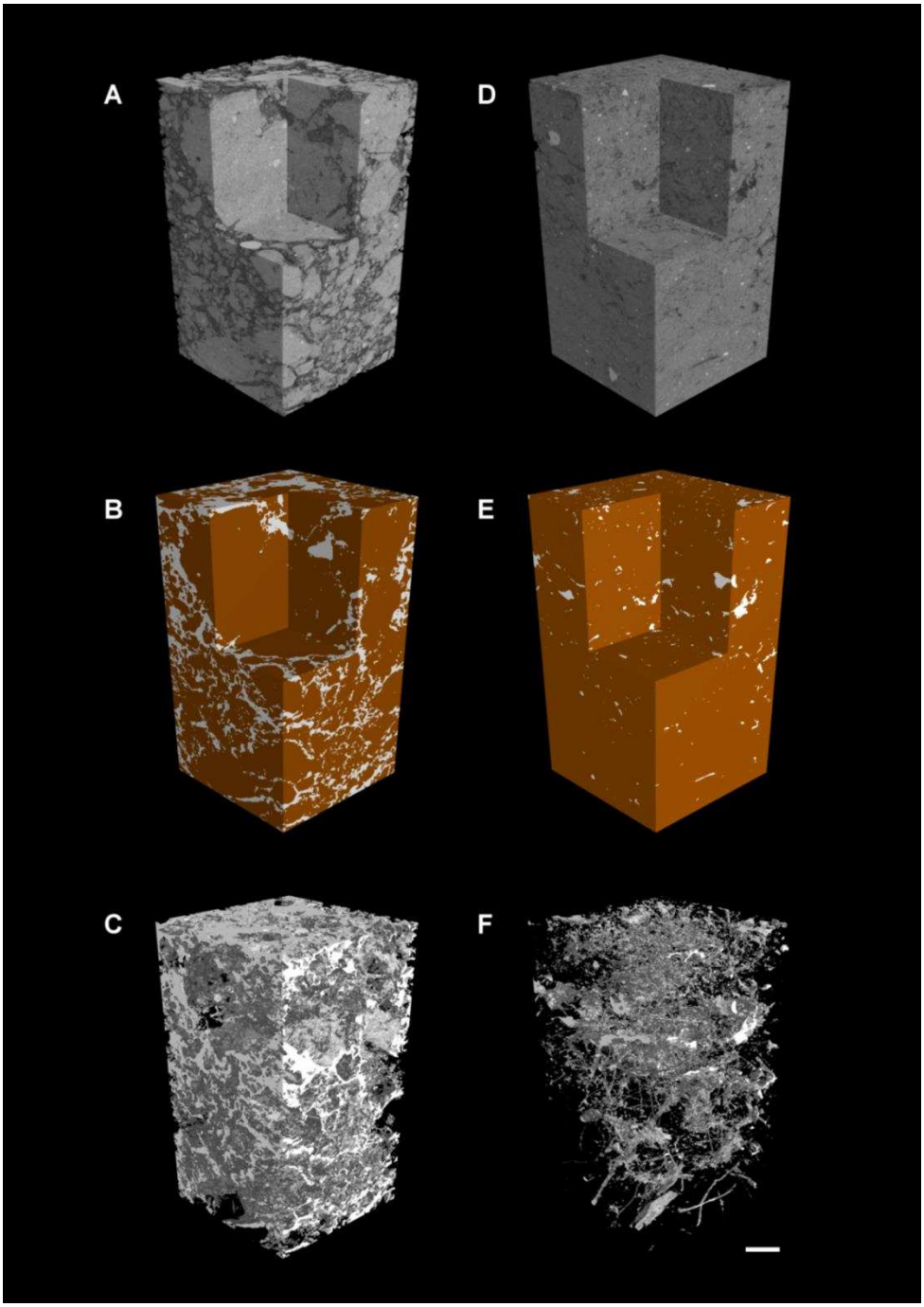

Fig. 4.6. Non-destructive 3D imaging of soil. A-C Thurlby (Tilled), D-F Wragby (Zero Tilled). A&D: 3D rendered grayscale density map of soil cores showing a virtual 'cut-out' to the revealing clear differences soil structure between the two treatments. B&E: Thresholded 3D image highlighting 'solid' soil in brown and 'void' pore space in white. C&F: Visualisation of pore space only highlighting it's connectivity and the presence of numerous bio-pores in the zero tilled soil. Scale bar = 10 mm.

the ‘fill holes’ function was used to remove noise. Measurements on soil physical features were obtained on the binary images which included porosity, number of pores, pore size and surface area of pores.

4.2.4 Soil chemical and biological properties

Various soil poroperties studied include soil pH, total soil organic matter, ammonium and nitrate nitrogen, microbial biomass carbon and microbial biomass nitrogen. Detailed description of procedures are provided in appendix.

4.2.5 Fluxes of greenhouse gases

Cores were removed from the 4^{o}C environment and kept at a constant temperature of 16 ^{o}C for 48 hours to activate and stabilise the biological activity. Gas sampling was performed by placing cores in 1.5 litre plastic jars (20 cm height and 10 cm diameter) with a septum on the top to aid gas sampling using a 20 ml syringe. The detailed description of procedure is given in appendix.

4.2.6 Statistical analysis

Each site consisted of a pair of fields; one of which was ploughed and the other had been zero tilled for a number of years. The sites were in areas consisting of a range of soil types. The sites were located in different geographical regions although at each site the tilled and zero tilled plots were located adjacent to each other (always separated by <10m). Samples were taken at random locations in each field and at two soil depths (0-10 and 10-20 cm). The variation in soil properties in response to tillage and soil depth was analysed as split-split plot design in a linear mixed model with site, field and location within fields as random effects. Tillage, soil depth and their interaction were

considered as fixed effects. To further test the effect of number of years since adoption of zero tillage and to account for differences with respect to soil texture, the clay content of the soil and, for each zero-tilled field, the number of years since conversion to zero tillage and their interactions with soil depth were included as fixed effects in the model. Multiple linear regressions were used to predict the best model describing the fluxes of GHGs from soil. The maximal model consisted of all the physical, chemical and biological properties studied in this experiment. By using a stepwise backwards elimination process, only the variables that contributed significantly to the model and reduced the residual sum of squares were retained in the model. For illustrative purposes we also carried out the single linear regression between the parameters that contributed to the multiple regression models. All tests were performed using Genstat (14th Edition, VSN International Ltd, Hemel Hempstead, UK).

4.3 Results

4.3.1 Soil physical properties

Soil texture varied substantially between the different sites. The soils at Bingham, Burton Lazars and Bourne were predominantly clayey in texture whereas the soils at Canwick, Lissington, Whitehall and Wragby were predominantly sand textured (Table 4.1). No significant variation was found in soil texture between paired fields ($P > 0.05$). Zero tilled soils had higher bulk density (1.16 Mg m^{-3}) than tilled soils (1.09 Mg m^{-3}) (Fig. 4.7a, $P < 0.001$) while the duration (from 5 to 10 years) under zero tillage did not influence bulk density ($P > 0.05$). Zero tilled soils had an increased average shear strength of 28.0 MPa compared to 12.0 MPa under tilled fields (Fig. 4.7b, $P < 0.001$), but the duration of zero tillage did not affect the shear strength ($P > 0.05$).

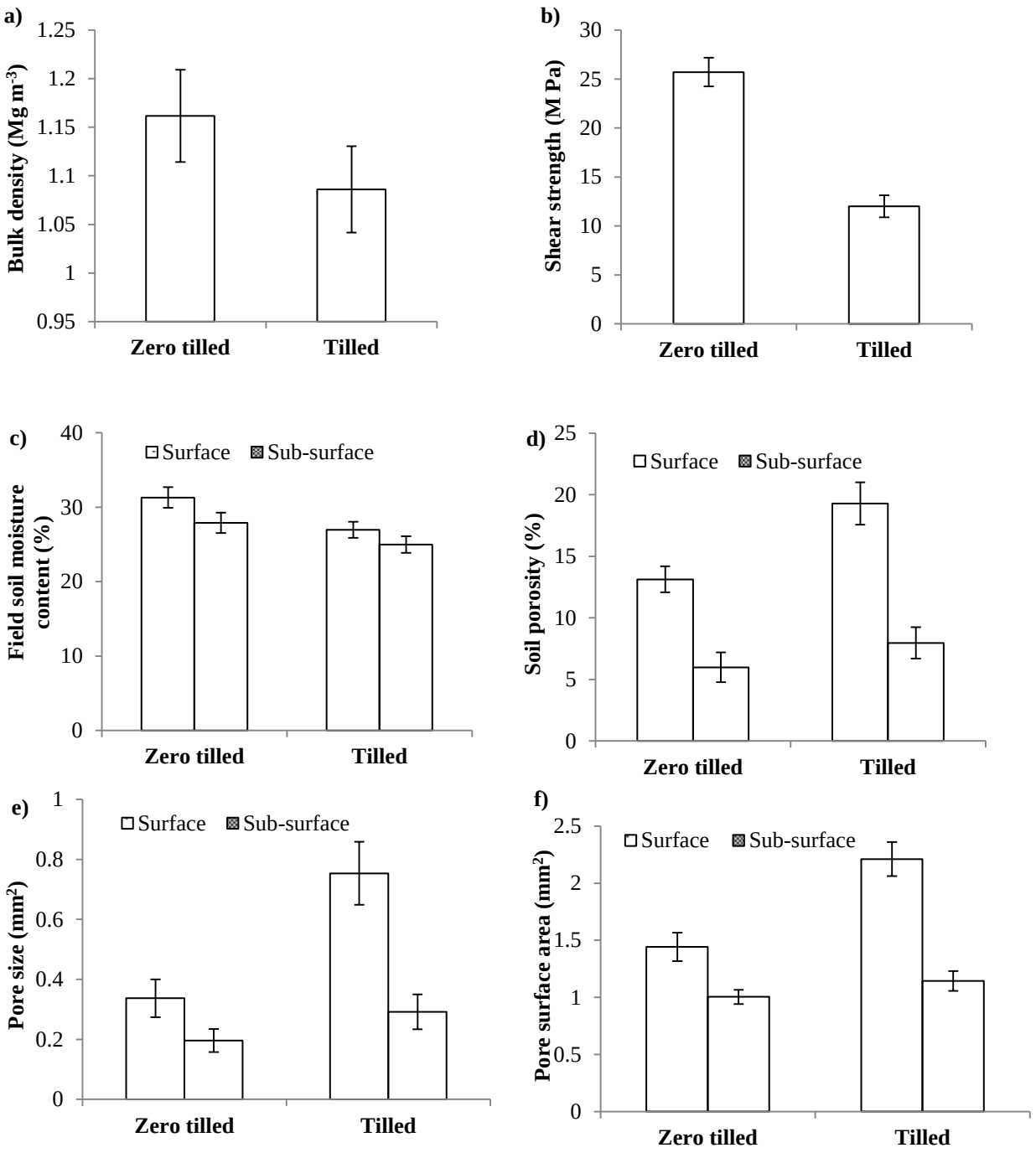

Fig. 4.7. Selected soil physical property results for zero tilled and tilled managed soil. (a) and (b) depicts the bulk density and soil shear strength under zero tilled and tilled soils. Figures from (c) to (f) shows the field soil water content (c), soil porosity (d), soil pore size (e) and surface area of soil pores (f) at the surface (0-10 cm) and sub-surface layers (10-20 cm) in zero tilled and tilled soils (average values for different sites and standard error of the mean are shown, n = 33). Figures d-f measured by X-ray CT.

Average soil moisture content (volumetric) was significantly higher under zero tilled soils (29.3%) compared to tilled soils (26.0%) ($P < 0.01$), although, the duration of zero tillage did not have a significant effect on soil moisture content ($P > 0.05$).

4.3.2 Soil pore characteristics

X-ray CT measured soil porosity was significantly higher under tilled soil (14.0%) than zero tilled soil (9.0%) ($P < 0.001$, Fig. 4.7d). The porosity in the surface layer (0-10 cm) of tilled soils were 32% higher than under zero tilled soils and in the 10-20 cm layer the porosity of tilled soils were 29% higher compared to zero tilled soils ($P < 0.001$). The duration of tillage and its interaction with depth was not statistically significant (Table 4.2).

Soil pore size followed a similar pattern to soil porosity (Fig. 4.7e). Pore size significantly varied with tillage type and soil depth with increased pore size at the surface layers of tilled soil (Table 4.2, $P < 0.05$). Tilled soils had larger pores (0.52 mm^2) compared to zero tilled soils (0.27 mm^2) ($P < 0.01$) with the largest pore sizes recorded in the 0-10 cm layer (0.55 mm^2) as opposed to the 10-20 cm layer (0.24 mm^2) ($P < 0.001$).

The surface area of the soil pore system was higher under tilled soil (Fig. 4.7f, $P < 0.001$). The surface area of pores was also greater in the 0-10 cm depth (1.83 mm^2) than the 10-20 cm samples (1.07 mm^2) across both tilled and zero tilled soils ($P < 0.01$). Duration of zero tillage did not influence the pore surface area (Table 4.2, $P > 0.05$).

4.3.3 Soil chemical and biological properties

Tillage practice did not have an effect on soil pH ($P > 0.05$) while soil pH was higher in the 10-20 cm layer than in the 0-10 cm layer (Table 4.2 and 5.3, $P < 0.001$). Zero tilled sites contained significantly more SOM than tilled fields ($P < 0.001$). Soil from the 0-10 cm layer contained more SOM than soils from the 10-20 cm layers in both zero tilled (7.81 and 7.41% at surface 0-10 cm and subsurface 10-20 cm respectively) and tilled soils (6.60% at surface and 6.15% at subsurface) (Table 4.3, $P < 0.001$). There were no significant effects for duration of tillage on soil organic matter (Table 4.2).

Neither NH_4-N nor NO_3-N content in the soil was affected by tillage. Soil from the upper 10 cm contained significantly higher NH_4-N than the 10-20 cm layer (Table 4.3, $P < 0.01$). Nitrate (NO_3-N) followed a similar trend to that of NH_4-N. Tillage type and tillage duration did not influence the NO_3-N content. Soil depth significantly influenced NO_3-N content ($P < 0.001$) with the highest amount in the surface layer (0-10 cm) under both zero tillage and conventional tillage.

Zero tilled soils contained significantly more microbial biomass carbon than tilled soils ($P < 0.001$). The mean microbial biomass carbon under zero tilled soil was 510.4 mg kg^{-1} soil against 403.2 mg kg^{-1} soil in tilled soils. Microbial biomass carbon was significantly higher in the 0-10 cm layer (591.8 mg kg^{-1} soil) than the 10-20 cm layer (442.2 mg kg^{-1} soil) under zero tillage ($P < 0.001$, Fig. 4.8). However there was no significant effect of duration of zero tillage (Table 4.2).

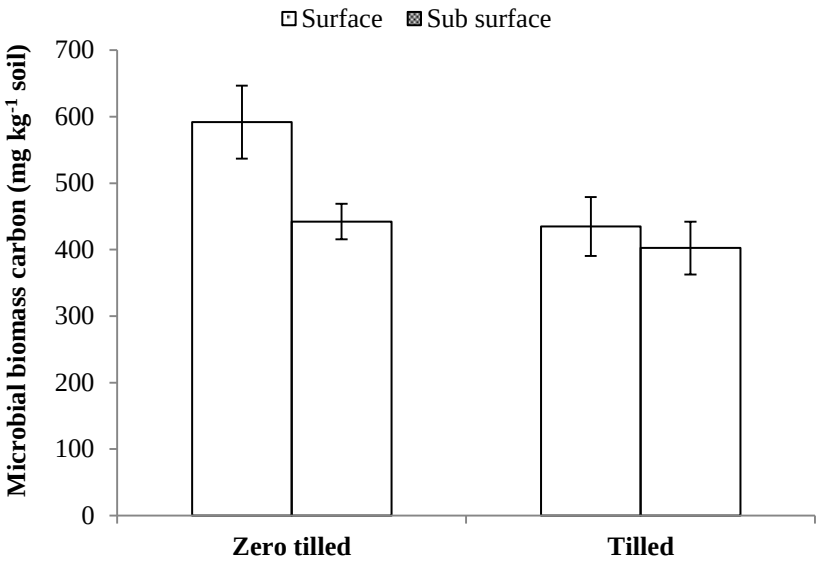

Fig. 4.8. Microbial biomass carbon at surface and sub-surface layers in zero tilled and tilled soils (average values for different sites and standard error of the mean are shown, n = 33).

Tillage and soil depth significantly influenced soil microbial biomass nitrogen (Table 4.2 and 4.3). Zero tilled soils contained a higher microbial biomass nitrogen (91.1 mg kg^{-1} soil) than tilled soil (70.0 mg kg^{-1} soil) ($P < 0.001$). Surface layers (0-10 cm) maintained more microbial biomass nitrogen than sub surface layers (10-20 cm) under both zero tilled soils and tilled soils.

4.3.4 Fluxes of greenhouse gases

CO_2 flux was higher from tilled soils than zero tilled soil ($P < 0.05$, Fig. 4.9a). CO_2 fluxes under zero tilled soil ranged from 47 to 216 mg m^{-2} h^{-1} with a mean value of 141 mg m^{-2} h^{-1} whilst under tilled sites it ranged from 119 to 236 mg m^{-2} h^{-1} with a mean value of 171 mg m^{-2} h^{-1}. The CO_2 flux on a per soil weight basis was also higher under tilled soil (873 ng g^{-1} h^{-1} soil) compared to zero tilled soil (688 ng g^{-1} h^{-1} soil) ($P < 0.01$, Fig. 4.9b).

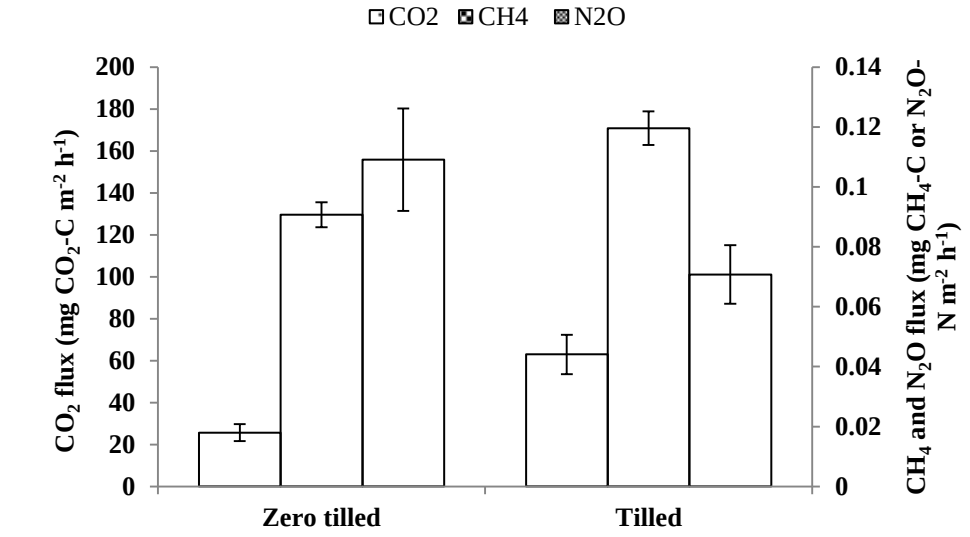

Fig. 4.9a. Fluxes of CO_2, CH_4 and N_2O expressed per surface area under zero tilled and tilled soils (average values for different sites and standard error of the mean are shown, n = 33).

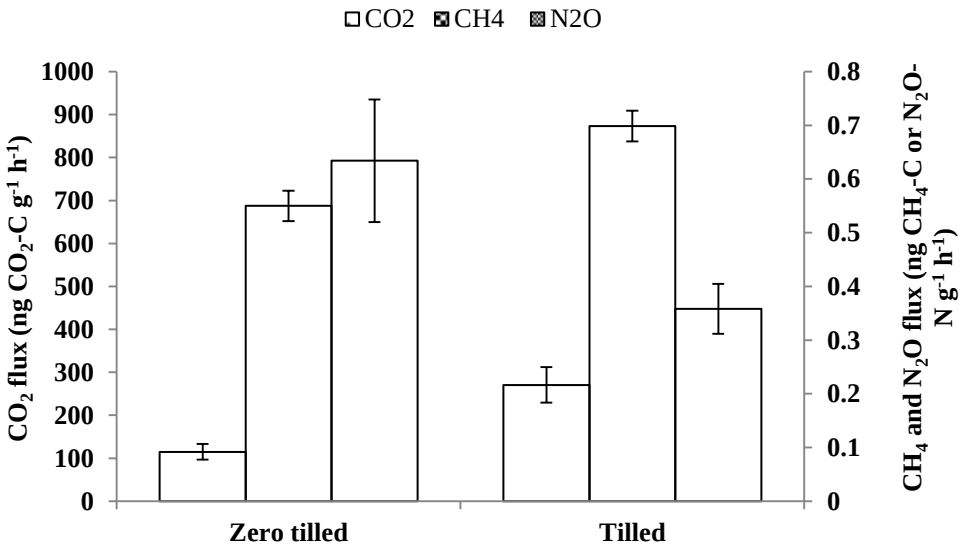

Fig. 4.9b. Fluxes of CO_2, CH_4 and N_2O expressed per gram of soil under zero tilled and tilled soils (average values for different sites and standard error of the mean are shown, n = 33).

CH_4 fluxes were generally positive and higher from tilled soils (0.044 mg m^{-2} h^{-1} or 0.22 ng g^{-1} soil) compared to zero tilled soil (0.018 mg m^{-2} h^{-1} or 0.09 ng g^{-1} soil) ($P < 0.05$, Fig. 5.4a and 4.9b). In contrast, N_2O emissions were higher under zero tilled soil (0.63 ng g^{-1} h^{-1}) than tilled soils (0.36 ng g^{-1} h^{-1}) (68% higher under zero tilled soils when measured on a soil area basis and 77% on a soil dry weight basis) ($P < 0.01$, Fig. 4.9a and 4.9b).

The net global warming potential calculated was significantly higher from tilled soil than zero tilled ones. Tilled soil produced 20% on area basis or 26% on weight basis greater global warming potential (GWP) than zero tilled soil ($P < 0.05$, Fig. 4.10). There was no evidence to suggest that the duration of zero tillage considered in this study affected net emissions of greenhouse gases.

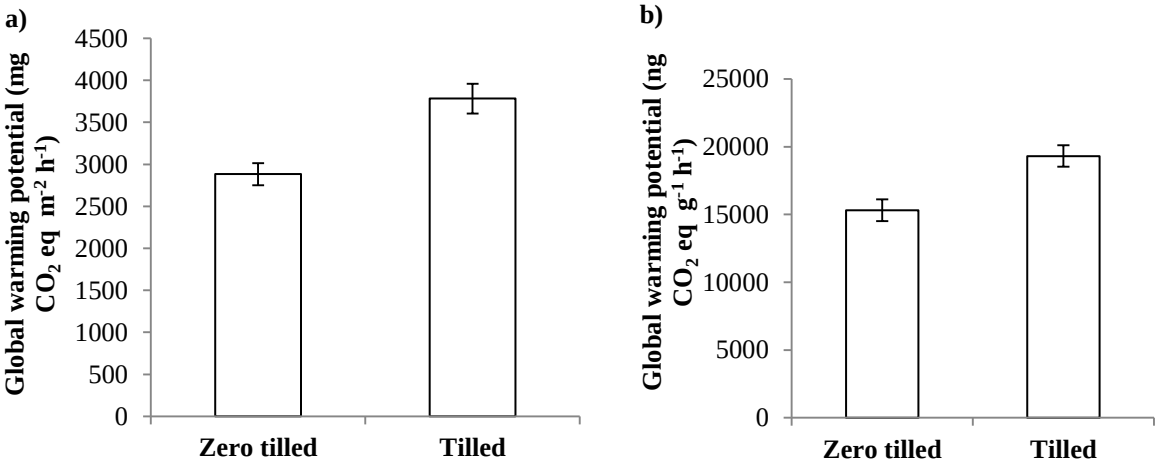

Fig. 4.10. Global warming potential under zero tilled and tilled soils (average values for different sites and standard error of the mean are shown, n = 33). (a) GWP expressed in terms of mg m^{-2} $h^{-1}$and (b) GWP expressed in terms of ng g^{-1} h^{-1}.

4.3.5 Relationship between greenhouse gas fluxes and soil properties

CO_2 fluxes were predicted by a multiple regression model ($P < 0.001$) including bulk density (BD), microbial biomass carbon (MBC) and soil porosity (P) which accounted for 69.9% of the variation. The optimal model for CO_2 flux is provided in the equation 1.

$$CO_2 \text{ flux (mg } m^{-2} h^{-1}) = 124.1 - 39.1BD + 0.0412MBC + 3.689P \quad (1)$$

In this model the soil porosity contributed to c.40% of variation, much higher than the individual contribution by any other parameter, as illustrated by retaining the parameter when fitting last from the model. Microbial biomass carbon and bulk density contributed to 30% of the total variation (Figures 4.11a, 4.11b and 4.11c).

Only soil shear strength, as a measure of soil density (SS) explained variation (18.0%) in CH_4 flux (Equation 2, Figure 4.11d, $P < 0.01$).

$$CH_4 \text{ flux (mg } m^{-2} h^{-1}) = 0.05344 - 0.001078SS \quad (2)$$

The optimal model (equation 3) for N_2O flux accounted for 62.0% of the variation and included soil moisture (SM), microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC) (Figures 4.11e and 4.11f, $P < 0.001$).

$$N_2O \text{ flux (mg } m^{-2} h^{-1}) = -0.0746 + 0.002057SM - 0.00049 \quad (3)$$

$$MBN + 0.0003104MBC$$

Individually microbial biomass carbon explained the greatest proportion (20.8%) of the total variation when fitted last in the model. Removing soil moisture and microbial biomass nitrogen separately from the model did not

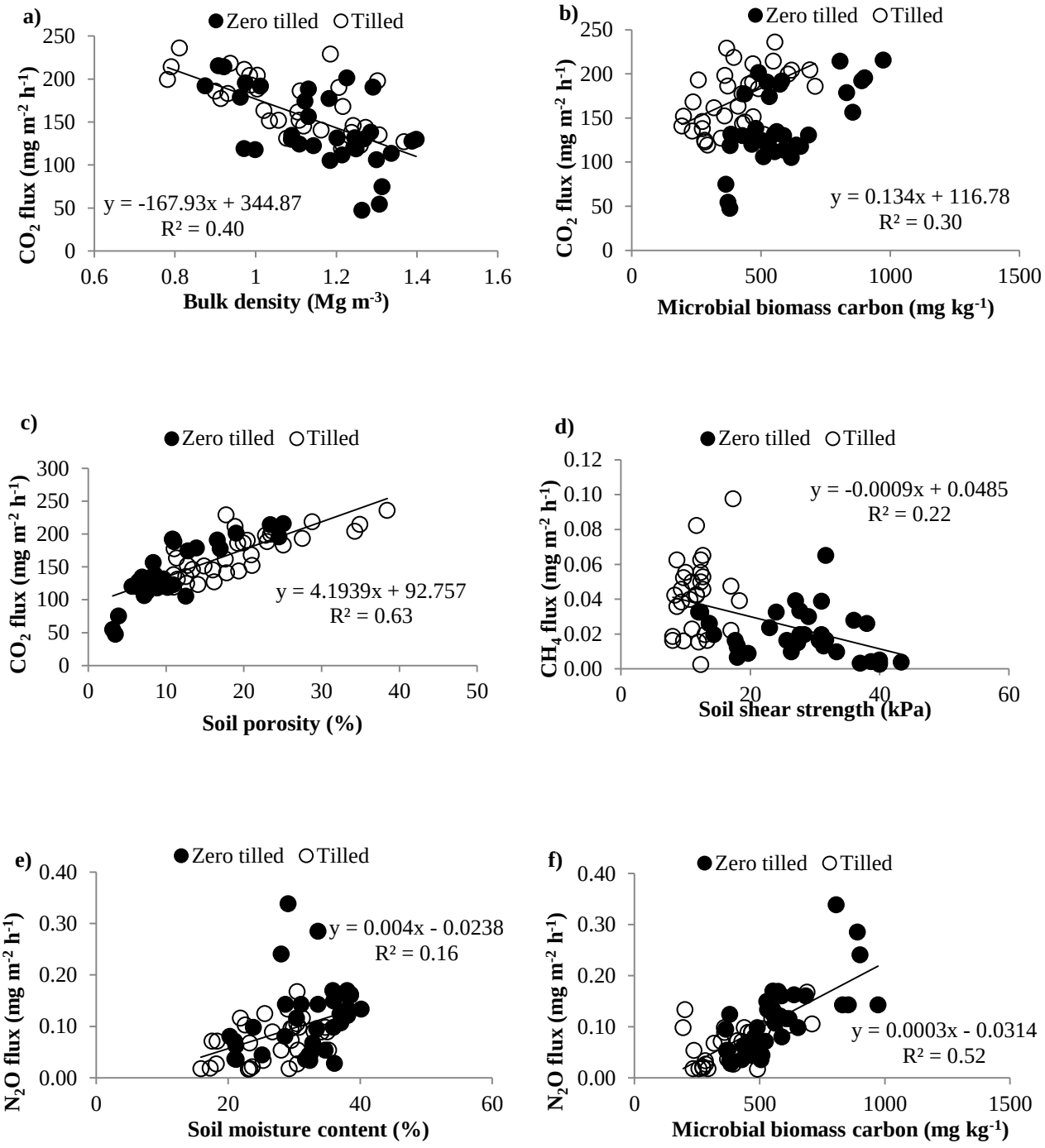

Fig. 4.11. Illustration of important relationships between soil bio-physical properties and GHG release. (a) soil bulk density and CO_2 flux from soil; $F_{1,64}$ = 42.08, P <0.001 (b) microbial biomass carbon and CO_2 flux; $F_{1,64}$ = 5.89, P <0.05 (c) soil porosity and CO_2 flux; $F_{1,64}$ = 110.14, P <0.001 (d) soil shear strength and CH_4 flux; $F_{1,64}$ = 14.08, P <0.001 (e) soil moisture content and N_2O flux, ; $F_{1,64}$ = 12.62, P <0.001 and (f) microbial biomass carbon and N_2O flux; ; $F_{1,64}$ = 69.5, P <0.001.

substantially decrease the amount of variation explained suggesting that these factors were confounded.

4.4 Discussion

Here we have demonstrated tillage practice has the potential to strongly influence release of CO_2, CH_4 and N_2O, through its impact on soil biophysical properties. However, the main driving factors and the direction of change varied among the three GHGs measured. The higher CO_2 release found in response to tillage highlights the role of ploughing in the turnover of soil aggregates and exposure of organic materials for microbial decomposition (Ussiri et al., 2009b). Soil pore characteristics such as overall porosity were a stronger predictor of CO_2 flux than soil organic matter and microbial biomass carbon, which has not previously been reported. The effect of zero tillage was to reduce soil porosity by 29%, which lead to 21% reduction in CO_2 efflux. These results demonstrate that the increased soil porosity under conventional tillage favours the activities of aerobic organisms by improving movement of water and air through the soils (Udawatta et al., 2008) with important implications for CO_2 emissions. In parallel, strong effects of soil bulk density on CO_2 productions was shown by Beare et al. (2009) who found 2.3 times more CO_2 production under uncompacted soil than in compacted soil. The CO_2 flux data presented here (47 to 235 mg m^{-2} h^{-1}) and is in the range of that reported for arable land (47 mg m^{-2} h-^{1}) and grassland (186 mg m^{-2} h-^{1}) for European soils by Schaufler et al. (2010). Similar effects of tillage on CO_2 fluxes were shown by Ball et al. (1999) who attributed the greater CO_2 efflux to the larger pores created by tillage.

CH_4 flux ranged from 0.0025 to 0.16 mg m^{-2} h^{-1}, which is high compared to values reported by Schaufler et al. (2010): e.g. average CH_4 flux in arable land was 0.0014 mg m^{-2} h^{-1} and in grassland it was 0.0005 mg m^{-2} h^{-1}. The reduced CH_4 flux under zero tillage was best predicted by soil shear strength which reflects the reduced porosity and high bulk density in zero tilled soils (Wu et al., 1992; Schjønning et al., 2000; Bhattacharyya et al., 2006). Furthermore increased bulk density in soil can prevent flow of CH_4 in soil and the resulting enhanced retention of CH_4 in soil may improve its oxidation by methanotrophs (Smith et al., 2001). The development of methanotrophic populations is negatively affected by tillage (Mosier et al., 1997) which are slow to recover (Hütsch, 1998; Nazaries et al., 2011). Despite the less porous and wetter status of zero tilled soils, which normally promote CH_4 production (Yu et al., 2007), the opposite was the case here which may be due to increased activity of methanotrophic bacteria (Ussiri et al., 2009a).

N_2O fluxes were comparable to those of Regina et al. (2010) in Finnish soils after 5-7 years of zero till management (0.003 to 0.23 mg m^{-2} h^{-1}) with significantly higher N_2O fluxes under zero till soils. They reported 21 to 86% higher N_2O flux in zero till soils when compared to tilled soils. The average increased emission of N_2O flux under zero tilled soils obtained by Oorts et al. (2007) was 39% for a 30 year experiment. As with CH_4, N_2O is also produced under reducing conditions in water logged and poorly aerated soils (Gregorich et al. 2008, Choudhary et al. 2002), the increased N_2O emissions from zero tilled soils was attributed in part to the wetter and denser soils found under this management regime. In contrast to the CO_2 and CH_4 fluxes, the production of N_2O was most strongly related to the total soil microbial biomass. The greater

total soil microbial biomass found under zero tillage may play very important role for N_2O release. One important aspect of zero tillage is enhanced crop residue retention resulting in greater SOM content. Given the importance of an adequate supply of labile substrates for the denitrifying bacteria (Choudhary et al., 2002), it may also be that the crop retention under zero tillage drives greater N_2O release.

Tilled soil produced 20% greater net global warming than zero tilled soil indicating a potential for zero tillage system to mitigate climate change after only 5 to 10 years since conversion (earlier than this was not measured here). In parallel with this Del Grosso et al. (2005) also reported a 33% reduction in global warming potential under zero tillage (0.29 Mg C ha^{-1} y^{-1}) compared with tilled soil (0.43 Mg C ha^{-1} y^{-1}) for major non-rice cropping systems in US. However some contradictory research was reported that increased global warming under zero tillage (Robertson et al., 2004; Piva et al., 2012).

Zero tilled soils had enhanced SOM, microbial biomass carbon and nitrogen compared to tilled soils. Importantly, the time during which the soils had been under conservation tillage did not influence the SOM content in the soil, suggesting that a steady state is reached (although only changes between 5 and 10 years were measured). Although West and Post (2002b) in similar work recorded a large increase between 5-10 years. The time required to reach a steady state for carbon sequestration will vary with respect to climate, soil types and the management practices followed (Post et al., 2004).

A very important question remains is how the impact of the change in soil porosity brought by tillage/zero tillage on net GHG release and the GWP varies spatiotemporally across a greater range of soils types, crops and climate than

those explored in our study. With reduced tillage practices becoming more prevalent globally, it is important to further understand the impacts of this on the biophysical evolution of the soil environment at both micro and macroscales. It is clear from this study that the modification of soil structure by tillage plays a crucial role for GHG release. Our study was based on analysis on undisturbed cores, and to fully account for the impact of zero tillage on GHG release it is important to extend this work to insitu field measurement through the year to account for variation in weather and crop development. In conclusion, we have shown soils under zero tillage increased N_2O emissions, but this is counterbalanced by a substantial reduction in CO_2 and CH_4 emissions which is closely linked to the geometry of the soil pores. To evaluate the potential of zero till as a tool for mitigation of climate change there is a need to assess the impact of zero till on yield to ensure a balance between climate change mitigation and food security is achieved.

This chapter addressed the sub aims 1 and 3 of the thesis; to evaluate the changes in soil pore characteristics under different tillage practices and to investigate climate change mitigation capabilities of zero tillage. This chapter demonstrated that zero tilled soils exhibited less global warming potential compared to tilled soils and the greenhouse gas emissions from soil is affected by the physical characteristics to a considerable extent.

List of tables

Table 4.1. Selected soil and management characteristics of the experimental sites.

Location	Site 1 Bourne-A	Site 2 Bourne-B	Site 3 White hall	Site 4 Lissington	Site 5 Oakham-A	Site 6 Oakham-B	Site 7 Burton L- A	Site 8 Burton L-B	Site 9 Bingham	Site 10 Canwick	Site 11 Wragby
Elevation (m)	45	62	48	21	75	94	54	43	19	32	26
Years under zero till management	7	7	10	10	7	7	7	7	8	5	5
Cropping activity at tilled site	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat/Peas	Wheat	Sugar beet	OSR*
Cropping at zero tilled site	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat	Wheat/OSR	Wheat	Wheat	Wheat/OSR
Soil texture	Clay	Clay	Sandy loam	Sandy clay	Silt loam	Silty clay loam	Clay	Silty clay	Clay	Sandy loam	Sandy clay

*Oil seed rape

Table 4.2. Statistical output from linear mixed modelling (texture, tillage, duration, depth) for the physico-chemical characteristics of soils under zero tillage and conventional tillage (F statistic).

Parameter	Clay (%)	Tillage	Duration of tillage	Depth	Tillage x depth	Duration of tillage x depth
Moisture content	**6.97 (58)***	**17.86 (10)****	0.0 (10)^{ns}	**52.29 (63)*****	3.27 (63)^{ns}	0.65 (63)^{ns}
Porosity	**6.70 (32)***	**16.49 (14)*****	0.02 (14)^{ns}	**59.3 (63)*****	**15.86 (63)*****	1.61 (63)^{ns}
Pore size	**11.31 (21)****	**14.21 (15)****	0.38 (15)^{ns}	**17.26 (63)*****	**4.89 (63)****	0.37 (63)^{ns}
Surface area of pores	**14.71 (36)*****	**17.01 (13)*****	0.15 (13)^{ns}	**47.71 (63)*****	**8.36 (63)****	0.25 (63)^{ns}
Soil pH (1:2)	**6.72 (46)***	0.40 (17)^{ns}	1.83 (17)^{ns}	**38.49 (63)*****	**15.78 (63)*****	1.64 (63)^{ns}
Soil organic matter	0.07 (53)^{ns}	**33.24 (10)*****	0.02 (10)^{ns}	**84.13 (63)*****	0.22 (63)^{ns}	0.12 (63)^{ns}
NH_4-N	**3.86 (44)** *	1.21 ^{ns}	0.73 ^{ns}	**7.52 (63)****	0.10 (63)^{ns}	3.97 (63)^{ns}
NO_3-N	2.35 (40)^{ns}	0.04 (17)^{ns}	6.45 (17)^{ns}	**29.8 (63)*****	**5.03 (63)***	0.57 (63)^{ns}
Microbial biomass carbon	0.25 (57)^{ns}	**33.96 (10)*****	2.12 (10)^{ns}	**37.14 (63)*****	**35.67 (63)*****	**4.82 (63)***
Microbial biomass nitrogen	0.11 (33)^{ns}	**25.85 (10)***	1.96 (10)^{ns}	**20.42 (63)*****	**7.44 (63)****	0.59 (63)^{ns}

(Figures in parenthesis indicate degrees of freedom), ns: non-significant.
*** $p < 0.001$.
** $p < 0.01$.
* $p < 0.05$.

Table 4.3. Selected chemical properties of soils under zero tillage and conventional tillage*.

Tillage	Depth	Soil pH (1:2)	Soil organic matter (%)	NH_4-N (mg kg^{-1} soil)	NO_3-N (mg kg^{-1} soil)	Microbial biomass N (mg kg^{-1} soil)
Zero tilled	**Surface (0-10 cm)**	6.98±0.13	7.81±0.44	2.59±0.10	0.66±0.05	104.9±7.92
	Sub surface (10-20 cm)	7.32±0.10	7.41±0.42	2.42±0.08	0.45±0.04	77.3±5.11
Tilled	**Surface (0-10 cm)**	7.22±0.14	6.59±0.42	2.51±0.16	0.62±0.06	73.4±5.11
	Sub surface (10-20 cm)	7.29±0.13	6.15±0.40	2.30±0.14	0.54±0.06	66.6±3.79

***Mean**±Standard Error of mean (n=33)

5. Chapter 5: Microbial mechanisms governing soil carbon sequestration under conservation tillage in temperate soils

The previous chapters (2, 3 and 4) have shown that tillage practices significantly affect soil physical properties. The changed physical properties affected the soil carbon storage and emission of greenhouse gases from soil. The zero tillage practices have been found beneficial ti reduce overall greenhouse gas emission in comparison to conventional tillage practices. The carbon sequestration capabilities of zero tillage practices were also demonstrated in chapters 2 and 4. However the microbial and physico-chemical mechanisms of C protection or sequestration related to a change in soil management are less well understood. Therefore and experiment was formulated to assesses the microbial and biological basis of carbon sequestration for soils managed by conventional and conservation tillage. The fluxes of greenhouse gases were assessed in a disturbed condition, along with assessment of soil biochemical properties such as enzymes, functional groups of organic matter and active microbial functional diversity. This paper will be submitted to the **Soil Biology and Biochemistry** and is presented in an unpublished paper format.

Microbial mechanisms governing soil carbon sequestration under conservation tillage in temperate soils

S. Mangalassery[a], S.J. Mooney[a], D.L. Sparkes[a], Wesley Fraser[b], S. Sjögersten[a] *

[a]School of Biosciences, Sutton Bonington Campus, University of Nottingham, Sutton Bonington, Loughborough, Leicestershire, LE12 5RD, UK

[b]Department of Environment, Earth and Ecosystems, The Open University, Milton Keynes MK7 6AA, UK

Corresponding author at: Division of Agricultural and Environmental Sciences, School of Biosciences, University of Nottingham, Sutton Bonington campus, Loughborough, Leicestershire, UK, LE12 5RD. Tel.: +44 115 9516257.

E-mail address: sofie.sjogersten@nottingham.ac.uk (S. Sjögersten).

Key words: Carbon sequestration, Microbial biomass carbon, Greenhouse gases, Soil enzymes, Soil organic matter, Soil microbial functional diversity

Abstract

Studies on reduced tillage practices have indicated that a reduction in soil disturbance can be useful to preserve soil organic matter. In this study we explored the role of microbial processes and functional organic chemistry for C sequestration in soils that had been zero tilled for 7 years against annually tilled soils located adjacent to each other. Zero tilled soils contained 9% more soil C and 30% higher microbial biomass C than tilled soil. Increased CO_2 emission was observed in tilled soils compared to zero tilled. Overall the global warming potential was 69% less under zero tilled soil compared to tilled soils, although increased CH_4 and N_2O fluxes were recorded under zero tilled soils. Increased microbial activity was evident in zero tilled soils as observed from the increased activities of enzymes such as dehydrogenase, cellulose,

xylanase, β-glucosidase, phenol oxidase and peroxidase. Under zero tilled soils C is preserved in recalcitrant forms which are facilitated by the increased activities of microbes in the presence of increased accumulation of crop residues. These results suggest that a modified microbial regime plays a major role in sequestering carbon in zero tilled condition.

5.1 Introduction

Soil C sequestration has been suggested as a strategy to mitigate greenhouse gas emissions and improve soil quality (Bhattacharyya et al., 2009). It has been estimated agricultural soils have the potential to sequester about 5500 to 6000 Mt CO_2 –eq per year by 2030 (Chatterjee et al., 2009). The soil C stocks and the potential of soil to sequester C are affected by different environmental variables such as regional climate, soil physical and chemical properties and soil management (West et al., 2007). Maintaining and preserving soil organic matter is crucial given the major role it plays in controlling the physico-chemical and biological properties that affect crop production and sustainability of agricultural ecosystems (Denef et al., 2004).

Conservation tillage practices have been shown to improve or to maintain soil organic matter by helping to sequester C in soil (West et al., 2002). The increased C sequestration capabilities under conservation tillage practices have been attributed to the low degree of soil disturbance which minimise the decomposition of soil organic matter and develop a litter layer at the surface that modifies the soil physico-chemical and biological properties (de Rouw et al., 2010).

Organic matter in soil occurs as a complex heterogeneous mixture of organic compounds and consists of different fractions each of which varies in their stability against degradation. Management practices such as tillage alter the soil matrix by

manipulating soil porous architecture and subsequently influence the location of microorganisms around or within soil aggregates. The literature suggests physico-chemical protection of soil organic matter largely depends on soil aggregation (Golchin et al., 1994; Six et al., 2000b). The biochemically recalcitrant stable fraction of C is reported to have a turnover rate of many thousands of years while the labile fraction is characterised by more decomposition in response to soil management such as tillage and crop rotation (Zotarelli et al., 2007). A third intermediary fraction is stabilised by physico-chemical mechanisms by occupying within the soil aggregtes or by bonding to clay surfaces (Hermle et al., 2008). The amount of C sequestered in agricultural soil depends on how each of these fractions responds to tillage practices. Apart from the physical aspects of organic matter protection by soil aggregates (of various sizes), the chemical structure of organic matter itself is also another important determinant deciding the sequestration of C in soil. Traditionally organic matter dynamics in soil have been assessed by chemical fractionation into humic, fulvic acids and humin (Balesdent, 1996) or by physical methods such as physical fractionation and density fractionation (Six et al., 2000a). However, these methods provide no insight into the functional composition of the organic materials. The use of Fourier Transform Infrared spectroscopy has been used to study SOM characteristics in soil due to its ability to provide the information of functional groups and structural entities (Mao et al., 2008).

The microbial community structure in soils play an important role in determining the amount of C sequestered in soil or decomposed and released into the atmosphere. Microorganisms aid sequestration by re-synthesising the products of decomposition into stable organic matter compounds (Bausenwein et al., 2008). Due to the continuous addition of substrates under conservation tillage practices, the pattern of

microbial community structure may be distinctly different from the tilled soil (Plassart et al., 2008). Changes in microbial community with respect to increased arbuscular michorizal fungi and PLFA profiles were reported by Helgason et al. (2010). These changes in microbial community may be reflected in microbial functioning of the soil by affecting soil enzymatic activities (Acosta-Martínez et al., 2008). A number of soil enzymes are involved in the carbon dynamics in soil (Sardans et al., 2008). Cellulase and xylanase are important enzymes in carbon metabolism bringing decomposition of organic constituents in plant materials (Luxhøi et al., 2002). β-glocosidase is another important enzyme in the C cycle responsible for hydrolytic breakdown of organic constituents in plant litter (Madejon et al., 2003). Dehydrogenase activity in soil indicate the intensity of microbial metabolism in soil (Tabatabai, 1982), whereas oxido- reductive enzymes such as phenol oxidase and peroxidase performs lignin degradation, humification and carbon mineralisation (Sinsabaugh, 2010). Tilled soils have been reported to contain lower enzymatic activity than zero tilled soils (Melero et al., 2011) which is attributed to an increased availability of organic materials and organic carbon (Acosta-Martínez et al., 2007), changes in soil moisture, soil temperature, soil aeration, constitution of soil flora and fauna (Alvear et al., 2005).

The mechanism of enhanced C sequestration under conservation tillage practices have been largely attributed to the aggregation changes in soil by conservation tillage and microbial activities apart from increased availability of crop residues. However the microbial and physico-chemical mechanisms of C protection or sequestration related to a change in soil management are less well understood. The aims of this study were to characterise the components of soil organic matter under conventional and conservation tillage practices. The additional objectives were to identify and

explain the variations in microbial community structure using Biolog ecoplates and activities of selected enzymes involved in C metabolism such as cellulose, xylanase, β-glucosidase and oxido-reductase enzymes in soil such as dehydrogenase, phenol oxidase and peroxidase. We hypothesise that a reduction in tillage enhances biological activity in soil which will positively affect C stabilisation leading to its sequestration in soil.

5.2 Materials and methods

5.2.1 Sample preparation

A selection of previously sampled fields was chosen for further analysis. These were fields at Thurlby, Melton and Oakham. These fields were visited again on 14th November 2012 to collect fresh soil samples.

As in Experiment 3, all sampling sites comprised pairs of intensely tilled farms and farms where zero tillage practices are followed and care was taken to ensure we re-visited the same sites as the previous work. From each location, bulk soil samples were collected from two depths (surface 0 to 10 cm and sub surface10-20 cm), after harvest of the previous crop. The sampling was replicated in random locations five times at each site. These samples were labelled and sealed in plastic bags before being transported to the laboratory. In the laboratory the field moist samples were composited by mixing the replicates. They were then partitioned for various analyses and stored as required for analysis. The samples for the study of microbial community structure and soil enzymes were frozen at -20^{o}C and the samples were thawed at 4^{o}C prior to analysis over 5 days (Schinner et al., 2012). One set of samples were retained at 4 ^{o}C to study GHG flux and microbial biomass carbon. One

set of samples was air dried, passed through 2 mm sieve and ball milled for FTIR (Fourier Transform Infrared spectroscopy) analysis.

From each location, five bulk soil samples were collected from two depths (0 - 10 cm and 10-20 cm), after harvest of the previous crop, during November 2012. The pooled subsamples were used for analysis. Samples for the study of microbial community structure and soil enzymes were frozen at -20^{o}C and thawed at 4^{o}C prior to analysis over 5 days (Schinner et al., 2012). One set of samples were retained at 4 ^{o}C to study greenhouse gas (GHG) flux and microbial biomass C. One set of samples were air dried and passed through 2 mm sieve. These samples were then oven dried and subjected to ball milling using a planetary ball mill (Retsch, PM400) using an agate mortar with four balls, at a speed of 300 rpm for 4 minutes.

5.2.2 Soil chemical properties

The soil properties studied include tocarbon, total nitrogen and greenhouse gas fluxes (CO_2, CH_4 and N_2O), Absorption spectra was gathered using Fourier Transform Infrared spectroscopy (FTIR). The detailed description of these techniques and procedures are presented in appendix.

5.2.3 Soil biological properties

The soil biological properties estimated were microbial biomass carbon and microbial biomass nitrogen. The functional diversity of soil microorganisms were estimated using biolog eco plates. Also different soil enzymes were studied and these include dehydrogenase, cellulose, xylanase, β-glucosidase, phenol oxidase and peroxidase. The detailed description of procedures are provided in the appendix.

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5.2.4 Statistical analysis

The statistical software package Genstat (14th Edition, VSN International Ltd, Hemel Hempstead, UK) was used for the analysis of data. A two-way analysis of variance was applied to results obtained from laboratory analysis with soil texture and tillage as the two factors. The treatment means were compared at the $P < 0.05$ level using the LSD. Standard errors of means were calculated and provided as required. Simple linear regressions were carried out to understand the relationship between different parameters.

For Biolog plates Garland (1997) recommended choosing positive values higher than 0.25 absorbance could eliminate weak false positive response. Hence the statistical analysis was carried out on the mean colour intensity values greater than 0.25. A repeated measures ANOVA was carried out to assess the effect of incubation time on AWCD and other functional groups. A two way analysis of variance was performed to test the effect of tillage and depth on AWCD and different functional groups. For this a time point was chosen which had average well colour development values between 0.75 and 1.0 (Garland, 1997) and this was at 120 h of incubation. The substrate-utilization patterns were subjected to principal component analysis (PCA).

Multiple linear regressions were used to predict the best model describing the carbon content in soil. The maximal model consisted of all the chemical and biological properties studied in this experiment. By using a stepwise backwards elimination process, only the variables that contributed significantly to the model and reduced the residual sum of squares were retained in the model. For illustrative purposes we also carried out the single linear regression between the parameters that contributed to the multiple regression models.

5.3 Results

5.3.1 Soil chemical properties

5.3.1.1 Total carbon and nitrogen

Zero tilled soils contained 9% more total C (1.42%) than tilled soil (1.38%) which was statistically significant (Table 5.1, $F_{1,5}$ = 71.06, P <0.001). The total C content was higher in the surface layer (0-10 cm) than sub surface layers (10-20 cm) ($F_{1,10}$ = 13.30, P <0.01). In zero tilled soils the surface layer contained 14% more C than in the subsurface, whereas in tilled soil it was 16%. Total nitrogen followed a pattern similar to that of C with significantly higher content in zero tilled soil (0.25%) than tilled soil (0.16%) ($F_{1,5}$ = 10.99, P <0.05) and significantly higher values in the 0-10 cm layer than 10-20 cm layers ($F_{1,10}$ = 6.11, P <0.05).

5.3.1.2 FTIR

Fig. 5.1 shows the FTIR spectra of surface layer (0-10 cm) of zero tilled soil. The general patterns of spectra in these two soil management regimes were similar. In IR bands the absorption peaks were evident at 20 wave numbers and the corresponding functional groups were predicted by comparing with the published information (Glagovich, 2013). The information on peaks and functional groups are provided in Table 5.2. Statistically significant differences in frequencies were obtained on peaks at 2 wave numbers namely 709 cm^{-1} (aromatics) and 711 cm^{-1} (aromatics). Zero tilled soils produced significantly higher peaks corresponding to the aromatics functional groups (Fig. 5.2). Sub surface soils contained significantly higher absorption peaks at wave numbers 709 cm^{-1} (aromatics) and 711 cm^{-1} (Aromatics).

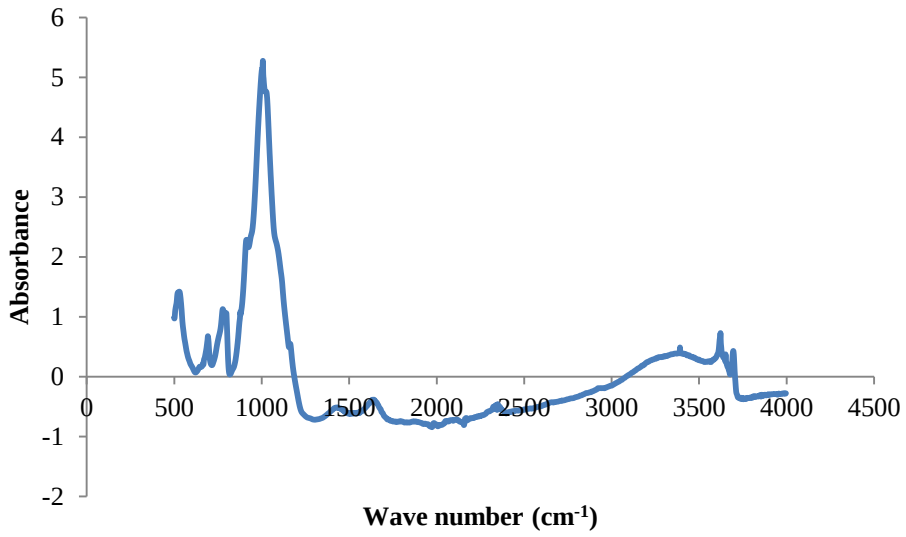

Fig. 5.1. Fourier Transform Infrared (FTIR) spectra of zero tilled soil (0-10 cm layer).

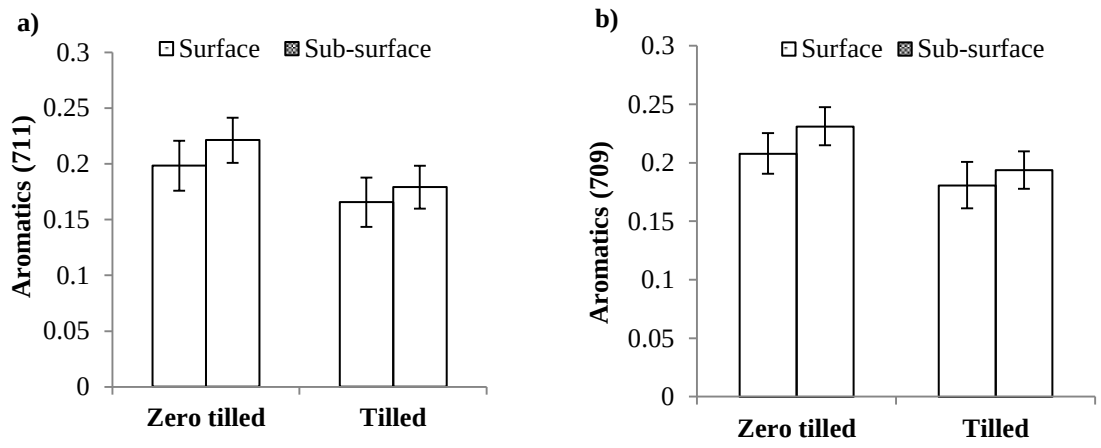

Fig. 5.2. Absorbance values at surface (0-10 cm) and sub surface (10-20 cm) layers under zero tilled and tilled soils at wave nmbers (a) 711, (b) 709.

5.3.1.3 Greenhouse gas flux

The highest CO_2 flux was from tilled soil (5.7 µg m^{-2} g^{-1} h^{-1}) which was significantly higher than from zero tilled soil (3.4 µg m^{-2} g^{-1} h^{-1}) (Table 5.3, $F_{1,5} = 6.9$, $P < 0.05$). A 41% increased flux was observed in tilled soil when compared to zero tilled soil. The CO_2 flux was higher from soil collected from 0-10 cm depth range than from soil in 10-20 cm layer in both zero tilled and tilled soil ($F_{1,10} = 14.44$, $P < 0.01$). The CH_4 fluxes varied significantly between tillage treatments (Table 5.3, $F_{1,5} = 18.99$, $P < 0.01$). The emission of CH_4 from zero tilled soils (0.85 ng m^{-2} g^{-1} h^{-1}) was 75% higher than from tilled soils (0.20 µn m^{-2} g^{-1} h^{-1}). The emission from surface and subsurface layers also exhibited significant variation ($F_{1,5} = 6.26$, $P < 0.05$). In general surface emission was 59% greater than from subsurface. There was increased N_2O flux from zero tilled soil (0.92 ng m^{-2} g^{-1} h^{-1}), although not significantly different (Table 5.3, $F_{1,5} = 1.49$, $P > 0.05$). Soil depth and its interaction with tillage did not affect the N_2O flux significantly.

When all the greenhouse gases were considered together the global warming potential was significantly higher from tilled soil (126 µg m^{-2} g^{-1} h^{-1}) than from zero tilled soil (74 µg m^{-2} g^{-1} h^{-1}) (Table 5.3, $F_{1,5} = 6.87$, $P < 0.05$). Tilled soil caused 41% higher warming than zero tilled soil on a CO_2 equivalent basis. The surface (0-10 cm) soil layer caused significantly higher warming than subsurface layer (10-20 cm) in both zero tilled and tilled soils ($F_{10} = 14.58$, $P < 0.01$). The surface layers caused 21% and 18% higher warming compared to subsurface layers in zero tilled and tilled soils respectively.

5.3.2 Soil biological properties

5.3.2.1 Microbial biomass carbon and nitrogen

Zero tillage increased microbial biomass C in soil significantly ($F_{1,5}$ = 10.88, P <0.05). Zero tilled soils contain as much as 30% higher microbial biomass C (538 mg kg^{-1} soil) than tilled soils (377 mg kg^{-1} soil) (Table 5.1). Depth of soil sampling also significantly influenced the microbial biomass C ($F_{1,10}$ = 20.61, P <0.001). The surface soils (0-10 cm) had 35% and 23% higher microbial biomass C than 10-20 cm subsurface layer in zero tilled and tilled soils respectively. Microbial biomass nitrogen also followed a similar trend to that of C (Table 5.1). There was a significant effect of both tillage ($F_{1,5}$ = 5.6, P <0.05) and depth ($F_{1,10}$ = 13.29, P <0.05) for microbial biomass nitrogen.

5.3.2.2 Soil microbial functional diversity

AWCD values in soil significantly increased with incubation time indicating the presence of active microbial flora in both zero tilled and tilled soils (P <0.001, Figure 5.3). Significantly increased AWCD values (P <0.05) were recorded for zero tilled soils (0.46) compared to the tilled soils (0.39). The surface 0 - 10 cm layer recorded the highest AWCD values in both zero tilled (0.50) and tilled soils (0.42) compared to subsurface 10 - 20 cm layer (0.43 in zero tilled and 0.35 in tilled) (P <0.05). Principal component analysis did not provide a clear separation of C substrate utilisation among different treatments.

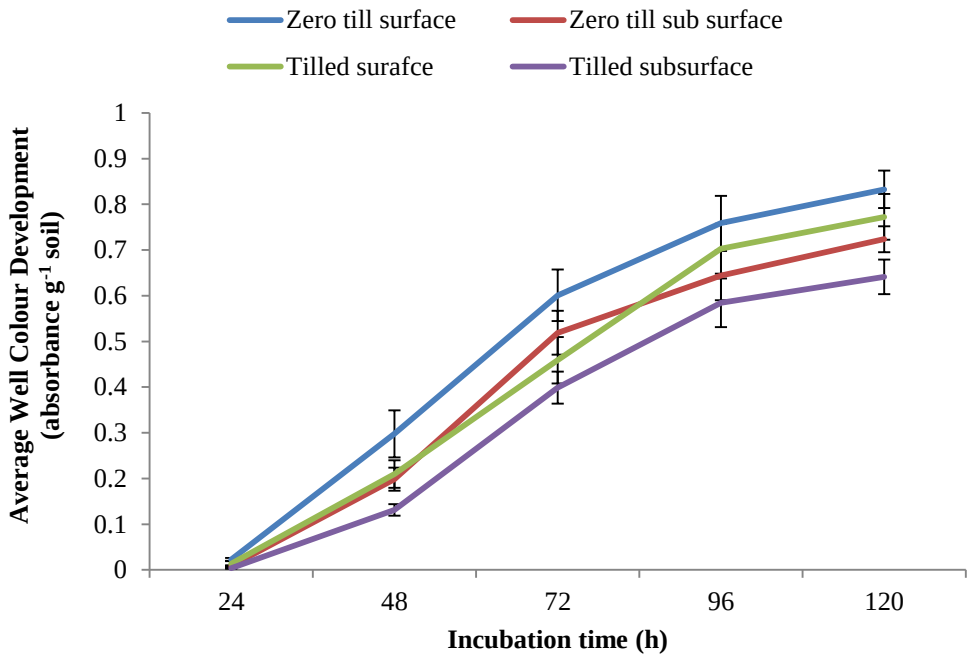

Fig. 5.3. Average Well Colour Development (AWCD) obtained by Biolog ecoplates. Error bars indicate standard error of means (n=6).

5.3.2.3 Soil enzymatic activities

Zero tilled soils had higher dehydrogenase activity (1.46 µg TPF g^{-1} h^{-1}) compared to tilled soils (0.91 µg TPF g^{-1} h^{-1}) ($F_{1,5}$ = 19.54, P <0.01). The surface 0-10 cm soil layer showed greater dehydrogenase activity than the subsurface layer (10-20 cm) in both zero tilled and tilled soil, but the effect was more prominent in tilled soils (Fig. 5.4a, $F_{1,10}$ = 148.08, P <0.001). Similar to dehydrogenase, zero tilled soils exhibited significantly increased cellulose activity (Fig. 5.4b, $F_{1,5}$ = 21.98, P <0.01) with mean values of 0.33 mg GE g^{-1} day^{-1} compared to 0.14 mg GE g^{-1} day^{-1} in tilled soils. Of the two soil depths studied the 0-10 cm layer recorded significantly higher cellulose activity in both zero tilled and tilled soil than 10-20 cm layer ($F_{1,10}$ = 24.42, P <0.001).

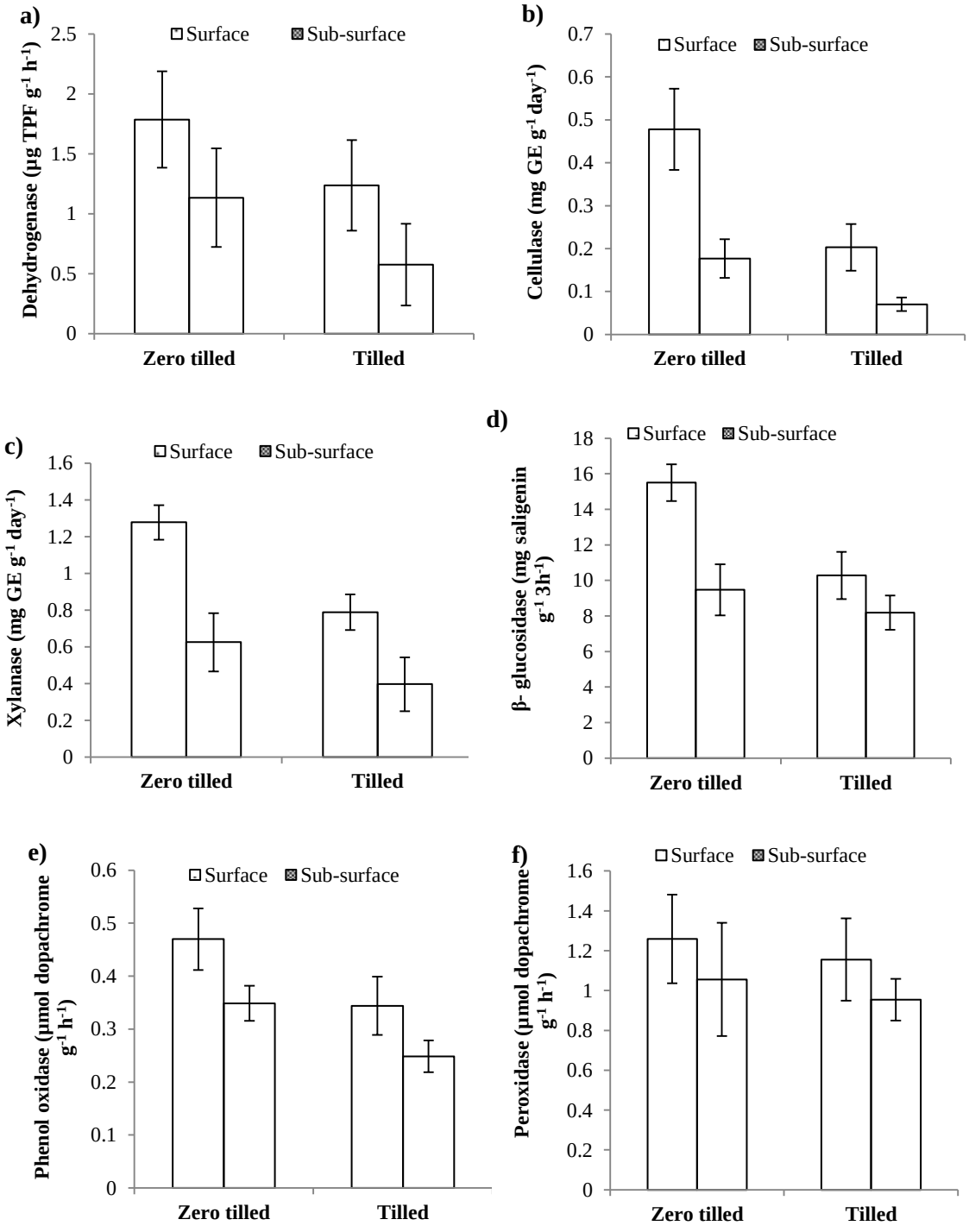

Fig. 5.4. Soil enzymes at surface (0-10 cm) and sub surface (10-20 cm) layers under zero tilled and tilled soils; (a) dehydrogenase, (b) cellulase, (c) xylanase, (d) β-glucosidase, (e) phenol oxidase and (f) peroxidase.

Xylanase activity followed a similar pattern to dehydrogenase. Zero tilled soil contained 38% higher activity than tilled soils (Fig. 5.4c, $F_{1,5} = 8.34$, $P < 0.05$). The upper 0-10 cm soil layer recorded increased xylanase activity than the subsurface 10-20 cm layer ($F_{1,10} = 21.95$, $P < 0.001$). The zero tilled upper layer contained 1.28 mg GE g^{-1} day^{-1} of xylanase activity which was 51% higher than 10-20 cm layer. In tilled soil surface activity was 0.79 mg GE g^{-1} day^{-1} that was higher by 49% above the 10-20 cm subsurface layer.

Tillage also significantly influenced the β-Glucosidase activity in soil with zero tilled soil recording an activity of 12.5 mg saligenin g^{-1} $3h^{-1}$ which was 26% higher than under tilled soil (Fig. 5.4d, $F_{1,5} = 14.28$, $P < 0.05$). Glucosidase activity was significantly higher in the 0-10 cm layer than the 10-20 cm layer ($F_{1,10} = 18.06$, $P < 0.01$). The surface increase was 39% in zero tilled soil and 20% in tilled soil. The tillage depth interaction was also significant ($F_{1,10} = 4.24$, $P < 0.05$).

Phenol oxidase activity was significantly affected by tillage ($F_{1,5} = 31.49$, $P < 0.01$) and depth ($F_{1,10} = 30.27$, $P < 0.001$), but the tillage depth interaction was not significant (Fig. 6.4e, $F_{1,10} = 0.42$, $P > 0.05$). The surface (0-10 cm) activity in zero tilled soil was 0.47 µmol dopachrome g^{-1} h^{-1} which was 26% higher than the activity in the 10-20 cm layer. The surface activity in the 0-10 cm layer in tilled soil was 28% higher than the 10-20 cm layer. However the tillage x depth interaction was not significant. There was no significant effect of either tillage or depth on the peroxidase activity in soil. However the activities were higher under zero tilled conditions and in the surface layers.

Soil enzymes were evaluated on, a per gram of carbon and a per microbial biomass carbon in soil basis to find out if the activity was due to increased availability of carbon substrates. These results followed a very similar pattern to that of enzymes

reported on a per soil basis indicating tillage also plays an important role in the activity of soil enzyme activities above and beyond soil C availability and its impact on the microbial biomass.

5.3.3 Factors affecting carbon content in soil

Carbon content in soil was predicted by a multiple regression model ($F_{5,18}$ =32.9, P < 0.001) including β-glucosidase (BG), dehydrogenase (DH), xylanase (X), soil water content (M) and clay content in soil (Clay) which accounted for 90.1% of the variation. The optimal model for C is provided in equation 1.

$$C\ (\%) = 0.981 - 0.00818BG + 0.1351DH + 0.3382X - 0.01462M + 0.01452Clay \quad (1)$$

In this model the soil clay content contributed to 19.1% of variation, estimated by dropping the parameter when fitted last from the model. The rest of the variation can be attributed to the soil enzymes and soil moisture availability (Figures 5.5a, 5.5b, 5.5c and 5.5d). However linear regression showed that, individually soil moisture content was not related to soil C (P <0.05). The multiple regression analysis of GHGs against different soil enzymes and other properties could not establish a significant effect between them.

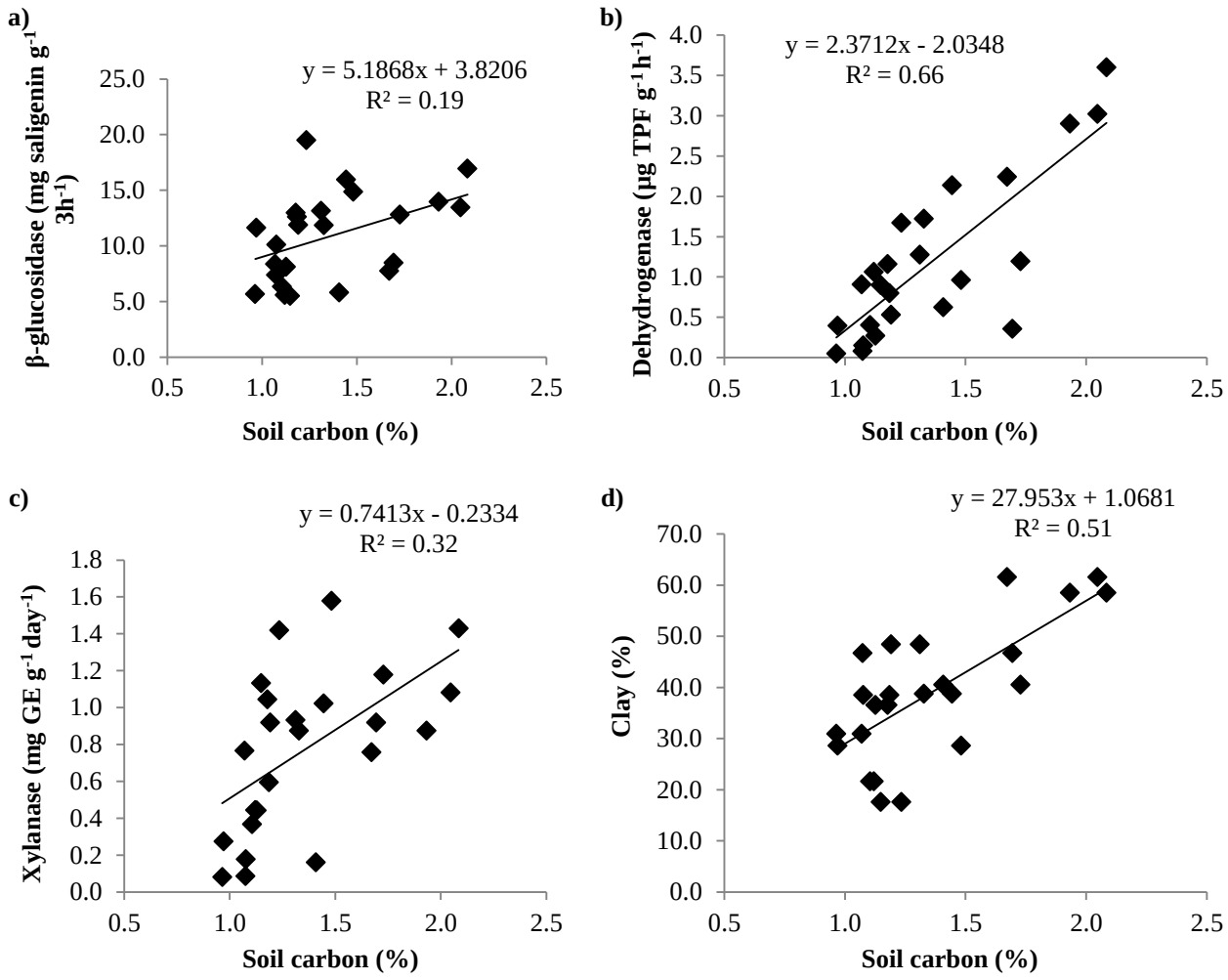

Fig. 5.5. Illustration of relationships between soil biophysical properties and soil C (a) β-glucosidase and soil C content; $F_{1,22}$=5.26, P <0.05 (b) dehydrogenase and soil C; $F_{1,22}$=41.91, P <0.001 (c) xylanase and soil C; $F_{1,22}$=10.27, P <0.01 (d) soil clay content and soil C; $F_{1,22}$=22.89, P <0.001.

5.4 Discussion

Zero tillage sequestered C both in the 0-10 cm and 10-20 cm soil layers, 9% over 7 years for total C. Ernst et al. (2009) found 8% higher total soil C under no-tilled soil than conventionally tilled soil within 12 years. Plaza et al. (2012) reported 16% more

organic C under no-tillage soils of 25 years than conventionally tilled soils. They attribute the increased C at surface to the retention of crop residues at surface layers and enhanced C at subsurface layers due to decomposition of root biomass left in soil year after year. The increased root biomass under zero tillage might also be due to the improved root growth by conserving soil moisture and regulating the soil temperature through the effect of crop residues left at surface, apart from providing nutrients by decomposition of crop residues left at surface (Acosta-Martínez et al., 2011). Sainju et al. (2005) reported increased root biomass and C and N accumulations at 0-15 cm depth in cotton and sorghum in zero tilled plots. In contrast to zero tilled sites the reduced contents of C and nitrogen in the tilled soils can be attributed to the increased decomposition of soil organic matter consequent to ploughing. The C protection in soil is also dependent on the form in which it is stored in soil. In this study zero tilled soils contained significantly higher absorption intensities for wave numbers corresponding to the functional groups such as aromatics, amines and carboxylic acids. Aromatic and alkyl C in soil are considered as a relatively recalcitrant fraction of soil C (Baldock et al., 1992). The accumulation or preservation of aromatics may be due to the preservation of lignin during decomposition of crop residues, where the quantity of crop and root residues are higher at the surface and subsurface layers in zero tilled soils compared to a tilled soil. Gregorich et al. (2001) reported increased aromatic C contents under legume based rotation compared to maize based monoculture in Ontario, Canada. These aromatic structures of C which are mainly plant derived (Krull et al., 2003) play an important role in the net C sequestered in soils owing to the higher turnover times of 10 to 100s of years as reported by Jenkinson et al. (1990). It indicates that increased C sequestration capabilities under zero tilled soils are also related to the chemical structure of the organic matter.

Absence of soil cultivation under zero tillage is beneficial to providing a continuous supply of organic materials to soil microorganisms and is reflected in the increased microbial biomass C and biomass nitrogen in zero tilled soils (Balota et al., 2003). Increased microbial activities under zero tilled soils were also evident in terms of the enzymatic activities which were higher under zero tilled soils than tilled soils and has been observed by others (Roldán et al., 2005; Melero et al., 2009). Acosta-Martinez et al. (2008) attributed the increased enzyme activities under non disturbed pasture soil to either the presence of active microbial biomass constituting intracellular enzymes or to extracellular enzymes which remained part of soil organic matter or both of these. Due to lack of disturbance in zero tilled soils, the biochemical environment is less oxidating compared to soils that are ploughed (Melero et al., 2009). The surface accumulation of crop residues and subsurface supply of organic materials through root biomass in zero tilled soils could further enhance the enzyme effect. A stable pool of enzymes are preserved in most humified organic portions by bonding soil enzymes to humic colloids and clays (Trasar-Cepeda et al., 2008). Soil dehydrogenase enzyme is linked to the C cycle and its increased presence under zero tilled soils indicates more water soluble C fractions under this management (Roldán et al., 2005).

The enzymes involved in C metabolism (cellulose, xylanase, β-glucosidase) were positively correlated with C content and microbial biomass C; this was also observed by Katsalirou et al. (2010) for cellulose and β-glucosidase. The increased activities of enzymes such as cellulose, xylanase and β-glucosidase in zero tilled soils indicate the predominance of microbes involved in degradation of cellulose and other polysaccharides. These enzymes act upon the polysaccharides in crop residues and root biomass and convert them into soil humus and recalcitrant C in different soil

aggregates and thus helping to sequester C in soil, apart from helping to release nutrients for plant uptake (Alvear et al., 2005). In other words it can also be stated that under zero tilled conditions, a pool of organic matter might be generated in which soil hydrolytic enzymes could be stabilised (Trasar-Cepeda et al., 2008) and so both soil enzymes and soil C are protected (Martens et al., 1992)

Lignin and other hydro C's in plant residues are regarded as an important rate limiting factor in the later stages of litter decomposition and subsequent sequestration of C in soil by the transfer of plant C to soil organic matter (Burns et al., 2013). Lignin degradation is brought about by oxidative enzymes such as phenol oxidase and peroxidase enzymes produced mainly by fungi. The lignin degradation by these enzymes leads to humification (Jastrow et al., 2007) leading to the formation of stable C compounds from plant remains. Increased activities of these enzymes in zero tilled soil were attributed to the absence of soil disturbance which allow fungal hyphae to make bridges of between soil and crop residues (Holland et al., 1987). Fungi are primarily responsible for the degradation of resistant components of organic matter in crop residues. They also form bridges between soil and crop residues which is important in zero tilled where soil-crop residues mixing is minimal. The resistant components of fungal cell walls such as chitin and melanin brought back to soil on fungal lysis may also be responsible for increased C sequestration due to their resistance to degradation. The increased activities of phenol oxidase under zero tilled conditions indicate zero tilled soils are more capable of sequestering C compared to tilled soils. The positive correlations between cellulase and phenol oxidase indicate that both hydrolases and oxidases are active in tilled and zero tilled soils owing to the availability of microbial resources for these different group of enzymes. The multiple regression models showed that soil enzymes such as β-

glucosidase, dehydrogenase and xylanase significantly contributed to the carbon model showing the importance of extracellular enzymes in converting C in crop residues to the soil C.

AWCD values also serve as good indicator of microbial activity (Mijangos et al., 2009). Increased AWCD values in zero tilled soils indicated higher metabolic activity in these soils to convert the available organic substrates into soil C. The reduced microbial functional diversity under tilled conditions were attributed to soil disturbance that adversely affect the soil organisms (Lupwayi et al., 2001). However the AWCD values were not correlated with soil organic matter or microbial biomass C, implying in part that the microbial community in soil was changed during the course of different soil management practices as has been observed by Wang et al. (2007). It may also be due to the inability of Biolog plates to include the whole microbial community (Waldrop et al., 2000). The substrate utilisation pattern provided by Biolog plates mainly account for fast growing aerobic bacteria (Govaerts et al., 2007). The positive correlation of AWCD values with enzymes such as cellulose and xylanase, indicating the physiological state of microbial cells (Alarcón-Gutiérrez et al., 2009). The AWCD values were not correlated to other enzymes such as phenol oxidase, peroxidase etc. which is attributable to the fact that biology plates cannot detect fungi as they are incapable of reducing tetrazolium violet used in the plates (Mijangos et al., 2009). However in contrast to the enzyme analysis, Biolog was unable to provide a clear treatment effect with regard to substrate utilisation among different functional groups.

The multiple regression analysis indicated that apart from soil enzymes, texture played a pertinent role in C sequestration. Clay soil tends to store more carbon since clay content significantly contributed to the total variation, indicating that higher

carbon sequestration is possible under fine textured soils than coarse textured soils. The increased C sequestration capacity of clay soils may be due to the possibility of absorption of organic carbon to clay surfaces, entrappment of carbon on pores of aggregates or encapsulation of organic carbon by clay particles (Nyamadzawo et al., 2009). Six et al. (2000a) proposed aggregate turnover is reduced under zero tillage leading to formation of stable micro aggregates. Under less disturbed conditions the microbial products might be better preserved in stabilised micro (<53 μm) and macro aggregates (<250 mm) (Powlson et al., 1981; Nyamadzawo et al., 2009). In contrast, tillage causes aggregates to breakdown (Six et al., 2000a) and increase soil temperature (Yang et al., 2008) both of which trigger microbial decomposition of soil organic matter leading to reduced sequestration of C and nitrogen. Tillage mediated aggregate changes might bring changes in carbon storage in soil depending on texture of soil as reported by Mangalassery et al. (2013).

Even though C content and microbial activities were higher under zero tilled soils, CO_2 flux, which should reflect the respiration status of soil, was lower under zero tilled conditions compared to tilled soils. The CO_2 flux in soil reflects overall respiration which includes soil fauna such as nematodes and other invertebrates. Under tilled conditions with continuous soil disturbance, the components of macro and micro fauna may be in a state of stress compared to the more stabilised and steady environment under zero tilled conditions. This might have enhanced the CO_2 flux from tilled soils apart from increased decomposition of crop residues and soil organic matter at a faster rate. Under zero tilled soils, carbon can be protected from the microbial activity, perhaps by forming stable microbial products of decomposition, whereas in tilled soils more readily degradable C is available for microbial action.

CH_4 fluxes in soil are related to the aeration status of soil. The increased compaction and moisture under zero tilled conditions creates more anaerobic conditions (Kimura et al., 2012) that might favour methanogenic microbes. Kiener et al. (1983) found many methanogens can survive several hours or longer on exposure to air. Negative CH_4 values indicating CH_4 oxidation/uptake in soil was recorded at only two locations under tilled sites in this study and this may be due to increased aerobic condition to which methane oxidisers responded quickly (Sey et al., 2008). There are many reports of increased N_2O emission under zero tillage compared to under conventional tillage (Ball et al., 1999; Chatskikh et al., 2007). This has been attributed to decreased water filled pore space, mineral nitrogen concentration (Oorts et al., 2007), reduced gas diffusivity and air-filled porosity (Chatskikh et al., 2007), increased water content (Blevins et al., 1971) and a denser soil structure (Schjønning et al., 2000; Beare et al., 2009).

This chapter addressed the sub aims 3 and 4 and investigated how effective zero tillage is in mitigating climate change when compared to tilled soil. This chapter demonstrate that the Carbon sequestration capabilities under zero tillage is both physically and microbially mediated as in the overarching hypothesis.

5.5 Conclusions

Tillage plays a major role in sequestration of C and emission of greenhouse gases. It was found that soils farms following zero tillage for 7 years had 9% higher C than tilled soil. The reduction of tillage can enhance sequestration of C by increased microbial activities and soil enzymes and accumulation of C in recalcitrant forms. The increased C sequestration capabilities are linked to the soil physical characteristics such as clay content and microbial properties. In contrast soil tillage enhanced decomposition of organic matter and emission of CO_2. Even with increased

emission of CH_4 and N_2O, the net warming potential was significantly reduced under zero tilled soils. The study indicates that zero tillage is beneficial for improving soil health and preserving soil organic matter.

Acknowledgements

S Mangalassery acknowledges the research funding by the Indian Council of Agricultural Research, New Delhi through International Fellowship programme and the University of Nottingham through Research Excellence Scholarship.

List of tables

Table 5.1. Microbial biomass C (MBC), microbial biomass N (MBN) and total C and N at surface (0-10 cm) and subsurface (10-20 cm) layers under zero tilled and tilled soils*.

Tillage	Depth (cm)	MBC	MBN	Total C	Total N
		mg kg^{-1} soil		%	%
Zero tilled	**0-10**	650±104	110.4±20	1.53±0.14	0.301±0.04
	10-20	425±69	66.4±15	1.32±0.14	0.202±0.02
Tilled	**0-10**	425±66	61.9±11	1.41±0.16	0.175±0.02
	10-20	328±67	46.3±11	1.18±0.10	0.149±0.02

***Mean**±Standard Error (n=6)

Table 5.2. F statistic from analysis of variance for the absorbance at different wave numbers

Wave number	Tillage	Depth	Tillage x depth	Functional group
2925	1.99 (5) ns	1.29(10) ns	0.09 (10) ns	Aliphatics
2850	0.13 ns	1.93 ns	0.07 ns	Aliphatics
1801	0.0 ns	0.49 ns	0.30 ns	C-O, C=O or N
1799	0.0 ns	0.5 ns	0.27 ns	C-O, C=O or N
831	5.13 ns	0.55 ns	0.15 ns	CH_2, Aromatic
829	5.16 ns	0.52 ns	0.25 ns	CH_2, Aromatic
827	5.17 ns	0.51 ns	0.34 ns	CH_2, Aromatic
825	5.32 ns	0.50 ns	0.48 ns	CH_2, Aromatic
823	5.55 ns	0.48 ns	0.62 ns	CH_2, Aromatic
821	5.85 ns	0.50 ns	0.76 ns	CH_2, Aromatic
819	6.1 ns	0.58 ns	1.02 ns	CH_2, Aromatic
761	2.06 ns	0.55 ns	2.58 ns	Aromatics
759	2.01 ns	0.66 ns	2.70 ns	Aromatics
711	10.11*	10.19**	0.69 ns	Aromatics
709	8.23*	9.06*	0.75 ns	Aromatics
671	0.45 ns	0.76 ns	0.93 ns	Aromatics
669	0.40 ns	1.1 ns	0.78 ns	Aromatics
665	0.88 ns	1.09 ns	0.09 ns	Aromatics
651	0.51 ns	3.57 ns	1.73 ns	Aromatics
649	0.36 ns	3.75 ns	2.07 ns	Aromatics

(Figures in parenthesis indicate degrees of freedom), NS: non-significant.
*** $p < 0.001$.
** $p < 0.01$.
* $p < 0.05$.

Table 5.3. CO_2 flux, CH_4 flux, N_2O flux and net global warming potential at surface (0-10 cm) and subsurface (10-20 cm) layers under zero tilled and tilled soils*.

Tillage	**Depth (cm)**	**CO_2-C flux**	**CH_4-C flux**	**N_2O-N flux**	**Net warming potential**
		µg m^{-2} g^{-1} h^{-1}	**ng m^{-2} g^{-1} h^{-1}**		**µg m^{-2} g^{-1} h^{-1}**
Zero tilled	**0-10**	3.78±0.67	1.098±0.23	1.03±0.64	83.57±14.80
	10-20	2.98±0.43	0.593±0.16	0.8±0.22	65.86±9.45
Tilled	**0-10**	6.29±1.01	0.388±0.34	0.71±0.26	138.61±22.22
	10-20	5.17±1.23	0.021±0.24	0.46±0.20	113.76±27.09

***Mean**±Standard Error (n=6)

6 Chapter 6: General discussion and conclusions

6.1 Introduction

The main objectives of this research were to establish the physico-chemical and biological basis of greenhouse gas emissions under different crop cultivation regimes namely conventional and zero tillage and to assess the climate mitigation opportunities of zero tillage. Initially, the project evaluated conventional tillage against soil managed as part of a grassland stewardship programme as a pilot study. Later, the impact of soil aggregate sizes (<0.5 to 4 mm), so as to mimic the effect of tillage on greenhouse gas flux was studied. The subsequent experiment studied how the soil biophysical properties such as soil pore structure affected GHG emissions. Finally a detailed characterisation of the functional composition and microbial functional diversity of SOM to assess the carbon sequestration potential was undertaken.

6.2 Effect of tillage/zero tillage on physico-chemical properties

After 5 to 10 years under zero tillage, it is clear that the soil surface becomes higher in soil shear strength compared to conventional tillage. The increased soil bulk density following adoption of conservation tillage has been widely reported. Dam et al. (2005) reported a 12.4% increase in bulk density in zero tilled soils of 11 years over tilled soil. With residues left at the surface of zero tilled soil, one might expect a lower bulk density compared to tilled soil, but this was not the case in this project as the tillage impact outweighed the residue effect. However over a longer term the residue retention might support a reduced bulk density (Lal et al., 1994).

Neither increased bulk density nor soil strength under zero tillage limited water storage in soil as significantly higher volumetric soil moisture content was found in

zero tilled soils compared to tilled soils. Higher water contents under zero tilled fields may have been influenced by enhanced retention of crop residues. Crop residues can reduce surface runoff, evaporation losses and increase water infiltration (Lampurlanés et al., 2006; Yoo et al., 2006). Also the continuity of macro pores can be destroyed by tillage, which restricts water movement from surface to the subsurface (Osunbitan et al., 2005).

X-ray CT data indicated greater macro porosity under tilled soils as tillage loosens soils and physically creates more macropores (Gupta et al., 1986; Gantzer et al., 2002). However, Zhou et al. (2009) found no difference in total soil porosity between tilled and untilled plots (21 years of no-tillage) at 0-20 cm layer, while reporting increased macro porosity in the surface layers (0-10 cm) of tilled soil. While porosity was higher under tilled soils, the pore connectivity and pore size distribution was better in zero tilled condition as indicated by the enhanced water retention. The packing of soil aggregates is responsible for the gross soil porous architecture. In the soil column study (Chapter 3), total soil porosity increased with decreasing aggregate size in clay loam soil whereas the reverse was observed for sandy loam soil. With a reduction in aggregate size, the number of pores increased in both clay loam and sandy loam soils whereas surface area of pores and aggregate size followed a linear trend.

Soil texture and aggregate size significantly influenced the soil carbon content. Micro-aggregates contained higher organic matter in clay loam soil, due to their ability to conserve organic matter (Papadopoulos et al., 2009) and lower turnover (Six et al., 2002). Micro-aggregates offer protection of organic matter from degradation due to reduced access to soil bacteria and strong bonding of SOC to these

aggregates by sorption (Lugato et al., 2009). In the sandy loam soils large sized aggregates had the highest organic matter as reported by Fernandez et al. (2010).

6.3 Physical and microbial basis of carbon sequestration in soil

The higher soil organic matter content under zero tilled soils reported in this study could be due to increased residue retention on soil surface and/or non-disturbance of soil. The crop residues left at surface might have been slowly decomposed and contributed to the carbon pool in soil. Under reduced tillage, the turnover of soil aggregates is minimised compared to tilled soils which may protect carbon within aggregates (Six et al., 1999). Sequestration of carbon occurs in soil when carbon is protected from decomposition, with soil physico-chemical and biological properties playing a significant role in this. Soil aggregation significantly influences the carbon storage in soil (Six et al., 1999). It was shown here that both soil texture and soil management significantly influenced the soil organic carbon stock with more soil organic carbon stock under clay loam soil than sandy loam soil, similarly under grassland soil than tilled soils. Zero tilled soils and surface layers contained more soil carbon than tilled soil and subsurface layers as expected. Disturbance of soil is believed to enhance macroaggregate turnover leading to decomposition of protected soil organic matter (Six et al., 1999). They also reported slower turnover of carbon in micro aggregates compared to macro aggregates. This was also the case in this study where the smallest aggregates (<0.5 mm) in clay loam soil had the highest organic matter (8.7%) with the lowest SOM in the field structured soil (7.5%). In sandy loam soil the larger aggregates (1-4 mm) contained more organic matter (5.2 and 3.5% respectively) than small aggregates (<0.5 mm). The textural differences in carbon storage can be attributed to the differences in bonding mechanisms. The C sequestration capacity of clay loam soil is greater than in sandy soil due to absorption

of organic carbon to clay surfaces, entrapment of carbon on pores of aggregates or encapsulation of organic carbon by clay particles (Nyamadzawo et al., 2009).

Changes in soil microbiological properties brought by management practices play a major role in soil carbon storage, this is evident from the positive relationship between soil carbon and microbial biomass carbon. With soil management practices which input more crop residues to the soil such as grass land and zero tillage, microbial populations were higher. The microbial biomass carbon reported in this study were comparable to other studies (Alvarez et al., 1995). Under reduced disturbance systems a stable pool of extra cellular hydrolytic and oxido-reductases are also preserved. These enzymes act on crop residues and convert the carbon in crop residues and root biomass to soil humus and recalcitrant carbon, thus helping to sequester carbon. The absence of soil disturbance is also beneficial to extract and preserve carbon from resistant products of decomposition of crop residues. Lack of soil disturbances provide stabilised activity for soil microorganisms especially fungi which are important in degrading the resistant components of crop residues and are therefore helpful in extracting carbon which are preserved in soil aggregates.

6.4 Climate change mitigation under zero tillage

It was shown that changes in soil physico-chemical and biological properties, as affected by tillage, strongly influenced GHG fluxes. CO_2 fluxes were positively related to soil porosity and microbial biomass carbon and negatively to bulk density. The effect of soil pore characteristics on CO_2 flux has not previously been studied and it is striking, but perhaps as expected, to observe its influence from a soil management perspective. Similar to these findings Reicosky et al. (1997) observed that conservation tillage slowed down CO_2 release as air-filled porosity was reduced. Whereas in tilled soils the aggregates are protected and organically bound organic

matter is released upon tilling the soil, by increased aeration and microbial activity leading to increased emission of CO_2 (Elder et al., 2008). CH_4 emission was negatively influenced by soil strength indicating longer retention of CH_4 in zero tilled soils produced in anaerobic micro sites which leads to its oxidation by methanotrophs (Smith et al., 2001). Lower CH_4 oxidation in less porous soils has also been reported by Dutaur et al. (2007). In contrast in tilled soils the CH_4 will find an easier diffusion route before being subjected to oxidation. For undisturbed field core samples used for experiment in Chapter 4, increased emission of CO_2 and CH_4 was recorded in tilled soils whereas N_2O emission was higher from zero tilled soil. For disturbed samples used in experiment in Chapter 5, CO_2 flux was higher from tilled soil, but CH_4 and N_2O from zero tilled soil. For undisturbed samples CO_2 flux was higher by 17.5% in tilled soils compared to zero tilled soils, whereas for disturbed soil it was 40.9%. These differences may be due to the effect of soil structure and pore characteristics which influence the gas flux and are not considered in disturbed samples. Soil moisture, microbial biomass carbon and nitrogen were the main factors contributing to enhanced N_2O emission in zero tilled soils. Zero tilled soils are reported to emit more N_2O compared to conventionally tilled soils (Oorts et al., 2007; Regina et al., 2010). Anaerobic conditions necessary for the production of N_2O through denitrification are more readily available under zero tilled than tilled conditions apart from the increased N input. This is due to increased water storage indirectly facilitated by enhanced organic matter and increased soil firmness due to the absence of cultivation. Increased soil organic matter under zero tilled soil also favours a higher N_2O flux by providing substrates for denitrifying bacteria (Choudhary et al., 2002).

In addition to chemical and biological properties, the physical properties of soil such as porosity and soil strength also play an important role in fluxes of CO_2 and CH_4, although for N_2O flux the role played by physical properties is secondary compared to chemical and biological properties. The direct dependence of GHG flux as depicted in Fig. 4.11, on soil pore characteristics were not reported before. This relationship of gas flux with porosity stronger than with microbial biomass carbon and total carbon might be linked to the increased activities of soil organisms, facilitating water and air movement under increasingly porous conditions. When all the greenhouse gases were considered together (by converting into global warming potential in CO_2 equivalent), tilled soil produced 26% higher warming (on a weight basis) than zero tilled soils indicating the relative advantage of zero tilled over tilled soil in climate change mitigation. Increased global warming potential under tilled conditions has been reported by others also. Ussiri et al. (2009) reported 51 to 58% global warming potential under zero till system compared conventional tillage with a lower total N_2O flux under 43 years of zero tillage.

6.5 Zero tillage on soil biological properties

Tillage practices significantly influenced various biological properties in soil. Untilled grassland soils contained 40.4% higher microbial biomass carbon in clay loam soil and 28.3% higher in sandy loam soil in the upper 10 cm layer than the tilled cultivated fields. On comparing zero tilled soils of different duration and soil texture with the adjacent tilled fields, the zero tilled soils showed higher microbial biomass carbon both at the surface and subsurface layers. The increment in microbial biomass carbon in the surface 0-10 cm layer in zero tilled soil compared to tilled soil ranged from 26.5 to 34.6% and in subsurface 10 to 20 cm layer it was 8.98 to 22.8%. The increased microbial biomass carbon under zero tilled soils can be attributed to the

increased accumulation of labile organic carbon in soil (Purakayastha et al., 2009), more stable soil aggregation and increased soil moisture content in zero tilled soils (Balota et al., 2003). When the microbial biomass carbon data for the two sampling years were compared, in zero tilled soil the microbial biomass carbon was increased by 10% at surface whereas in tilled soils it was decreased by 2%. Similar to microbial biomass carbon, the microbial biomass nitrogen was higher under zero tilled soils and the annual effect was also similar . Soil enzymatic activities were higher under zero tilled soils. This may be attributed to the increased carbon availability at the surface and root organic matter in the subsurface soil meeting the substrate requirements of a diverse microbial flora in zero tilled soils compared to a few dominant microbial types in tilled soil. The microbial decomposition products are re-oriented into soil aggregates where they are protected from further decomposition in the absence of soil disturbances. The increased soil enzyme activities in zero tilled soils indicate a predominance of microbes involved in degradation of a variety of components in plant and root residues and ultimately their addition to the soil carbon pool.

Even though C content and microbial activity were higher under zero tilled soils, CO_2 flux, which should reflect the respiration status of soil, was lower under zero tilled conditions compared to tilled soils. The CO_2 flux in soil reflects overall respiration which includes soil fauna such as nematodes and other invertebrates. Under tilled conditions with continuous soil disturbance, the components of macro and micro fauna may be in a state of stress leading to enhanced respiration compared to a stabilised and steady activity under zero tilled condition. This might have enhanced the CO_2 flux from tilled soils apart from increasing decomposition of the crop residues and soil organic matter at a faster rate.

6.6 Conclusions

The major conclusions from this research are:

- Tilled soils emitted more CO_2 (21% higher) and CH_4 (58%) when intact soil cores were sampled, whereas in a disturbed condition, CO_2 flux alone was higher (41%) from tilled soil. N_2O flux was always higher from zero tilled soils compared to tilled soil but in all cases, the zero tilled soils had a lower net emission of greenhouse gases on a CO_2 equivalent basis (20% higher under tilled soil) indicating potentially that zero tillage can be used to mitigate climate change in comparison to conventional tillage.
- Soil pore characteristics such as porosity and pore size played a significant role in the emission of greenhouse gases such as CO_2 and CH_4 amongst other factors such as microbial biomass carbon, bulk density and shear strength. Soil porosity alone accounted for 39.7% of the variation in the CO_2 flux, larger than any other parameter including microbial biomass carbon and soil carbon. This indicates that soil pore characteristics under the influence of soil management are a significant factor controlling greenhouse gas emissions. N_2O emission from soil can be largely explained by soil moisture, microbial biomass carbon and microbial biomass nitrogen.
- Continuous zero tillage increases soil bulk density and soil strength up to 20 cm depth of soil compared to tilled soils. However, when a comparison was made between a tilled soil and a grassland soil, the greater increased bulk density was recorded with tilled soil.
- Tilled soils are more porous (36% higher) compared to zero tilled soils. Tilled soils contained larger pores (0.52 mm^2).

- The texture of soil and size of aggregates play a crucial role in deciding the soil pore characteristics such as number of pores, pore size, porosity and pore area. Number of pores were higher in clay loam soil and located in smaller sized aggregates. Increased porosity (intra-aggregate) was observed with small sized aggregates in clay loam soil and with large sized aggregates in sandy loam soil. This subsequently impacted on GHG release.
- Soil aggregate size significantly influences soil organic matter retention. Among various aggregate sizes the smallest aggregates (>0.5 mm) contained more organic matter in a clay loam soil and in 1-2 mm and 2-4 mm in sandy loam soils.
- Zero tilled soils were more microbiologically active compared to the tilled soils and sequestered more carbon not only at surface 0-10 cm, but in 10-20 cm layer as well. Increased microbial and soil enzymatic activity was recorded in zero tilled soils which might have also influenced the sequestration of carbon in soil. The non-disturbance of soil might have facilitated stable microbial activity in zero tilled soils leading to the preservation of C in recalcitrant forms as observed by the increased presence of aromatic compounds in FTIR.
- Continuous availability of organic materials under zero tillage led to increased microbial biomass carbon and microbial biomass nitrogen at the surface and subsurface layers which bears a positive relationship with organic matter content in the soil. Zero tilled soils contained 16% higher SOM than tilled soil.

6.7 Future work

Some potential future directions of work as a continuation to this research are indicated below.

6.7.1 Studies on soil pore characteristics on micro scale

Our study used micro Computed Tomography for the characterisation of the soil pore network. Since we have used larger soil cores to better represent the field conditions, the resolution of CT scanning was compromised. The lowest resolution used in the study was 27.5 µm which means, in these images, only the pores larger than this size are only accounted for and the finer micro-pores are not included. However, these finer micro-pores are also likely to play an important role in soil physico-chemical and biological properties such as CH_4 and N_2O emissions as well as C stabilisation.

6.7.2 In situ studies in the field involving crop component

The work undertaken here was largely based in the laboratory, although the experiment in Chapter 5 was based on intact core sampled in the field. The results from this study need to be extended to the field, assessing the temporal and spatial variability in relation to different crops, soil textures and seasons in order to study the climate change mitigation potential of zero tillage. The introduction of a crop component will be helpful to allow detailed characterisation of inflow and outflow of organic matter in soil and make the data directly relevant to the farm situation. Simultaneous field measurement of GHG and soil structure in situ could be beneficial in precisely assessing the gas flux dynamics.

6.7.3 Effect of conservation tillage on crop yields vis a vis climate change mitigation

An important aspect to be considered while studying the climate change mitigation opportunities of conservation tillage is to take into account the yield decline or improvement by such practices. Although long term studies investigating the impact of conservation tillage on crop yield have been carried out in many parts of the world, integrated location specific studies combining the climate change aspect and crop yield are urgently needed.

6.7.4 Carbon sequestration

The obvious advantage of sequestration of carbon under zero tilled soil in comparison to tilled soil and grassland compared to cultivated land is demonstrated in this study. The influence of aggregates in sequestration was also studied. The detailed characterisation of functional components of carbon that is sequestered need further study. The forms and mechanisms in which carbon is sequestered in soil aggregates are still not clearly understood.

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8. APPENDICES

1. Shear strength

Soil strength in the surface 50 mm was recorded in the field using Pilcon 120 kPa hand vane (Fig. A.1). The vane is then rotated carefully until the soil fails and the force recorded. Four readings were collected from each plot to provide an average.

Fig. A.1. Estimation of shear strength in the field using hand held shear vane

2. Ponding limit

Time to ponding was determined using a peristaltic pump (Chemlab). Polythene tubes with flow rates 1.0, 1.2, 1.4, 2.0, 2.5, 2.9, 3.4 and 3.9 cm^3 min^{-1} were used to add water at pre-determined flow rate. The water flow was monitored and the time it took until surface ponding occurred was recorded in minutes.

3. Saturated hydraulic conductivity

Saturated hydraulic conductivity was quantified using the constant head method (Klute et al., 1986). For this, constant water head was created at 2 cm above the soil level in the soil column which had previously been saturated

with water. The water draining through the column for a period of 5 minutes was collected and measured and the saturated hydraulic conductivity was calculated using Darcy`s law as given below and was expressed in cm s^{-1}.

$$\frac{Q}{A} = Ksat\left[\frac{Hp + l}{l}\right] \quad (1)$$

Where Q is the rate of flow i.e. volume of water collected per time unit (cm^3 s^{-1}), A the cross sectional area of the soil column (cm^2), l is the length of soil column (cm).

4. Field soil water content

Field moist soil samples collected in the field were packed in polythene bags and transported to the laboratory and gravimetric soil moisture content was determined by oven drying at 105^{o}C (Page et al., 1982). Volumetric soil moisture content was estimated in the field using Delta-T Theta Probe. The rods of Theta Probes were inserted into the soil surface (upper 5 cm) and four readings were recorded at each site.

5. Dry bulk density

Intact soil cores were collected in steel cylinders of known diameter and height and placed in polythene bags before being transported to the laboratory. Soil cores were trimmed to remove excess soil for the top, bottom and sides of cylinders. They were then oven dried at 105^{o}C before being weighed and the bulk density was expressed as Mg m^{-3}.

6. Particle size analysis and soil texture

Particle size analysis was performed using the hydrometer method (Bouyoucos, 1961), 50 grams of <2 mm sieved, air-dried soil was treated with 6% H_2O_2 and gently heated to remove organic matter. The coarse sand and fine sand was separated by sieving before making up to one litre for recording the hydrometer reading. A 5% solution of sodium hexa metaphosphate was used to bring about dispersion and soil mixing was ensured using a plunger before recording the hydrometer readings for silt and clay and clay. The sand content was determined after oven drying. Soil textural classification was made according to European classification using 60 µm as the upper limit for silt (Rowell, 1994).

7. Soil pH

A combined pH electrode was used to measure pH of 1:2 soil water suspension of air dried 2 mm sieved soil sample.

8. Soil organic matter

Soil organic carbon was estimated using Walkley and Black method (Nelson et al., 1982). Briefly, the soil samples were air dried and finely ground to pass through 0.5 mm sieve. The organic matter was mixed with potassium dichromate and sulphuric acid and the residual dichromate was titrated with ferrous ammonium sulphate. Soil organic matter content was also estimated using the method of loss on ignition, by igniting the humus content of an oven dried soil at 550^oC in a muffle furnace. Soil organic carbon stock was determined by multiplying organic carbon content with thickness of soil core and bulk density and was expressed as Mg C ha^{-1} using the following equation (Batjes, 1996).

$$\text{Mg C ha}^{-1} = \frac{\%\text{C} \times \text{B.D.}\,(\text{Mg m}^{-3}) \times \text{depth (m)} \times 10^4\ \text{m}^2\ \text{ha}^{-1}}{100} \quad (2)$$

9. Total carbon and nitrogen

The soil samples were first air dried and passed through 2 mm sieve. These samples were subjected to ball milling using a planetary ball mill (Retsch, PM400). Oven dried (105^{o}C) soil samples were ground using an agate mortar with the help of four balls, at a speed of 300 rpm for 4 min. About 15 mg of ball milled samples were weighed into a silver capsule followed by addition of 5 mg of vanadium pentoxide. Total C and N analysis was determined using a CN analyser (Flash 112 series, CE instruments) set at a furnace temperature of 900^{o}C, carrier gas flow of 140 ml min^{-1} and oxygen flow of 250 ml min^{-1}. An organic soil with a known C and N content was used as standard.

10. Ammonium and nitrate nitrogen (NH_4-N and NO_3-N)

For the measurement of ammonium and nitrate (NH_4-N and NO_3-N) concentration, 6g of field moist soil was extracted in 40 ml of 2M KCl. Ammonium in the extracts was determined colourimetrically (Kempers, 1974). One millilitre filtrate was mixed with phenol and hypochlorite to form a blue indophenol complex in solution. The concentration of ammonium in solution was measured colourimetrically at 635 nm using a spectrophotometer.

For the determination of NO_3-N, nitrate in a suitable aliquot of KCl extract was reduced to nitrite using spongy cadmium, which was further complexed to form a red azo-species in solution using sulphanilamide and N-1-naphthylethelenediamine dihydrochloride. The concentration of NO_3-N was

measured by comparing the absorbance with known standards of KNO_3 at a wavelength of 543 nm (Jones, 1984).

11. Greenhouse gas estimation from soil

Greenhouse gas measurements were conducted by placing the soil cores in glass jars of known volume (1.5 dm^3 for experiment in Chapters 2, 3 and 4; 0.25 dm^3 for experiment Chapter 5). The glass jars were fitted with rubber septa in the lid for head space gas sampling with a syringe. To calculate the headspace volume of glass jars, the volume of soil cores were subtracted from the volume of glass jars. The soil cores were placed inside the jar. Soon after closing the lid ambient air equivalent to removal by sampling later was added to the jar. The gas sampling was undertaken after ensuring adequate mixing of the air within the jar using a magnetic stirrer. BD Plastipak polypropylene syringes were used for gas sampling. The gas sampling was repeated at 15 minute intervals until one hour. The collected gas samples were stored in pre-evacuated 12 ml air tight glass vials closed airtight. On the day of analysis gas samples were taken with a syringe inserted into the vials and were analysed for concentration of CO_2, CH_4 and N_2O using gas chromatography equipped with a thermal conductivity detector (TCD), flame ionization detector (FID) and an electron capture detector (ECD) (GC-2014, Shimadzu). Nitrogen was used as carrier gas. The fluxes of these samples were calculated using linear regression of the gas concentration against sample time. The GHG data was converted to mass per volume and mass per weight basis by the use of ideal gas equation and the molecular mass of each gas (Denef et al., 2007).

$$n = \frac{PV}{RT} \quad (3)$$

Where n = number of moles of CO_2, N_2O or CH_4, P is atmospheric pressure (≈1 atm), V is the volume of head space (dm^{-3}), R is the ideal gas constant (0.08205746 L atm K^{-1} mol^{-1}) and T is the temperature of sampling (273.15 + room temperature in ^oC).

$$E = \frac{nm}{at} \times 1000 \quad (4)$$

Where E= flux of each gas in mg m^{-2} hr^{-1}, n = number of moles of CO_2, N_2O or CH_4, m = molar weight of CO_2 (44.01), N_2O (44.01) or CH_4 (16.04), a = area of the soil core used and t is the time in hour. The gas flux was also expressed on a per mass basis of soil.

Total greenhouse balance or net global warming potential was calculated in CO_2-equivalents as per IPCC (2001) using the following equation.

$$GWP = \left|\left(\frac{CO2 \times 44}{12}\right) + (CH4 \times 23) + (N2O \times 296)\right| \quad (5)$$

12. Microbial biomass carbon and nitrogen

Field moist soil samples were used for the estimation of microbial biomass carbon and nitrogen by the chloroform fumigation-extraction technique as per Vance et al. (1987). Fifteen grams of field moist samples were incubated under chloroform environment in presence of soda lime for 24 hours. Both fumigated and unfumigated control samples were extracted using 60 ml of 0.5 M K_2SO_4. Microbial biomass carbon and nitrogen in the extracts was analysed using a Shimadzu CN analyser (TOC-V CPH Shimadzu). The results were corrected using the value of 0.45 for both carbon and nitrogen as suggested by Jenkinson et al. (2004). Microbial biomass carbon was then determined as follows.

$$Cm = \left[\left(\frac{TOCf}{Wfsoil}\right) - \left(\frac{TOCb}{Wbsoil}\right)\right] \times \left|\frac{Vext}{kEC}\right| \quad (6)$$

Where C_m the microbial biomass carbon (mg C kg^{-1} soil), TOC_f the total organic carbon measured in the fumigated soil extract (µg mL^{-1}), TOC_b the total organic carbon measured in blank soil extract (µg mL^{-1}), V_{ext} the volume of K_2SO_4 extract (mL), W_{fsoil} the dry weight equivalent of soil for fumigated, W_{bsoil} the dry weight equivalent of soil for control, and kEC a coefficient to convert chloroform liable carbon to microbial biomass carbon. The microbial biomass nitrogen was calculated in the same way.

13. Microbial functional diversity in soil

The carbon utilisation pattern in soil collected from the field was studied using Biolog GN2 microplates (Biolog Inc., California, USA, supplied by Techno-path Distribution Ltd, Limerick, Ireland). Biolog systems measure the functional ability of bacterial communities to utilise specific C substrates (Preston-Mafham et al., 2002). The plates consisted of 95 different carbon substrates in wells along with a control well without any substrate. The complete list to this carbon substrates are provided in Table A.1. The colourless redox dye (tetrazolium violet) present in each well gets reduced following the substrate utilisation in each well and turns into purple colour. The intensity of colour was measured with plate reader with a filter (595 nm). Initially the soils stored at -20^oC were thawed over 48h. One gram dry weight equivalent of soil was suspended in 100 ml of ¼ Ringer's solution (2.25 g NaCl, 0.105 g KCl, 0.12 g $CaCl_2$ and 0.05 g $NaHCO_3$ dissolved in 1 litre of distilled water to make a full strength Ringers solution) to get a soil dilution of 10^2. The suspension was thoroughly mixed before transferring 120 µL of suspention to each well of biolog plates using a multichannel dispensing pipette. The biolog plates were then incubated at 20 ^oC for 5 days. The

absorbance of each wells of biolog plates were measured at 595 nm using a microplate reader (BioTek ELX 808, BioTek Instruments, Vermont, USA) initially within 2 h of inoculation and then at 24h intervals for 5 days. The colour intensity was measured using the software Gen5 (BioTek instruments, Inc, USA). The absorbance values were corrected by subtracting them from initial absorbance values recorded within 2 h of inoculation to account for the differences in absorption created by soil particles. Negative readings after the correction were adjusted to zero (Lupwayi et al., 2001). The average well colour development (AWCD) was calculated by dividing colour response of each well by the sum of the optical density data for all the 95 wells. According to the type of carbon substrates used in the well, they were further grouped into different functional guilds, eg. polymers, carbohydrates, carboxylic acids etc. The average colour development for each guild was then computed. Garland (1997) recommended that choosing positive values higher than 0.25 absorbance could eliminate weak false positive response. Hence the statistical analysis was carried out on the mean colour intensity values greater than 0.25. A repeated measures ANOVA was carried out to assess the effect of incubation time on AWCD and other functional groups. A two way analysis of variance was performed to test the effect of tillage and depth on AWCD and different functional groups. For this a time point was chosen which had average well colour development values between 0.75 and 1.0 (Garland, 1997) and this was at 120 h of incubation. The substrate-utilization patterns were subjected to principal component analysis (PCA) using AWCD as a co-variable to understand the patterns of substrate use.

14. Dehydrogenase

Dehydrogenase activity was determined based on modification of Thalman (1968) suggested by Ohlinger (1995). For this, 5 g of field moist soil samples were incubated with 5 ml of 1% solution of 2,3,5-triphenyltetrazolium chloride at 25 ^{o}C for 16h. The triphenyl formazan (TPF) formed was extracted with 25 mL of acetone by shaking vigorously for 2h in the dark. The solution was filtered in semi dark rooms and the intensity of TPF was measured at 546 nm against the known standards and was expressed as µg TPF g^{-1} h^{-1}.

15. Cellulase

Field moist soil (10 g) was incubated in 15 ml acetate buffer (2M, pH 5.5) using carboxy methyl as substrate (15 mL, 0.7% w/v) for 24 h at 50°C in a stoppered Erlenmeyer flask. The control was similarly incubated after adding only the acetate buffer, but without substrate. After incubation, 15 mL of substrate solution was added to the controls, and the control and samples were filtered immediately. Reducing sugars released during the incubation period was made to react with potassium hexacyanoferrate (III) in an alkaline medium. The reduced potassium hexacyanoferrate (II) was then allowed to react with ferric ammonium sulphate in an acid medium to form a coloured complex of ferric hexacyanoferrate (II). The intensity of the colour was read at 690 nm using a spectrophotometer. The activity of cellulase was expressed as mg GE (glucose equivalents) g^{-1} day^{-1} (Schinner et al., 1990).

16. Xylanase

Field moist soil (5 g) was incubated in 15 ml acetate buffer (2M, pH 5.5) using xylan as substrate (15 mL, 1.2% w/v) for 24 h at 50°C in a stoppered

Erlenmeyer flask. The control was similarly incubated after adding only the acetate buffer, but without xylan. After incubation, 15 mL xylan solution was added to the controls, and the control and samples were filtered immediately. Reducing sugars released during the incubation period was made to react with potassium hexacyanoferrate (III) in an alkaline medium. The reduced potassium hexacyanoferrate (II) was then allowed to react with ferric ammonium sulphate in an acid medium to form a coloured complex of ferric hexacyanoferrate (II). The intensity of the colour was read at 690 nm using a spectrophotometer. The activity of xylanase was expressed as mg GE (glucose equivalents) g^{-1} day^{-1} (Schinner et al., 1990).

17. β- Glucosidase activity

The measurement of β- Glucosidase activity was based on the method modified from Hoffman and Dedeken (1965) reported by Schinner et al. (2012). Briefly 5 g of field moist sample was incubated with 20 mL of acetate buffer (2M) and 10 mL of salicin (35 mM) at 37 ^{o}C for 3 h. The release of saligenin was determined colorimetrically using 2,6-dibromchinone-4-chlorimide at 578 nm using spectrophotometer. The β- Glucosidase activity was expressed as mg saligenin g^{-1} $3h^{-1}$.

18. Phenol oxidase and peroxidase

The measurement of phenol oxidase and peroxidase was based on Dick (2011). For measurement of phenol oxidase activity, 0.5 g of field moist soil was incubated with 3 mL of acetate buffer and 2 mlL of 10 mM L-DOPA (L-3,4-dihydroxy phenylalanine). Incubation was done at 25 ^{o}C in a shaking environment (100 rev min^{-1} for 10 minutes). This was followed by

centrifugation for 10 min at 5^{o}C. The reaction product (dopachrome) was read at 475 nm using a spectrophotometer. The method for peroxidase was the same as phenol oxidase, but with an additional step of addition of 0.2 mL of 0.3% H_2O_2, just before incubation. These enzymes were expressed as µmol dopachrome g^{-1} h^{-1}.

19. Fourier Transform Infrared spectroscopy (FTIR)

ATR-FTIR absorption spectra were obtained with a Bruker Tensor 27 FTIR equipped with N2 purge gas generator and a MCT detector. Initially and after every 8 samples, a background spectrum was run. Oven dried ball milled soil samples were placed on the crystal spot and the arm is rotated over and turned down to press the sample down to the crystal face. A total of 128 scans were performed for each soil samples in the data collection range of 400 to 4000 cm^{-1} at a resolution of 1 cm^{-1}. All spectra were normalised using total mean an standard deviation, before being subjected to analysis. In IR bands, the wave numbers corresponding to the absorption peaks were identified and corresponding functional groups were assigned by comparing with the published information (Glagovich, 2013). Analysis of variance was performed on the frequencies corresponding to these wave numbers.

Table A.1. Carbon Sources in Biolog GN2 microtitre plates

Yellow = Polymers ; Blue = Carbohydrates ; Lime green = Carboxylic Acids ; Pink = Amino Acids ; Orange = Esters ; Peach = Brominated chemicals ; Purple = Amides ; White = Aromatic chemicals ; Red = Amines ; Green = Alcohols and Grey = Phosphorylated Chemicals

	1	2	3	4	5	6	7	8	9	10	11	12
A	Water	α-Cyclo-dextrin	Dextrin	Glycogen	Tween 40	Tween 80	N-Acetyl-DS-Galactosamine	N-Acetyl-D-Glucosamine	Adonitol	L-Arabinose	D-Arabitol	D-Cellobiose
B	i-Erythritol	D-Fructose	L-Fucose	D-Galactose	Gentio-biose	α-D-Glucose	m-Inositol	α-D-Lactose	Lactulose	Maltose	D-Mannitol	D-Mannose
C	D-Melibiose	β-Methyl-D-Glucoside	D-Psicose	D-Raffinose	L-Rhamnose	D-Sorbitol	Sucrose	D-Trehalose	Turanose	Xylitol	Pyruvic Acid Methyl Ester	Succinic Acid Mono-Methyl-Ester
D	Acetic Acid	Cis-Aconitic Acid	Citric Acid	Formic Acid	D-Galactonic Acid Lactone	D-Galactu-ronic Acid	D-Gluconic Acid	D-Gluco-saminic Acid	D-Glucuronic Acid	α-Hydroxy-butyric Acid	β-Hydroxy-butyric Acid	γ-Hydroxy-butyric Acid
E	p-Hydroxy Phenylacetic	Itaconic Acid	α-Keto Butyric Acid	α-Keto Glutaric Acid	α-Keto Valeric Acid	D,L-Lactic Acid	Malonic Acid	Propionic Acid	Quinic Acid	D-Saccharic Acid	Sebacic Acid	Succinic Acid
F	Bromosuccinic Acid	Succinamic Acid	Glucuronamide	L-Alaninamide	D-Alanine	L-Alanine	L-Alanyl-glycine	L-Asparagine	L-Aspartic Acid	L-Glutamic Acid	Glycyl-L-Aspartic	Glycy-L-Glutamic Acid
G	L-Histidine	Hydroxy-L-Proline	L-Leucine	L-Ornithine	L-phenylalanine	L-Proline	L-Alayl-Glycine	D-Serine	L-Serine	L-Threonine	D,L-Carnitine	γ-Amino Butyric Acid
H	Urocanic Acid	Inosine	Uridine	Thymidine	Phenyethylamine	Putrescine	L-Pyro-glutamic Acid	2,3-Butanediol	Glycerol	D,L-α-Glycerol Phosphate	α-D-Glucose-1-Phosphate	D-Glucose-6-Phosphate