# Applied soybean and maize residue contributions to soil organic matter in a temperate soybean/maize intercropping system

by

Amanda Bichel

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Environmental Studies

in

Environment and Resource Studies

Waterloo, Ontario, Canada, 2013

© Amanda Bichel 2013

# AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

# ABSTRACT

Intercropping, defined as two or more crops grown on the same land area at the same time, is a sustainable alternative to sole crops. Intercropping has been associated with multiple benefits, such as increased nutrient and soil organic carbon (SOC) cycling, decreased soil erosion and increased carbon (C) sequestration. A common intercropping practice is to integrate cereal and legume crops such as maize (*Zea mays* L.), and soybean (*Glycine max* (L.) *Merr*.). Most studies on intercropping have focused on yield, weed control, and land use efficiency in the tropics. Few studies have researched C and nitrogen (N) dynamics in temperate intercrops, with respect to soybean and maize residue stabilization. Soil from Balcarce, Argentina, was incubated for 140 days with soybean, maize, or no residue. Throughout the incubation, results illustrated the effect of residue application upon the soil, specifically through significantly higher amounts of light fraction (LF) C and $LF_N$ concentrations, soil microbial biomass (SMB) C and $SMB_N$ concentrations, higher microbial diversity, lower $N_2O$ production rates, in addition to distinct isotopic values in soil fractions and $CO_2$ ($p<0.05$). Furthermore, it was observed from $\delta^{15}$N-TN and $\delta^{15}$N-LF that treatments with soybean residues included had higher N cycling ($p<0.05$), emphasizing the importance of including N-fixing legumes in complex agroecosystems. Significant changes over time in SMB and SMCS characteristics, and isotope values ($p<0.05$) indicated the preferential utilization of relatively young and easily accessible litter. Furthermore, the loss of labile material over the incubation resulted in more recalcitrant forms (such as older C and lignin) to be utilized. Slightly higher SOC, TN, $LF_C$ and $LF_N$ concentrations, as well as lower $CO_2$ production rates suggested 2:3 (rows of maize:rows of soybean) as a more desirable intercrop design for C sequestration. The 1:2 intercrop design was observed to be more beneficial for microbial community structure, furthering the idea that intercropping is a beneficial alternative to sole cropping. This study improves knowledge in residue stabilization and C sequestration in complex agroecosystems, providing encouragement for the implementation of more sustainable management practices.

# ACKNOWLEDGEMENTS

I would like to express my appreciation to many people who supported me through this thesis. First to my advisor Maren Oelbermann for giving me welcomed feedback whenever it was needed, and who made this thesis possible. I would also like to thank my committee member Stephen Murphy (for assisting me with statistics) and my external reader Paul Voroney, who both took time out of their busy schedules to read and comment on my thesis.

Meaghan Wilton, my lab and travel mate who never hesitated to offer her assistance, always adding amazing creative input. A giant thank you to people who helped me with lab work. Myles Stocki from the University of Saskatchewan who cared about each and every sample, and made sure I understood each column of results. Richard Elgood over in EIT was never obligated to help, but always did, and was a huge encouragement throughout.

Thanks to all of my friends and family. My new amusing and intelligent friends in Waterloo who made the last two years memorable ones. Thanks also to the folks at Sustainable Waterloo Region for getting me out of the house every Tuesday, I learnt so much with you all. Thanks to my family, especially my parents, for your support and love that I always know I can count on. Thank you also to my partner's family for treating me as a member and being curious about my thesis. Also to the rest of my friends and family for understanding that I had to live across the country to complete the next chapter of my life, and for understanding that I had to be on a student's budget for two more years. Your support means a lot to me.

Thanks especially especially to my partner Pieter for countless edits, helping me in the lab and with calculations with no hesitation, and pure selflessness, but most of all, for the limitless moral support that you gave me. I can only hope that I did the same for you.

To everyone I mentioned and to anyone who I did not list, there is no way I could have done it without you. Thank you forever!

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# LIST OF ABBREVIATIONS

C               Carbon

$CO_2$          Carbon dioxide

GHG             Greenhouse gases

LF              Light fraction

N               Nitrogen

$N_2O$          Nitrous oxide

SMB             Soil microbial biomass

SMCS            Soil Microbial Community Structure

SOC             Soil organic carbon

SOM             Soil organic matter

TN              Total nitrogen

# GENERAL INTRODUCTION

## i. Introduction

Current widespread agriculture methods are recognized by the use of sole crops, pesticides and machinery, which has led to decreases in soil organic carbon (SOC), total nitrogen (TN), and ecosystem diversity, as well as increases in soil erosion and greenhouse gas (GHG) emissions (Horrigan et al. 2002). A possible management technique to reduce these affects is intercropping, where two or more crop types are grown on the same land area simultaneously (Hauggaard-Nielsen et al. 2001). Due to biological diversity and increased interactions above and below-ground, intercropping is known as a complex agroecosystem, and has been shown to increase SOC and TN stocks, decrease erosion and increase diversity (Wall et al. 1991; Beaudette et al. 2010; Zhou et al. 2011). For example, the integration of legume and cereal crops produces benefits in nutrient cycling for both crops, such as increased N cycling from the legume to the cereal crop (Kurdali 2009). Many studies have focused on intercrops in tropical ecosystems but few have studied intercropping in temperate climate zones (Leite et al. 2007; Makumba et al. 2007; Oelbermann and Echarte 2011; Dyer et al. 2012). For example, to reduce soil degradation and SOC losses, the use of intercrops is increasing in the Argentine Pampas and thus requires research to determine the viability of such management practices in the temperate climate zone (Oelbermann and Echarte 2011).

## ii. General Objectives

This study was designed to determine how effective $C_3$ and $C_4$ crops contribute to stabilized carbon (C) in a temperate intercropping system. The overall objective of this thesis was to enhance our understand of the role of intercropping in soil C and N transformations. Specifically, it focused on the measurements of C and N transformations in soils amended with

either soybean or maize residues to soil fractions with a mixed $C_3/C_4$ origin in an incubation study. This was accomplished throughout Chapters 3, 4 and 5 through the following objectives:

(a) To determine transformations and pathways of C and N from crop residues, through an evaluation of SOC, TN and light fraction (LF)

(b) To determine the influence of the soil microbial pool on residue stabilization by quantifying soil microbial biomass (SMB) and soil microbial community structure (SMCS)

(c) To quantify GHG production rates and $^{13}C$ of respired $CO_2$ to understand residue stabilization and C sequestration.

## iv. Hypotheses and Null Hypothesis

*Hypothesis*

In a laboratory incubation experiment where soybean and maize residue was added to soils collected from sole and intercropped fields, it was hypothesized that:

(a) SOC, TN, $LF_C$ and $LF_N$ concentrations would be higher in intercrops than sole crops, and distinct isotopic values would be observed from each residue type.

(b) $SMB_C$, $SMB_N$, diversity, density and activity would be higher in intercrops than sole crops, and distinct isotopic values in the SMB would be observed from each residue type

(c) GHGs emitted from intercrops would be lower than from sole crops, and distinct isotopic values would be observed from each residue type in respired $CO_2$.

*Null Hypothesis*

(a) No differences would be observed in SOC, TN, $LF_C$ and $LF_N$ concentrations or isotopic values between sole and intercrops due to the application of soybean and maize residues.

(b) No differences would be observed in $SMB_C$, $SMB_N$ concentrations and isotopic values, or diversity, density and activity between sole and intercrops due to the application of soybean and maize residues.

(c) No differences would be observed in GHG flux rates or isotopic values of respired $CO_2$ between sole and intercrops due to the application of soybean and maize residues.

## v. Benefits and Applications of Research

This research provides further insight into the potential for introducing intercropping systems as a long-term and sustainable agroecosystem management practice. This will be achieved through further understanding of long-term incorporation of soybean and maize residues in complex agroecosystems, and the role of C sequestration and GHG mitigation in this process. Insight is also provided on the most effective intercrop ratio (design) that could be applied to farms in the temperate zone.

# 1. LITERATURE REVIEW

**1.1 Soil Organic Matter**

*1.1.1 The Global Carbon Cycle*

Carbon (C) is a ubiquitous element that, in its reduced form, makes up half of the organic material on Earth (Chapin et al. 2002). There are four major pools through which C circulates; oceans, atmosphere, soil and vegetation, and sediments and rocks (Field and Raupach 2004). Carbon dioxide ($CO_2$) in the ocean exists mainly as a pH-controlled equilibrium of carbonate, bicarbonate, and $CO_2$; cycles by photosynthesis and detritus settling, and is released through upwelling (Chapin et al. 2002). Soil and vegetation use photosynthesis and microbial and plant respiration to cycle $CO_2$ into and out of the smaller atmospheric pool. Turnover times for each component of the terrestrial C cycle vary from seconds (photorespiration) to thousands of years (humus breakdown). Storage of C in rocks, the largest C pool, has turnover times up to millions of years (Chapin et al. 2002).

*1.1.2 Soil Organic Matter*

Soil organic matter (SOM) is formed by the decomposition of fresh plant matter and organism decay (Melillo et al. 1989; Gardiner and Miller 2008). Fractions of SOM can be found at several stages of decomposition, including living organisms, partially decomposed plant and animal residues (labile fraction), and humus (recalcitrant fraction) (FAO 2005). SOM is important to soil quality, productivity and sustainability as it provides and stores nutrients for plants, retains air and water, reduces soil erosion, and controls pesticide movement (Gregorich et al. 1994; He et al. 2008). Many factors influence SOM quality, including soil pH, temperature, moisture, texture, quality and quantity of added residues, and especially microbial activity (FAO 2005;

Samahadthai et al. 2010).  The quantity of SOM mostly depends on the balance of organic matter inputs and decomposition, increasing when input rates are higher than decomposition rates (Post et al. 1982).

Together, SOM and plants store more than twice the amount of C than the atmosphere (Cao and Woodward 1998).  Furthermore, long-term C sequestration depends on turnover times of SOM (Pendall and King 2007).  This is important for climate change because as soil fractions are decomposed large amounts of $CO_2$ are released to the atmosphere (Merino et al. 2004).  In the agricultural setting, land use practices, such as complex agroecosystems can limit this process by delivering more plant litter to the soil, enhancing SOM levels and increasing C sequestration (Oelbermann et al. 2006a).  Important components of SOM are the soil light fraction (LF), soil organic carbon (SOC) and soil total nitrogen (TN).  The LF is a labile form of SOM, while SOC and TN are considered to be an inventory of the total SOM (Gregorich et al. 1994).

*1.1.3 Soil Organic Carbon*

Soil organic carbon holds approximately two-thirds of all terrestrial C and is important in determining the physical and chemical make-up of soil (Schimel et al. 1994; Oelbermann et al. 2006b).  Soil organic carbon can be affected by decomposition rates, climate, and soil characteristics, although it has been estimated that it can take anywhere from 3 to 13 years to be observed in croplands, depending on soil properties and depth (Schrumpf et al. 2011).  The majority of SOC is found in the top 60 cm of soil and is found to decrease with depth (Manjaiah et al. 2000).  Increasing SOC quantity can decrease soil erosion, help filter pollutants before they reach water, increase crop nutrients and yield, and allow less $CO_2$ into the atmosphere (Kimble et al. 2002; Merino et al. 2004; Ghimire et al. 2012).

Land management techniques considerably affect SOC quantity. For instance, in complex agroecosystems, reduced tillage and increased plant residues left on the soil, increased SOC levels and C sequestration (Diels et al. 2004; van Groenigen et al. 2011). A study by Studdert and Echevrría (2000) found that SOC in crop rotations of wheat, maize, soybean and sunflower all decreased after 11 years. Furthermore, soybean crops had greater SOC losses than that of maize due to higher residue inputs from maize. However at relatively high soil C concentrations, which varies depending on soil type, it has been found that C saturation occurs, at which point soils stop sequestering C and reach a steady state (Stewart et al. 2009). The amount of SOC sequestered unquestionably affects the amount of $CO_2$ entering the atmosphere. For example Marland et al. (2003) found that in the U.S. if a no-tillage practice replaced conventional tillage, an additional 337 kg C/ha/year would be sequestered for 20 years, after which, the system would reach an equilibrium in 20 more years.

*1.1.4 Soil Light Fraction*

Light fraction, described as material that has a density between 1.5 and 2.0 $g/cm^3$, is made up mainly of partly decomposed plant litter, but can also contain microbial biomass, charcoal, humus and other plant materials such as seeds (Gregorich et al. 1994; Crow et al. 2006; Soon et al. 2009). In addition, the LF has a high C/N ratio, is more depleted in $^{13}C$ and holds more labile C relative to heavier fractions, as it is primarily made up of plant material (Crow et al. 2006). Furthermore, because the LF is the most labile component of SOM, it can strongly influence SOC, TN, soil respiration, and microbial diversity. The LF is more sensitive to land use changes than heavier fractions, since it is more easily decomposable and is in transition from fresh litter to humus (Soon et al. 2009). Therefore when SOM is added or removed, LF is more affected than SOC (Leite et al. 2007). This allows the LF to illustrate effects of a land use change on SOM and possibly predict future changes to slower pools (He et al. 2008). For example, there was a

larger LF when plant residues were added in the field and in an incubation experiment (Bolinder et al. 1999; Oyedele et al. 1999).

*1.1.5 Soil Nitrogen*

Nitrogen undergoes many transformations into, within and out of the soil (Chapin et al. 2002). It is initially brought into the soil system through fixation, usually by legumes, where N gas ($N_2$) is taken from the atmosphere and transformed into ammonium ($NH_4^+$).  In the soil, N may undergo nitrification ($NH_4^+$ is transformed into nitrite ($NO_2^-$) and nitrate ($NO_3^-$)), mineralization (organic N is transformed into inorganic N available to plants), or immobilization ($NH_4^+$ and $NO_3^-$ are transformed into organic N).  Nitrogen can leave the soil system through denitrification ($NO_3^-$ is transformed into $N_2$ or $N_2O$), assimilation (plants use the available inorganic form of N), or leaching (National Research Council 1993; Chapin et al. 2002).

Nitrogen, found almost everywhere in the environment, is mainly stored within SOM in terrestrial ecosystems.  It is one of the most important nutrients for both plants and microbes, and is continuously cycled through soil, plants and atmosphere (National Research Council 1993).  The addition of fertilizers, erosion and decomposition can change the amount of soil TN. A lack of soil TN (a high C/N ratio) can also influence C due to less microbial activity and decomposition (Gregorich et al. 1994).  Levels of N are also strongly affected by land use.  For example, it was shown that when intercropping legumes with non-legumes, there was higher N cycling and more efficient N uptake by crops (Kurdali 2009).  In an alley crop study, where soybean was planted between rows of trees, TN levels were found to decrease after ten years in a sole crop, but stay stable in the alley crop (Oelbermann et al. 2006a).

## 1.2 The Soil Microbial Pool

Soil microbial activity can significantly affect C, N and nutrient cycling, soil formation and overall soil quality (Spedding et al. 2004; Sun et al. 2009). Soil microbes offer the important ecosystem function of decomposing 60-90% of all terrestrial plant litter (Giller 1996). Microbial diversity is most affected by soil conditions such as pH, temperature, moisture and SOM quantity, but is also affected by soil pore size, food sources, habitat variability, seasonal crop variability, and disturbances (Giller 1996; Spedding et al. 2004). Soil microbial biomass (SMB) and activity have been shown to be useful indicators of environmental stress, like heavy metal contamination or changes in soil management, such as tillage (Barajas-Aceves et al. 1999; Drijber et al. 2000). When comparing short and long-term changes in the soil environment, microbes are better suited as an indicator of short-term studies, due to their relatively fast response time to changes (Hargreaves et al. 2003). Using multiple parameters such as soil microbial biomass carbon $SMB_C$ and SOC, has also been proven a useful indicator of soil quality (Barajas-Aceves 2005; Suman et al. 2006).

Human activities, such as land use management, contribute to altering microbial communities. In an alley cropping system, as more residues are added to the soil, more C and N is added as a food source, increasing SMB and potential C sequestration, while creating better soil quality when compared to sole crops (Suman et al. 2006; Rivest et al. 2010). Furthermore, when intercropping cereal and legume crops, it has been shown that SMB increases in the legume crop as well as in the rhizosphere, performing the service of fixing N for plants (Sun et al. 2009). Studies have also showed that more active microbes feed on more labile materials such as the LF, and less active microbes feed on more recalcitrant material (Hassink 1995). These studies suggest that sustainable cropping practices such as intercropping are beneficial to the microbial population, and in turn enhance soil quality (Suman et al. 2006).

## 1.3 Modern Tools for Evaluating Soil Carbon and Nitrogen Dynamics

Stable isotopes are elements that differ in their number of neutrons and atomic mass, and do not undergo radioactive decay (Fry 2006). They have been used since the 1930s in geology but are now strongly incorporated into plant and soil sciences (Ehleringer and Vogel 1993).

*1.3.1 C$_3$ and C$_4$ Plants - The Natural Abundance Method*

Early stable isotope research in botany revealed that plant matter was slightly depleted in $^{13}$C when compared to parent material, and that different plant species varied in the amount of $^{13}$C (Nier and Gulbransen 1939). A paper published by Bender in 1968 recognized a difference in the amount of $^{13}$C due to differing photosynthetic pathways in C$_3$ and C$_4$ plants. Almost all temperate plants and tree species (90%) are C$_3$, while C$_4$ plants are mostly grasses and make up 2% of all plant species (Balesdent and Mariotti 1987; Glaser 2005). Both plant types discriminate against $^{13}$C when consuming $CO_2$ but differ in their internal processes. A more efficient carboxylation reaction in C$_4$ plants results in lower discrimination against $^{13}$C than C$_3$ plants which creates distinct differences in $\delta^{13}$C of C$_4$ plants (-8‰ to -18‰), C$_3$ (-22‰ to -33‰) and the atmosphere (-7‰) (O'Leary 1988; Boutton et al. 1998; Schweizer et al. 1999). The pronounced difference between plant types is used as an isotopic tracer and is referred to as the natural abundance method (Balesdent and Mariotti 1987; Lynch et al. 2006). This allows scientists to track changes in the make-up of plant communities through time (Boutton et al. 1999) and to explore the incorporation of soybean and maize residues and turnover of these residues once incorporated into the soil carbon pool (Martin et al. 1990; Bernoux et al. 1998; Costantini et al. 2007). For example, in Argentina, a baseline field study was conducted to evaluate contributions from soybean and maize sole and intercrops to C$_3$/C$_4$ mixed soil (Oelbermann and Echarte 2011). They found that after one season of intercropping, C$_4$ was the main C contributor, due to previous land use (Oelbermann and Echarte 2011).

*1.3.2 Stable Isotopes from Respired Carbon Dioxide*

Stable C isotopes from soil respired $CO_2$ have been measured to study exchanges of $CO_2$ between the atmosphere and terrestrial ecosystem, as well as $CO_2$ variations in space and time (Flanagan and Ehleringer 1998; Yakir and Sternberg 2000). Furthermore, stable C isotopes of $CO_2$ have been used to study fractionation or discrimination of $^{13}C$ during microbial respiration (Ekblad et al. 2002; Šantrůčková et al. 2000), or to attempt to distinguish contributions of respired $CO_2$ from light and heavy fractions or from $C_3$ and $C_4$ plants (Ekblad and Högberg 2000; Crow et al. 2006). Šantrůčková et al. (2000) determined that discrimination during microbial metabolism depends on the growth stage of the microbial community, whereas Ekblad et al. (2002) determined that microbial discrimination of $^{13}C$ was negligible during respiration, by measuring $\delta^{13}C$ from respired $CO_2$ after changing microbial substrates from $C_3$ to $C_4$. Furthermore, $^{13}C$ from respired $CO_2$ has been used to distinguish between contributions from $C_3$ and $C_4$ crops throughout the season in a rotation or between different soil substrates (Liang et al. 1999; Griffis et al. 2005). The separation of three sources to respired $CO_2$ (native SOM and two applied sources) was even possible using substrates with distinct decomposition time and $\delta^{13}C$ values, and the natural abundance method (Kuzyakov and Bol 2005). In $C_3/C_4$ intercropping studies, $\delta^{13}C$ values from respired $CO_2$ could aid in determining both contributions from $C_3$ and $C_4$ sources as well as the flow of C between soil fractions such as SOC, SMB and $CO_2$.

*1.3.3 Stable Isotopes from Soil Organic Carbon*

Soil organic carbon is shown to be more enriched in $^{13}C$ than the plant litter it originates from (Pendall and King 2007) while also becoming more enriched with depth (Feng 2002). Many possibilities exist for the cause of this fractionation, yet there appears to be no universal consensus. Some of this fractionation can be explained by a decrease (approximately 1.5‰) in

atmospheric $^{13}C$ since the 1800s due an increase in fossil fuel burning and deforestation (Francey et al. 1999). Deeper SOC evolved when the atmosphere was more enriched in $^{13}C$ while SOC closer to the surface, formed today, when the atmosphere is less enriched (Francey et al. 1999; Boström et al. 2007). Other possible fractionation factors include discrimination of $^{13}C$ during microbial respiration, which states that microbes respire $CO_2$ depleted in $^{13}C$, thereby making C in the soil enriched (Schweizer et al. 1999). However, other researchers have found that there is no, or negligible fractionation during microbial respiration (Ekblad et al. 2002). Alternatively, preferential decomposition has been found to occur, meaning microbes prefer to use food sources that are more depleted in $^{13}C$, or newer C, and leave more enriched C in the SOM (Šantrůčková et al. 2000; Blagodatskaya et al. 2011). Another explanation to this fractionation, due to similar $\delta^{13}C$ values in respired $CO_2$ and microbes at lower depths, is a larger contribution to deeper SOC from soil microbes and fungus than earlier thought (Boström et al. 2007). Soil isotope studies could potentially be useful in intercropping studies with $C_3$ and $C_4$ crops. Some previous intercrop studies with $^{13}C$ isotopes, focus on water-use efficiency and discrimination of $^{13}C$ in the intercrops (Kurdali 2009; Makoi et al. 2010).

*1.3.4 Stable Nitrogen Isotopes*

The $^{15}N$ natural abundance method has also been used for isotopic measurements within soil. It assumes that with no other sources of N, $N_2$-fixing plants will have a $\delta^{15}N$ value determined by the atmosphere and soil (0‰), whereas non-$N_2$ fixing plants will have a $\delta^{15}N$ value similar to the soil (Unkovich et al. 2008). This method has been used to measure $N_2$-fixation in plants and to determine contributions from the soil and atmosphere to N in different plant components (Kurdali 2009). It has also been found that the stable N isotope composition of microbes are enriched compared to their surrounding substrate, but that amount of enrichment varies with soil age (Coyle et al. 2009). However, it was found to be difficult to use the $^{15}N$ natural

abundance method with decomposable material because of $^{15}N$ heterogeneity in the substrate (Lynch et al. 2006).

The amount of $^{15}N$ in the N cycle is also affected by fractionation factors. For instance, depletion of N isotopes in plant matter and soil were seen due to lower water use efficiency in wetter environments (Peri et al. 2012). Furthermore, loss of N from leaching, mineralization, plant uptake and volatilization removed lighter isotopes, enriching the substrate in the heavier isotope, $^{15}N$ (Högberg 1997). Within the N cycle, mineralization is less discriminate against $^{15}N$ than nitrification resulting in more $^{15}N$-enriched $NH_4^+$ and more depleted $NO_3^-$ (Högberg 1997). Crop type can also change the isotopic make-up of the soil, since certain plants prefer $NH_4^+$ to $NO_3^-$, cycling $^{15}N$-enriched $NH_4^+$ back to the soil in plant litter. Due to the complexity of the N cycle and different fractionation factors, it is difficult to measure a single component, making it beneficial to measure $^{15}N$ under controlled conditions (Högberg 1997).

The N natural abundance method is useful in intercropping systems with a legume and a cereal because of the N-fixing legume. It has been used in intercropping studies to evaluate N transfer from the legume to the cereal crop as well as how sole and intercrops are affected by applied N fertilizer (Giller et al. 1991; Ghaley et al. 2005). Furthermore, it has been used to evaluate the interspecific competition for N between the cereal and legume crops as well as weeds (Hauggaard-Nielsen et al. 2001).

*1.3.5 $C_3$ and $C_4$ Crop Residue Decomposition*

The decomposition of $C_3$ and $C_4$ crop residue and its effect on soil characteristics has been thoroughly studied. Soil organic carbon turnover times, and decomposition rates of SOC and LF due to a shift in vegetation from $C_3$ to $C_4$ crop has been evaluated (Martin et al. 1990; Wynn and Bird 2007; Marschner et al. 2008; Oelbermann and Echarte 2011). The introduction of a $C_4$ species on previously $C_3$-dominated soil illustrated that TN, N mineralization was higher with

$C_3$ species, and that $C_4$ species had a higher biomass and lignin concentration (Mahaney et al. 2008). Changes from $C_3$ to $C_4$ residue input, or vice versa, along with isotopes have been used to evaluate soil microbial preferential utilization of new C sources and discrimination against $^{13}C$ during microbial respiration (Ekblad et al. 2002; Blagodatskaya et al. 2011). A study by Kramer et al. (2012) assessed the flow of C from $C_3$ and $C_4$ root and shoot residue to SOC and SMB pools. They found that amounts of incorporated $C_4$ residue were most in the SMB and least in SOC in a shift from $C_3$ to $C_4$ vegetation.

Respired $CO_2$ from the decomposition of $C_3$ and $C_4$ residues has also been studied. In southern Manitoba, Glenn et al. (2011) evaluated the amount of maize residue lost to respiration after harvest on a previously $C_3$ dominated soil. They found that at first maize contributed to $CO_2$ far more than older sources, however after 6 months the maize residue had turned over and contribution came mostly from older SOC (Glenn et al. 2011). The decomposition of $C_3$ legumes has been found to be correlated with $CO_2$ production rates in a field experiment in India (Arunachalam et al. 2003). It has also been found that decomposition will not necessarily decline with higher $CO_2$ concentrations as previously thought (Ross et al. 2002). Potthoff et al. (2005) analyzed how a mixture of residues and the addition of N affected decomposition and $CO_2$ and $N_2O$ production rates. It was found that adding the mixture of residues increased $CO_2$ production rates, and created sites for denitrification, increasing $N_2O$ production (Potthoff et al. 2005). The effects on soil characteristics and GHGs due to decomposition of $C_3$ and $C_4$ residues on a $C_3/C_4$ mixed soil in a temperate intercropping system has been studied in the field, but not in an incubation (Vachon and Oelbermann 2011).

## 1.4 Climate Change

Only in the past few decades has climate change gained wide recognition, even though scientists could demonstrate the greenhouse effect more than a hundred years ago. The greenhouse effect is the warming of Earth's surface and lower atmosphere (troposphere),

through thermal radiation that is either absorbed or reflected by greenhouse gases (GHGs) such as water vapour, carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$) and ozone (IPCC 2007). Greenhouse gases are emitted anthropogenically through the combustion of fossil fuels and biomass, but can also be emitted naturally through volcanic eruptions and solar variations. The Earth can naturally absorb GHGs produced by ecosystems, but increased GHGs produced from the beginning of the industrial era is thought to have put this system out of balance. Greenhouse gases are being released at a greater rate than can be naturally absorbed (Table 1), causing a more rapid increase in Earth and tropospheric temperatures than in the past 650,000 years (IPCC 2007). For instance, before the industrial era there were 280 ppm $CO_2$, and 0.260 ppm $N_2O$ in the atmosphere compared to 379 ppm, and 0.319 ppm respectively in 2005 (IPCC 2007). Although lower in amount than $CO_2$, $N_2O$ is equally important, as it is more efficient at absorbing radiation (Hillel 1998). $CO_2$ and $N_2O$ are stored and cycled though ecosystems naturally and anthropogenically.

**Table 1.1** Natural and anthropogenic sources and absorption of greenhouse gases and the net atmospheric increase globally in 2001 (adapted from Lindstrom et al. 2001 and IPCC 2007).

| Greenhouse Gas | Sources | | | Natural Absorption | Annual atmospheric increase |
|---|---|---|---|---|---|
| | Natural | Anthropogenic | Total | | |
| $CO_2$ (Mt C) | 210,000 | 6,300 | 216,300 | 213,100 | 3,200 |
| $N_2O$ (Mt of gas) | 9.5 | 6.9 | 16.4 | 12.6 | 3.8 |

Carbon dioxide can be stored in oceans, soils, rocks and the atmosphere. It is taken out of terrestrial and marine ecosystems through photosynthesis and is stabilized in ecosystems by decomposition or plant uptake. Carbon dioxide enters the atmosphere naturally through root and microbial respiration, but also anthropogenically by the burning of fossil fuels, and

deforestation. Nitrous oxide, the longest living GHG in the atmosphere, absorbs radiation about 300 times more efficiently than $CO_2$ (Smith 2010). Nitrous oxide is produced naturally through microbial activity and anthropogenically through the addition of inorganic N fertilizers to crops, and other industrial sources. It is removed from the atmosphere primarily via ultraviolet and ozone photolysis in the stratosphere (Smith 2010). Together, these processes influence the GHG effect and its control on temperature.

Physical observations of climate change have been well documented. These include increases to global mean surface temperature by 0.13°C per decade in the last 50 years (up from 0.7°C per decade from the previous 50 years) with twice the temperature increase in the Arctic over the past 100 years (IPCC 2007). This correlates with a decrease in snow cover at latitudes above 65°N, an increase in mass loss for many glaciers, and shorter frozen ground times and depths in the last 100 years. Oceans have also seen a 0.10°C warming of the top 700 m of ocean water, changes to distribution of fresh and saline waters, an increase in approximately 118 Gt of inorganic C since 1750 and an increase in global sea level by about 1.8 mm per year since the 1960s (IPCC 2007). Furthermore, an increase in precipitation for latitudes over 30°N, concomitantly with an increase of drought in tropical zones (due to decreased precipitation), have been observed since the 1970s. Additionally, intense storms and cyclone events have increased since the 1970s, especially in tropical regions (IPCC 2007).

Possible outcomes of persisting climate change with a 'business-as-usual' attitude have been estimated by numerous models. Even with the most ideal long-term strategies, it is estimated that a doubling of $CO_2$ (which could very likely be reached by 2100) could lead to a global mean temperature increase from 2°C to 4.5°C (IPCC 2007). Regional predictions estimate that warming will be higher than the global mean everywhere except Central and South America, Australia and New Zealand and small islands. Predictions also state that with no mitigation for climate change, global effects will not only continue, but be more extreme than in the past century (IPCC 2007).

## 1.5 Agriculture Management Practices

Agriculture and climate change form an intricate feedback loop that will continue to affect food, fuel and fibre supply for the worlds population. Depending on practices adapted, agriculture can affect climate change by either increasing or decreasing the amount of GHGs in the atmosphere. To understand different management practices today, it is necessary to review a condensed history.

### 1.5.1 A Brief History of Agriculture

Agriculture, or plant cultivation and animal breeding, began approximately 10,000 years ago in the Neolithic Era (Mazoyer and Roudart 2006). This corresponded with a transition away from a nomadic and predatory lifestyle. Two thousand years later, many crops and animals that we still see today were domesticated (wheat, lentils, pigs and cattle). Slash and burn agriculture was introduced later in the Neolithic Era, in which forest was cut down and burnt to temporarily provide nutrient rich land. This method is still practiced today, mainly in tropical forests in Africa, Asia and South America. In centuries to follow, agriculture spread to Mexico, South America and North America respectively, leading to the formation of many agricultural societies (Mazoyer and Roudart 2006).

The first modern agricultural revolution coincided with the industrial revolution from the 16th to the 19th centuries (Mazoyer and Roudart 2006). Farmers began to rotate annual grain crops with fodder crops, resulting in continuously used soil. This doubled crop production leading to a surplus of food, allowing people dependent upon agriculture to concentrate on other occupations (such as mining). Throughout the 18th and 19th centuries, equipment was advanced and distributed through improved transportation (Mazoyer and Roudart 2006).

The invention of the internal combustion engine and synthetic fertilizers led to the second modern agricultural revolution. Farmers could now use fossil fuels to replace animal

driven tools, while using inorganic fertilizers to increase crop yield. This allowed specialization of one crop or animal product, which replaced the diversity that existed before the 20th century (Mazoyer and Roudart 2006). The next shift in agriculture started in the 1950s and is termed the Green Revolution. It involved the selection of only high-yielding varieties of wheat and rice in developing countries, and relied upon synthetic fertilizer inputs to increase crop yields. This practice replaced traditional agriculture in many areas (Parayil 2003). Increases in productivity derived from the Green Revolution slowed significantly in the 1980s opening the door for the Gene revolution in the 1990s. Its focus is the use of biotechnology to alter the genetic make-up of plants to insert desirable traits, like pest or weed resistance (Parayil 2003).

Each agricultural revolution caused a spike in global population resulting in greater anthropogenic impacts upon ecosystems than ever before. For example, the second modern agricultural revolution increased population, crop yield and spread on a scale ten or a hundred times larger than before (Mazoyer and Roudart 2006). A new system subsequently developed, where more food was produced at a lower cost. This left a small number of large farms, instead of many small family owned farms, leading to what is currently known as 'industrial agriculture' (Mazoyer and Roudart 2006).


*1.5.2 Industrial Agriculture - The Current State of Agriculture*

Industrial agriculture relies on mechanized equipment, inorganic fertilizers, irrigation systems and genetics in efforts to increase yield to feed the growing population (Horrigan et al. 2002). Unfortunately these processes result in a tradeoff between efficiency and environmental quality. Several negative impacts are associated with industrial agriculture. Large areas of sole crops that are prevalent today reduce plant and animal biodiversity due to habitat destruction and ecosystem dominance of one crop species (Horrigan et al. 2002). Agriculture also contributes to climate change by using large amounts of fossil fuels in fertilizer, machinery, and meat

production (Horrigan et al. 2002). Excess soil tillage has increased the rate of erosion in the last 50 years, losing soil 17% faster than nature can replenish it (Kimbrell 2002). Furthermore, fertilizers and pesticide inputs needed for industrial agriculture increase every year due to increasing tolerance of weeds and pests to recurring chemicals (Kimbrell 2002). These chemicals end up leaching into nearby surface and ground waters, causing eutrophication and fish kills, or directly on the food that humans consume. Pesticides can also affect surrounding ecosystems and kill unintended insects and birds. It is estimated that at most, only half of applied fertilizer is taken up by crops (Tilman 1998). The use of biotechnology in agriculture is a highly contentious issue, some claiming that benefits of increased production highly outweigh potential human health and environmental risks in order to feed the growing population (Borlaug 2004). Others are concerned about the complexity of the issue and potential risks such as biodiversity loss and undesired health affects from manipulated genes (Conner et al. 2003; Abah et al. 2010). Despite conflicting information about industrial agriculture, it makes up a large fraction of our food system today, and is growing in developing nations like Brazil and Argentina (FAOSTAT 2011).

### 1.5.3 Agriculture and Climate Change

Agriculture has gained new recognition as a large GHG contributor, responsible for approximately 20% of all anthropogenic sources (Hillel 1998). Since the 1960s, agricultural area has increased by 461 million hectares and now occupies 37% of all land area (IPCC 2007; Smith et al. 2008). Clear cutting for agricultural land, flooding for rice and sugarcane crops, biomass burning, animal waste, addition of inorganic fertilizers and use of fossil fuels all contribute to the emission of $CO_2$ and $N_2O$ (Hillel 1998). Agriculture is responsible for approximately 84% of $N_2O$ while it is thought to emit approximately one-third of global $CO_2$ through deforestation and the burning of fossil fuels (Johnson et al. 2007; Smith et al. 2008).

Nitrous oxide is released from soil due to fertilizers (chemical and manure), tillage, irrigation, land use change, residue retention, biomass burning, and manure management resulting in anaerobic conditions (Johnson 2009). In an IPCC report on agriculture and climate change (2007), it was found that from 1990 to 2005, agriculturally produced $N_2O$ increased by 17%. In agricultural practices, $CO_2$ is regularly emitted through soil respiration, biomass burning and fossil fuel combustion, and taken out of the atmosphere through photosynthesis and SOC sequestration. Therefore, depending on management practice, agriculture can result in either a C sink or source (Organization for Economic Co-operation and Development 2001; Smith et al. 2008).

The type of agricultural practice implemented affects the amount of GHGs emitted. In a study by Merino et al. (2004) where GHGs were measured on a pasture, cropland and forest, it was found that $N_2O$ emissions were the lowest from the forest. In addition, the highest emission rates of $CO_2$ were observed after tillage, fertilization and manure application (Merino et al. 2004). In a study by Evers et al. (2010), a tree-based intercropping it was found that increased plant litter input compared to the sole crop resulted in more surface C sequestration and therefore less $CO_2$ released to the atmosphere. Furthermore, it was concluded that in the intercrops, $NO_3^-$ from fertilizers was taken up by trees, resulting in less $NO_3^-$ for denitrification, and therefore less $N_2O$ emissions (Evers et al. 2010).

The affect of climate change on agriculture is also apparent. Without considering external sources of variation, photosynthesis and plant growth increased with $CO_2$, especially at higher temperatures (Mendelsohn and Dinar 2009). Although this seems promising for crops in a changing climate, for more accurate data, variables such as soil moisture, nutrients, and crop type must also be considered. For example, rice paddies are limited by N uptake when $CO_2$ and temperature increase, whereas soybean crops are not (Cure et al. 1989; Kim et al. 2003). Climate change has also been predicted to create longer and warmer periods for weeds (Ziska and Bunce 2007). It is predicted that $C_3$ plants will benefit most from an increase in $CO_2$. This could

mean that $C_3$ crops will outcompete $C_4$ weeds, or that $C_3$ weeds will outcompete $C_4$ crops, when they are inadvertently mixed (Patterson et al. 1999). Furthermore, in an intercrop study by Reza Miri et al. (2012), higher levels of chlorophyll, and higher root, stem and leaf weights were seen in $C_3$ crops than $C_4$ crops in response to elevated $CO_2$ concentrations. The competitive ability of soybean crops also increased while the cereal crops and weeds decreased which could change weed and crop dynamics in intercrops with increased $CO_2$ (Reza Miri et al. 2012).

*1.5.4 Complex Argroecosystem Management Practices*

Land use change can ultimately determine underlying soil quality and productivity. In a land use change from natural grasslands to highly tilled agricultural lands, C and N were severely decreased, with over half of the nutrients lost in the first eight years (Zhao et al. 2005). The same study also showed that after cultivated areas were abandoned, C and N increased quickly after 6 years but did not return to natural grassland levels even after 50 years (Zhao et al. 2005). This shows the importance of long-term thinking when changing to more sustainable land use and management techniques.

Land use change from current agricultural practices to sustainable agriculture may be a way to counter associated problems of industrial agriculture. Sustainable agriculture can be defined as agriculture that is efficient in resource use and production, preserves the environment, competes economically, and improves the quality of life for farmers and society (Ikerd 1993). Many forms and techniques of sustainable agriculture can help bring back diversity to ecosystems in and around the crop, alleviate pests, weeds and disease (LaMondia et al. 2002; Anderson 2010) and increase residue quality and quantity returned to the soil which can increase SOM, or minimize SOM loss (Lal et al. 2007). These practices also have the potential for C sequestration to help offset GHG emissions (Lal et al. 2007). In agroforestry, increases in soil residue additions provide more organic material to the soil, increasing the

amount of SOC that can be stored (Oelbermann et al. 2004). It is estimated that by using the sustainable practices, anywhere from 400 to 800 Mt of C could be sequestered globally each year (Lal et al. 2007). Intercropping is one example of sustainable agriculture being adopted in the Rolling Pampas that could have a smaller ecological footprint (Oelbermann and Echarte 2011).

For example, in temperate regions, intercropping is recognized as a sustainable land management practice (Hauggaard-Nielsen et al. 2001). Intercropping is defined as two or more crops grown on the same land area at the same time (Hauggaard-Nielsen et al. 2001). For example, a common type of intercropping in the temperate zone is integrating legume and cereal crops. This leads to increased N-cycling between the two crops (Kurdali 2009). Due to formation of root nodules in symbiosis with soil bacteria called *Rhizobium*, legumes are one of few plant families able to fix atmospheric N (Freiberg et al. 1997). As a limiting nutrient, fixed N is subsequently transformed to $NH_4^+$ and taken up by the non N-fixing crop in the intercrop (Chapin et al. 2002).

Land equivalent ratio, an index that shows the amount of yield for a certain space, has been shown to be higher in intercrops than sole crops (Yilmaz et al. 2008). This means that an intercrop could produce more food than a sole crop on the same amount of space. Furthermore, intercrop yields have been found to be higher than in sole crops (Li et al. 2001; Yilmaz et al. 2008). Resilience of yields under stress, (such as drought) were reported to be greater in intercrops than sole crops (Natarajan and Willey 1986). With the combination of crops adapted to the environment, decreased pest activity has been observed, as well as increased weed suppression (Banik et al. 2006). Economic benefits have also resulted from growing more than one crop, since income becomes less sporadic (Raji 2007). In addition, because legumes supply N to the surrounding crops, less inorganic fertilizer is needed (Bedoussac and Justes 2010).

Microbial diversification has been found in intercrops, as more than one plant type provides a more diverse food source and habitat for microbes (Sun et al. 2009). Furthermore, $SMB_C$ and $SMB_N$ has been shown to be higher in intercrops when compared to sole crops,

leading to higher decomposition and respiration rates from intercrop soil (Suman et al. 2006; Oelbermann and Echarte 2011). Substantially lower soil erosion and runoff rates were reported when comparing red clover and maize intercrops to maize sole crops, and cassava-based intercrops to cassava sole crops (Wall et al. 1991; Iijima et al. 2004). This was due to higher amounts of plant residue and crop cover in the intercrops, which reduced the impact of rainfall and wind on the soil (Wall et al. 1991; Iijima et al. 2004). Nutrient acquisition and efficiency were higher in intercrops than in sole crops, thought to be due to legumes fixing more N to compensate for the competitiveness of cereal crops for N (Hauggaard-Nielsen et al. 2009). Soil organic carbon and C sequestration have also been shown to increase in intercropping systems. When maize was intercropped with a woody legume, SOC increased in the intercrop treatment and decreased in the maize sole treatment (Makumba et al. 2007). This was explained by increased plant litter input and below-ground biomass (roots) in intercrops. Furthermore, they found that C was sequestered deeper in intercrops than sole crops (Makumba et al. 2007). A study in the temperate zone found that soybean/maize intercrops had lower $CO_2$ production rates that soybean sole and maize sole crops (Dyer et al. 2012).

### 1.5.5 Agriculture in Argentina and the Rolling Pampas

The shift to industrialized agriculture to use heavier inputs, larger sole crops and genetically modified crops, occurred in the 20th century in Argentina (Filloy and Belloqc 2007). Fertilizer use from 1962 to 2002 increased by approximately 600% (FAOSTAT 2011) while genetically modified crops went from occupying 6.7 million ha in 1999 to 11.8 million ha in 2001 (Nap et al. 2003). Agriculture has grown intensely, focusing on the export of soybean, sunflower, maize and cattle production (Table 1.2). The majority of crops are sole crops, however alternative forms (such as certified organic) are beginning to spread.

The Pampas (50 million ha in area) are composed of five regions that stretch from northeast Argentina to Uruguay (Herrera et al. 2009; Hall et al. 1992). The region comprising the Rolling Pampa has fertile soils that allow for 10 million ha of agriculture, where wheat, maize and soybean are the most common crops (Alvarez and Grigera 2005). Grassland in the Rolling Pampa has been reduced by 924,000 ha between 1988 to 2002 due to agricultural expansion (Bilenca and Miñarro 2004). A study by Caride et al. (2012) found that 54% of the Rolling Pampas are currently under continuous agriculture and of this, 61% is comprised of only two different crop sequences which are soybean sole crops and a rotation between soybean, wheat and soybean and maize.

**Table 1.2** Agricultural data on production of soybean oil, maize, sunflower oil, bovine meat and amount of export from Argentina in 1961, 1985 and 2009 (from FAOSTAT 2011).

| Agricultural product | 1961 | 1985 | 2009 |
| --- | --- | --- | --- |
| Soybean oil production (tonnes) | 6 | 64,498 | 109,639 |
| Maize production (tonnes) | 59,153 | 183,118 | 393,947 |
| Sunflower oil production (tonnes) | 114,100 | 269,439 | 393,584 |
| Bovine meat production (tonnes) | 1,748,961 | 2,487,000 | 2,168,934 |
| Agricultural exports (billion $US) | 0.91 | 5.65 | 26.64 |

Continuous soil use, and decreasing soil quality has prompted the need for more sustainable practices in the Argentine Pampa. To decrease soil degradation, many farmers in the Rolling Pampa adopted no-tillage practices in the 1990s (Taboada et al. 1998). Results have been conflicting, showing that no-tillage practices can increase yields, SOC, and water content of soil (Bono et al. 2008), but that more compaction can occur with no-tillage, thereby reducing crop yield (Ferreras et al. 2000). Another technique used in Argentina to reduce soil

degradation and SOC losses is intercropping (Oelbermann and Echarte 2011). An early use of intercropping was the 'three sisters' where corn, beans and squash were planted together (Wang et al. 2010). Corn grew first which allowed the bean to grow up the corn stalk, while the squash would aid in weed control (Wang et al. 2010). Although it is not a new agricultural practice, intercropping is currently re-gaining recognition in the temperate region of South America (Oelbermann and Echarte 2011).

## 1.6 Future Research Needs

Although intercropping legumes and non-legumes has been extensively studied, focus has been on tree-based intercropping (agroforestry) (Peichl et al. 2006; Beedy et al. 2010) or in the tropics (Leite et al. 2007), and on yield, root interactions, and competition of the combined crops (Giller et al. 1991; Li et al. 2001; Raji et al. 2007). To date, few studies have evaluated C and N dynamics in temperate intercropping systems (Oelbermann and Echarte 2011). Additionally, no incubation studies have evaluated the effect of soybean or maize crop residue incorporation using stable isotopes as a tool to understand the mechanisms of C and N stabilization in temperate maize-legume intercropping systems. Therefore, the goal of this study was to quantify changes in SOC, TN, soil LF, SMB and GHGs as a result of soybean or maize residue incorporation in sole and intercropped agroecosystems in the temperate zone, using the natural abundance method to trace C and N isotopes using a long-term incubation study.

# 2. STUDY SITE AND FIELD SAMPLING

## 2.1 Study Site

Soil and crop residue samples were obtained from at the Instituto Nacional de Tecnología Agropecuaria (INTA), located in the Rolling Pampas near Balcarce in the Province of Buenos Aires, Argentina (37°45′S, 58°18′W) (Ferreras et al. 2000). This research centre is located 130 m above sea level and experiences a sub-humid climate characteristic of temperate grasslands (Hall et al. 1992). From 1982 to 2011, average annual rainfall was 916 mm and the mean annual temperature was 14.1°C (Table 1) (INTA 2012). Study site soil is of the *Mar del Plata* series, and is a loess soil that developed from wind blown silt (Domínguez et al. 2009; Nosetto et al. 2012).

**Table 2.1** Average precipitation, monthly high, low and mean temperatures for the Instituto Nacional de Tecnología Agropecuaria (INTA) at Balcarce, Argentina. Data are an average of 30 years from 1981-2011 (INTA 2012).

|  | Monthly mean precipitation (mm) | Monthly mean high temperature (°C) | Monthly mean low temperature (°C) | Monthly mean temperature (°C) |
|---|---|---|---|---|
| January | 113.57 | 27.64 | 14.09 | 20.86 |
| February | 81.05 | 26.41 | 13.57 | 19.99 |
| March | 91.74 | 24.34 | 12.29 | 18.31 |
| April | 74.91 | 20.35 | 8.97 | 14.66 |
| May | 60.59 | 16.39 | 6.21 | 11.30 |
| June | 52.06 | 12.95 | 3.79 | 8.37 |
| July | 50.10 | 12.23 | 3.04 | 7.63 |
| August | 50.22 | 14.32 | 3.91 | 9.12 |
| September | 59.72 | 16.20 | 4.95 | 10.58 |
| October | 92.42 | 19.38 | 7.57 | 13.48 |
| November | 94.29 | 22.65 | 9.90 | 16.28 |
| December | 95.43 | 25.81 | 12.19 | 19.00 |
| **Total/Mean** | **916.10** | **19.89** | **8.37** | **14.13** |

Soil at the site was classified as a Typic Argiudoll (USDA) (Andrade 1995) and Luvic Phaeozem (FAO) (Taboada et al. 1998) with a soil texture of 41.1% sand, 35.8% silt, and 23.1% clay. The average SOC concentration at the site is 33 g/kg (Domínguez et al. 2009). These soil properties and favourable climate characteristics make the Rolling Pampa (approximately 10 million hectares) relatively fertile, and therefore do not require high inputs of inorganic fertilizers (Alvarez and Grigera 2005).

The study site area was historically grassland ($C_3$/$C_4$ mixture), and was converted to cropland approximately forty years ago. Since cultivation, 50-70% of the land area has been continually used for grain cropping, causing soils to become N deficient and prone to crusting due to losses of SOM, develop a plough pan (compaction at the bottom of the plough) and be susceptible to erosion (Hall et al. 1992). To prevent further soil degradation, most agriculture in the region changed from conventional tillage to no-tillage in the 1990s (Alvarez et al. 2009). Currently, the main crops in the rolling Pampa region are wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and soybean (*Glycine max* (L.) *Merr*.). The rotation between intercrops (such as wheat and soybean) with maize sole crops is common in the area (Alvarez and Grigera 2005).

To control for variation, the study site was arranged in a randomized complete block design (RCBD) with three replicates of each of the following treatments; soybean sole crop, maize sole crop, 1:2 intercrop and 2:3 intercrop (Figure 2.1) (Gomez and Gomez 1984). The ratios represent the number of maize rows: the number of soybean rows and were chosen because they are commonly used in this region (Oelbermann and Echarte 2011). All intercrops have been sown in the same plot since the beginning of the study site in 2006, whereas the sole crops have been rotated since 2010 due to pest problems. From the sampled crops, maize was sown on October 19th, 2010 and soybean on November 17th, 2010. Phosphorus fertilzer (35 kg/ha) was applied to all treatments, and urea fertilizer (150 kg/ha) was applied to maize in sole crops, and to intercrops by hand at the bottom of the stalk. Soybeans in sole crops and intercrops were inoculated with *Bradyrhizobium japonicum*. Plant densities (plants per m²) were

**Figure 2.1.** Schematic of the randomized complete block design (RCBD) at INTA showing the three replicates of each soybean sole crop, maize sole crop, 1:2 (maize:soybean) and 2:3 (maize:soybean) intercrops, and measurements of the study site.

8 in maize sole crops, 30 in soybean sole crops, 4.4 in 1:2 intercrops and 5.3 in 2:3 intercrops. Maize was harvested on March 16th, 2011 and soybean on April 18th, 2011. Residue input ($g/m^2$) from each treatment was 904 C and 15.5 N from maize sole crops, 502 C and 14.2 N from soybean sole crops, 552 C and 10.4 N from 1:2 intercrops and 768 C and 13.3 N from 2:3 intercrops (Vachon 2008).

### 2.1.1 Soil and Plant Sampling

Soil was collected on May 3rd, 2011 from the top 20 cm, using a soil corer with an inner diameter of 5 cm. Five samples were taken from in the centre of each treatment in each replicate to avoid edge effects (Pennock et al. 2008). In intercropped treatments, soil was taken between all possible combinations of rows (between two maize rows, between two soybean rows and

between a maize and soybean row). Samples from each treatment in each replicate were weighed and combined. Before bringing soil to the University of Waterloo, it was air dried and passed through a 2 mm sieve to remove rocks, gravel and coarse crop residues and roots.

Soybean and maize residues were collected on May 12th, 2011 from each treatment in each replicate. Stems and leaves were collected to represent organic matter that remains on the field after harvest. Approximately 10 g of soybean residue was collected from random plants in each plot containing soybean, and combined. The same procedure was used for maize residues. Crop residues were dried for 24 h at 65°C, then ground to <2 mm (Wiley mill). Maize and soybean residues were ground separately as to not contaminate their $\delta^{13}C$ and $\delta^{15}N$ values (Table 2.2). Soybean residue is unexpectedly more enriched in $^{15}N$ than maize residue, possibly due to the lower $\delta^{15}N$ value of fertilizers, depleting maize residues but not soybean residues (Votoria et al. 2004).

**Table 2.2** Initial carbon and nitrogen characteristics for soybean and maize residues used in the laboratory incubation experiment.

| Characteristic | Soybean residue (C₃) | Maize residue (C₄) |
|---|---|---|
| C (%) | 44.8 | 42.2 |
| N (%) | 1.4 | 0.66 |
| C/N ratio | 32.0 | 63.9 |
| $\delta^{13}C$ (‰) | -28.62 | -11.89 |
| $\delta^{15}N$ (‰) | +3.14 | +2.47 |

## 2.2 Laboratory Incubation Experiment

The laboratory incubation experiment was conducted at the University of Waterloo with soil and crop residue samples collected in Argentina. In one set of 1 L jars, 1.5 g of soybean residue

was added to 60 g of soybean sole crop, 1:2 and 2:3 intercrop soil. In another set of 1 L jars, 1.5 g of maize residue was added to 60 g of maize sole crop, 1:2 and 2:3 intercrop soil. A set of control jars contained 60 g of each soil type with no added residue. Another control jar contained only ambient air (Figure 2.2 and Table 2.3). Each jar was replicated three times (corresponding to the plots in the RCBD) for each of five destructive sampling points (a total of 165 jars on day 1 of the incubation and 31 less after each destructive sampling point every 35 days). Residue addition was based on an experiment with soil and residue mixtures from the same site (Dyer 2010).
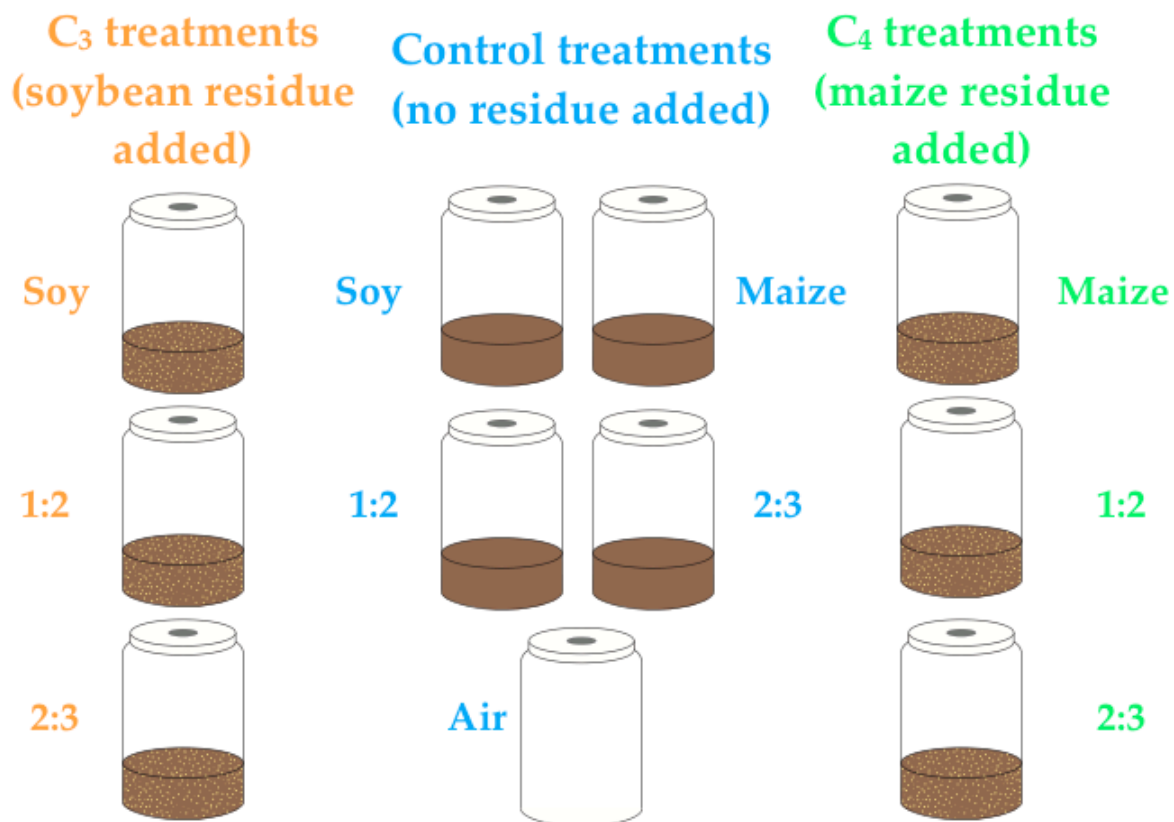


**Figure 2.2** Schematic of the incubation experiment set-up. Text beside the jars corresponds to the soil type (Soy=soybean sole crop, Maize=maize sole crop and 1:2 and 2:3=intercrop soil, ratios representing rows of maize: rows of soybean). The type of residue added to each jar is noted at the top.

**Table 2.3** Description of each treatment

| Treatment | Description |
|-----------|-------------|
| C$_3$-S | Soybean sole crop soil with added soybean residue |
| C$_3$-1:2 | 1:2 intercrop soil with added soybean residue |
| C$_3$-2:3 | 2:3 intercrop soil with added soybean residue |
| C$_4$-M | Maize sole crop soil with added maize residue |
| C$_4$-1:2 | 1:2 intercrop soil with added maize residue |
| C$_4$-2:3 | 2:3 intercrop soil with added maize residue |
| Cont-S | Soybean sole crop soil only (control) |
| Cont-M | Maize sole crop soil only (control) |
| Cont-1:2 | 1:2 intercrop soil only (control) |
| Cont-2:3 | 2:3 intercrop soil only (control) |

Before the incubation was initiated, soil water content was adjusted to 60% field capacity (10.5 ml for 60 g of soil) using deionized water (DI). Field capacity was quantified by placing 8 ml of water and 50 g of soil in a test tube, covering it with punctured paraffin paper and letting it sit for 12 h. A soil sample from the middle of the test tube was taken, weighed, dried at 105°C and reweighed. The amount of water to add to obtain a field capacity (FC) of 60% was quantified using the following equation from Stewart et al. (2009):

$$60\% \ FC = \left( \frac{wet \ soil \ (g) - dry \ soil \ (g)}{dry \ soil \ (g)} \right) \times 60\%$$

(1)

Each jar was tightly sealed using septa fixed with silicon gel, which made the experiment a closed system and allowed for gas samples to be taken from the headspace of the jar without

removing the lid. Jars were incubated at a constant temperature of 21°C (any variation on sampling days was recorded) and kept in the dark for 140 days.

Greenhouse gas samples ($CO_2$ and $N_2O$) were taken once every seven days (doubled for the first 14 days) using a syringe to extract 4 ml of gas from the headspace, and transferred to evacuated 3 ml Exetainer vials (Labco Limited, Ceredigion, UK) until ready for analysis (Chapter 5). The same amount of ambient air was then injected back into the jars. Once every 35 days (days 1, 35, 70, 105 and 140), 15 ml of gas was taken from the headspace, and transferred to evacuated 12 ml Exetainer vials (Labco Limited, Ceredigion, UK) until ready for $\delta^{13}C$-$CO_2$ analysis (Chapter 5). This was followed by destructive sampling for the analysis of water content, SOC and TN concentrations and $\delta^{13}C$-SOC and $\delta^{15}N$-TN, soil $LF_C$, $LF_N$ and $\delta^{13}C$-LF and $\delta^{15}N$-LF (Chapter 3), as well as $SMB_C$ and $SMB_N$ and $\delta^{13}C$-SMB and $\delta^{15}N$-SMB and soil microbial community structure using Biolog Ecoplates™ (Chapter 5).

Stable C and N isotopes were measured using a Costech ECS4010 elemental analyzer coupled to a Delta V mass spectrometer equipped with a Conflo IV interface at the Stable Isotopes Laboratory at the University of Saskatchewan. Delta $^{13}C$ values were compared to the Pee Dee Belemnite standard and $\delta^{15}N$ values compared to air. Isotope values were reported in per mill (‰) and quantified using the following equation:

$$\delta = 1000 \times \left( \frac{R_{sample}}{R_{standard}} \right) - 1$$

(2)

where

$$R = \frac{C^{13}}{C^{12}} \; or \; \frac{N^{15}}{N^{14}}$$

(3)

The maximum machine variation for each characteristic are; 1.8% for C and N concentrations, 0.5% for $\delta^{13}C$, 4.5% for $\delta^{15}N$, and 1.0% for $\delta^{13}C$-$CO_2$.

# 3. CARBON STABILIZATION: SOIL ORGANIC CARBON, SOIL TOTAL NITROGEN, AND SOIL LIGHT FRACTION

## 3.1 Introduction

Globally, soils store two-thirds of all terrestrial carbon (C) in soil organic matter (SOM) (Schimel et al. 1994). Soil organic carbon (SOC) can be affected by the rate of organic matter decomposition, climate, and soil chemical and physical characteristics, although changes can take many years to be observed (Oelbermann et al. 2006). Most terrestrial nitrogen (N) is also stored in SOM. The addition of fertilizers, erosion, and decomposition can increase or decrease the amount of soil N (National Research Council 1993). An important fraction of the SOM is the light fraction (LF) which mainly consists of partly decomposed plant matter, is extremely labile and is less physically protected than other fractions (Soon et al. 2009). The LF is influenced by the quantity and quality of residue input (Soon et al. 2009). A high residue quality is characterized by being accessible for decomposition by soil microbes, having a high N concentrations (low C/N ratio), and a low lignin concentration (Millar and Baggs 2004). (Alvarez et al. 1998). Therefore, changes in agricultural management practices which affect the nature and quantity of crop residues have a significant impact on soil $LF_C$ and $LF_N$.

Changes in land use and agricultural management practices strongly influence the quantity of SOC, total N (TN) and LF. For example, in a semiarid region in China, conversion of grassland to cropland decreased SOC stocks (Qiu et al. 2012). Furthermore, van Groenigen et al. (2011) found higher SOC stocks in crops with reduced tillage where crop residue remained, than in crops where conventional tillage and residue removal was used. Soil N quantity is also strongly affected by land use management. For example, TN stocks were significantly higher with zero-tillage, the addition of fertilizer and use of cover crops, than with conventional tillage and no cover crops (Mazzoncini et al. 2011). Furthermore, sequestration of SOC and N are seen

to be dependent upon management practice and quality of residue input to the soil (Wright and Hons 2005). An increase in residue inputs results in an increase in SOC stocks, which lowers loses of $CO_2$ to the atmosphere. Light fraction, closely related to residues, is a responsive fraction of SOM and can be used as an indicator of soil quality with changes in land use (He et al. 2008). For instance, a study by Malhi et al. (2003) compared cultivated fields and natural grasslands and found that $LF_C$ and $LF_N$ were 82 to 85% lower in cultivated fields than grasslands. Furthermore, SOC and TN were up to 34% lower in cultivated fields, showing the usefulness of the LF as an indicator of future affects on SOM.

In the southeastern part of the Rolling Pampa, where agriculture is prominent, SOC was 33.4 g/kg and organic N was 2.6 g/kg in 2009 (Domínguez et al. 2009). However, estimates by Alvarez (2001) state that anywhere from three 3 to 5 cm of topsoil (and 35% of topsoil C) have been lost due to cultivation. The use of intercrops is being adapted in temperate regions such as the Argentine Pampa in efforts to decrease SOC losses and soil degradation (Oelbermann and Echarte 2011). Intercropping has been shown to increase SOC stocks and sequester more C in the soil when compared to sole crops (Makumba et al. 2007). It has also been shown that intercropping a legume and a non-legume (for example, pea and barley) resulted in more soil N cycling. This may be due to increased uptake of N by the cereal crop, and more N fixation by the legume crop, to compensate for the competition (Hauggaard-Nielsen et al. 2009; Kurdali 2009). Furthermore, C and N in the LF were higher in intercropped treatments than sole crop treatments, as well as a lower $LF_C$: $LF_N$ ratio, indicating more available N for crops (Beedy et al. 2010).

The use of isotope techniques has been used to further understand the process of SOC stabilization. One approach is to determine contributions to SOC from sources with naturally different isotopic values, for example when there is a shift from $C_3$ (~-27‰) to $C_4$ (~-13‰) vegetation (Boutton et al. 1998). The change in $\delta^{13}C$ value of the soil will change at a rate depending on the decay rate of individual SOM fractions (Boutton et al. 1998). Integrating

isotopes for use in soil pools with different turnover times is also beneficial. Isotope ratios from the soil LF are also useful, since new plant material incorporated into the LF will show changes faster than heavier fractions (Boutton et al. 1998). Other nutrient cycles have also been analyzed by isotopes, for example by using N isotopes in intercropping to track N transfer between legumes and cereal crops (Giller et al. 1991).

In incubation studies to date, SOC, TN, and LF in intercrops have been studied simultaneously in research that focused on SOM dynamics in grasslands or sole crops (Accoe et al. 2004; Haile-Mariam et al 2008; Creamer et al. 2011). However, only one field study has assessed these characteristics together on intercrop soils from the temperate zone (Oelbermann and Echarte 2011). Similarly, C and N isotopes of SOC, TN, $LF_C$ and $LF_N$ have been used in incubation experiments, however the focus is on either C or N (Accoe et al. 2004; Crow et al. 2006; Creamer et al. 2011). Furthermore, isotopes of SOC, TN and the LF have been used in $C_3$/$C_4$ intercropping systems in the temperate region in a field study, but not in a controlled incubation (Oelberman and Echarte 2011). There is also currently a lack of knowledge on the affects of $C_3$ and $C_4$ crop residue decomposition in $C_3$/$C_4$ mixed soil on SOC, TN and LF. Soil organic carbon turnover times, and decomposition rates of SOC and LF due to $C_3$ and $C_4$ crop residue input have been evaluated in $C_3$/$C_4$ mixed soils (Martin et al 1990; Wynn and Bird 2007; Marschner et al. 2008; Oelbermann and Echarte 2011). The effect on TN due to the introduction of a $C_4$ grass onto a $C_3$ dominated soil has also been evaluated (Mahaney et al. 2008). However, the effects on SOC, TN, LF, and respective isotopes due to $C_3$ and $C_4$ crop residue input in $C_3$/$C_4$ mixed soils from a temperate intercropping system has never been studied.

This laboratory incubation experiment aimed to study C and N transformations and pathways resulting from the amendment of crop residues, to understand soybean and maize crop residue stabilization in sole crops and intercrops. This was accomplished by the following three objectives:

(a) To quantify changes in SOC, TN and LF concentrations, and $\delta^{13}C$ and $\delta^{15}N$ values due to soybean and maize crop residue input.

(b) To quantify C concentrations derived from soybean and maize crop residues into the whole SOC and the LF using $\delta^{13}C$ natural abundance.

(c) To quantify changes in soil TN and $LF_N$ concentrations, as well as $\delta^{15}N$-TN and $\delta^{15}N$-LF due to incorporation of soybean and maize crop residues.

## 3.2 Materials and Methods

### 3.2.1 Soil Organic Carbon and Total Nitrogen

Carbonates were removed prior to measurements of SOC, TN and isotopes, using the acidification method, which volatilizes C from the calcitic or dolomitic soil minerals. (Midwood and Boutton 1998). Soil was oven-dried at 40°C and ground to <250um using a ball mill (Retsch MM200). Carbonates were removed by adding 30 ml of 0.5M HCl to 5 g of soil and shaking the mixture, at 400 rpm for 30 minutes (Heidolph Unimax 1010 DT). This was repeated three times over 24 h, and was left to settle in between shaking times. HCl was then poured off, and soil was washed and shaken with DI every 12 h until soil stayed suspended in the DI. Soil was oven dried for 24 h at 40°C and reground to <250um using a ball mill (Retsch MM200) (Midwood and Boutton 1998).

Following carbonate removal, soil samples were packed into 9 mm x 5 mm tin capsules (each containing 15 to 20 mg soil) in preparation for analysis. Elemental (on all five sampling days) and isotopic analysis (only days 1, 70 and 140) of C and N were done at the University of Saskatchewan Stable Isotopes Laboratory (Costech ECS4010 elemental analyzer coupled to a Delta V mass spectrometer with Conflo IV interface). Soil organic carbon and TN concentrations were presented in g C/kg. SOC/TN ratios were also quantified. Soil moisture

content was quantified using the gravimetric method (Reynolds 1970) so that data could be expressed in an oven-dry weight basis.

### 3.2.2 Soil Organic Carbon and Total Nitrogen Stable Isotopes

Proportions of SOC on days 1, 70 and 140 derived from applied residue C and soil C sources when soybean or maize residues were applied, was quantified using a two end-member mixing model (Liang et al. 1999).

$$applied \cdot C(\%) = \left(\frac{\delta_{SOC} - \delta_{Cont}}{\delta_{residue} - \delta_{Cont}}\right) \times 100$$

(4)

and

$$soil \cdot C(\%) = 1 - applied \cdot C(\%)$$

(5)

where $\delta_{SOC}$ is $\delta^{13}C$ of SOC from soils with added residue, $\delta_{Cont}$ is $\delta^{13}C$ of SOC from the corresponding Control treatment with no residue added, and $\delta_{residue}$ is the $\delta^{13}C$ of the soybean (-28.62‰) or maize residue (-11.89‰) added. Contribution from applied residue C and soil C sources to SOC concentrations were found by multiplying applied and soil C proportions by SOC concentration (g C/kg). Nitrogen isotope values were reported and discussed in per mill (‰).

### 3.2.3 Soil Light Fraction

Light fraction was collected by density fractionation using the procedure described by Gregorich and Beare (2006). Approximately 10 g of soil was covered and let air-dry at room temperature (for seven days). Dried soil was placed into 100 ml glass jars and shaken with 35 ml of NaI with a specific density of $1.7g/cm^3$, for one hour at 400 rpm (Heidolph Unimax 1010 DT). The sides of the glass jars were then rinsed with NaI, covered at room temperature for 24 h. During this time the heavy fraction (above the specific density of $1.7g/cm^3$) sunk and the

light fraction (below the specific density of 1.7 g/cm³) floated.  The LF and top 2 cm of NaI were removed via aspiration onto a glass fibre filter.  NaI was washed from the LF using 75 ml of 0.01 CaCl₂, and CaCl₂ was washed from the LF using 75 ml of DI.  The LF was washed off the filter into a pre-weighed aluminum dish, dried at 40°C, weighed and ground to <250um using a ball mill (Retsch MM200).  It was then packed into 9 mm x 5 mm tin capsules (each containing 2 to 5 mg of LF) in preparation for analysis.  Elemental (on all five sampling days) and isotopic analysis (days 1, 70 and 140 only) of $LF_C$ and $LF_N$ were done at the University of Saskatchewan Stable Isotopes Laboratory (Costech ECS4010 elemental analyzer coupled to a Delta V mass spectrometer with Conflo IV interface).  Light fraction concentration was presented in g C or N/ kg.  The $LF_{C/N}$ ratio was also quantified.  Proportions of $LF_C$ derived from applied residue C and old C sources, were quantified using equations 4 and 5 described in section 3.2.2.  In the equations, $\delta_{SOC}$ was replaced with $\delta_{LFC}$.  Contributions from applied and soil C sources to LF concentrations were found by multiplying the applied and soil C source proportions by LF concentration (g C/kg).

## 3.3 Statistical Analysis

Data were tested for normal distribution ($p>0.05$; Shapiro-Wilk) and equal variances ($p>0.05$; Levene's).  When data were not normally distributed ($LF_C$ and $LF_C/LF_N$ ratio), the following statistical tests were performed on log transformed data. Although data were taken from separate jars, the same soil and applied residue was measured repeatedly over time (Swanston et al. 2002).  Therefore, differences between treatments on each day, between sampling days for each treatment, interaction effects from treatment by time, as well as overall means (averaged over the 140 d incubation), were analyzed using a two-way repeated measures analysis of variance (ANOVA).  Sampling day was used as the within subject repeated measure, and treatment type was used as the between subject main factor (Norman and Streiner 2008).  When

the ANOVA had significant main effects or interactions, a Tukey's post-hoc multiple comparison test with a Bonferroni correction was used to identify where differences were (simple effects). The Bonferroni correction was used to account for the dependence of samples in the repeated measures analysis (Rice 1989). For all statistical analyses the threshold probability level for determining significant differences was a p-value less than 0.05. All data analyses were carried out in IBM SPSS Statistics (version 21, 2012).

### 3.4 Results

*3.4.1 Soil Organic Carbon*

Initial values of SOC and TN did not differ significantly between treatments except for $C_4$-M, which was had significantly higher SOC than Cont-M (Table 3.1). Although not significant, SOC and TN in $C_3$, $C_4$, and Control intercrop treatments was higher than in the sole crop treatments. Initial $\delta^{13}C$-SOC values were significantly different between $C_3$, $C_4$ and Control treatments. $C_3$ treatments were the most depleted and $C_4$ treatments were the most enriched. Initial $\delta^{15}N$-TN values showed only significant differences between $C_3$ and corresponding Control treatments.

The interaction effect of treatment by day was not significant [$F(4,36) = 0.77$, $p = 0.81$] for SOC concentration (g C/kg). Main effects showed significant differences between treatments, and over time (Table 3.2). For example, there were significance differences between $C_3$-S, $C_4$-M and their corresponding Control treatments. $C_4$ intercrop treatments were higher than the maize sole crop on almost all days. Cont-2:3 was higher than sole treatments on all days but day 105. There were significant difference between sampling days in all $C_4$ treatments and the $C_3$-2:3 treatment, where day 1 was significantly higher than day 35. In all treatments, SOC initially decreased, then increased until day 140. Overall means of treatments with intercrop soil were higher than corresponding sole crop soil, but not significantly higher.

The interaction effect of treatment by day was not significant [$F_{(18,38)} = 1.07$, $p = 0.42$] for $\delta^{13}C$-SOC (‰). Main effects showed significant differences between treatments and between days (Table 3.3). All $C_3$ $\delta^{13}C$-SOC values (from -24.10‰ to -23.10‰) were significantly more depleted than $C_4$ (from -19.83‰ to -19.20‰), as were the overall means. All $C_3$ and $C_4$ treatments on all days (1, 70 and 140) differed from the corresponding Control treatments (from -21.88‰ to -21.61‰). All $C_3$ treatments became more depleted in $^{13}C$ between day 1 and 70 and became more enriched between day 70 and 140 (only significant for $C_3$-S). Control treatments were constant, with no significant differences between treatments or between days.

The interaction effect of treatment by day was not significant for SOC derived from new C sources (g C/kg) [$F_{(10,22)} = 0.87$, $p = 0.57$]. The main effects of treatment and time were not significant (Table 3.4a). Contributions from applied residues to SOC were higher in the $C_3$ treatments than the $C_4$ treatments after day 1, as were the overall means. $C_3$ intercrop treatments had less SOC derived from soybean residues than $C_3$-S. The interaction effect of treatment by day was not significant for SOC derived from old C sources (g C/kg) [$F_{(10,22)} = 0.34$, $p = 0.94$]. There was a significant main effect of time (day 1 was higher than day 70 in C3-1:2) but no significant main effect of treatment (Table 3.4b). $C_3$ intercrop treatments were consistently higher than $C_3$-S, as were the means. From day 1 to 140 all treatments decreased in proportion derived from old C sources except $C_3$-2:3. Soil organic carbon derived from old C sources was significantly higher than SOC derived from the applied residues (Appendix Table 1).

**Table 3.1** Initial soil organic carbon and total nitrogen concentrations (g C/kg) and C and N isotope values (‰) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added.

| Treatment | SOC (g C/kg) | TN (g N/kg) | $\delta^{13}$C-SOC (‰) | $\delta^{15}$N-TN (‰) |
|---|---|---|---|---|
| $C_3$-S | 34.77 (4.62)[a] | 2.34 (0.37)[a] | -23.62 (0.19)[a,*] | 6.74 (0.06)[a,*] |
| $C_3$-1:2 | 36.05 (2.22)[a] | 2.56 (0.09)[a] | -23.25 (0.05)[a,*] | 6.53 (0.01)[a,*] |
| $C_3$-2:3 | 35.37 (2.02)[a] | 2.52 (0.15)[a] | -23.32 (0.12)[a,*] | 6.65 (0.09)[a,*] |
| $C_4$-M | 35.40 (1.80)[a,*] | 2.37 (0.13)[a] | -19.20 (0.12)[b,*] | 6.60 (0.11)[a] |
| $C_4$-1:2 | 37.43 (1.44)[a] | 2.50 (0.12)[a] | -19.23 (0.07)[b,*] | 6.40 (0.16)[a] |
| $C_4$-2:3 | 37.74 (1.26)[a] | 2.73 (0.09)[a] | -19.81 (0.10)[c,*] | 6.61 (0.10)[a] |
| Cont-S | 26.30 (1.37)[z] | 1.94 (0.08)[z] | -21.63 (0.06)[z] | 6.93 (0.18)[z] |
| Cont-M | 21.81 (0.96)[z] | 2.32 (0.13)[z] | -21.73 (0.04)[z] | 6.90 (0.06)[z] |
| Cont-1:2 | 25.60 (2.19)[z] | 2.25 (0.17)[z] | -21.62 (0.04)[z] | 6.90 (0.09)[z] |
| Cont-2:3 | 26.72 (1.56)[z] | 2.34 (0.14)[z] | -21.66 (0.08)[z] | 7.12 (0.11)[z] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a) and between Control treatments (z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 3.2** Soil organic carbon concentration (g C/kg) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 34.77 (4.62)$^{a,A}$ | 36.05 (2.22)$^{a,A}$ | 35.37 (2.02)$^{a,A}$ | 35.40 (1.80)$^{a,*,A}$ | 37.43 (1.44)$^{a,A}$ | 37.74 (1.26)$^{a,A}$ |
| 35 | 29.78 (1.43)$^{a,*A}$ | 27.35 (1.44)$^{a,A}$ | 24.51 (0.30)$^{a,B}$ | 24.95 (2.90)$^{a,B}$ | 27.43 (1.50)$^{a,B}$ | 26.74 (2.01)$^{a,B}$ |
| 70 | 31.55 (2.53)$^{a,A}$ | 30.52 (0.67)$^{a,A}$ | 33.39 (2.89)$^{a,A}$ | 28.91 (1.60)$^{a,AB}$ | 29.13 (1.88)$^{a,AB}$ | 31.56 (1.98)$^{a,AB}$ |
| 105 | 32.18 (1.87)$^{a,A}$ | 29.67 (3.33)$^{a,A}$ | 32.10 (2.98)$^{a,AB}$ | 30.77 (2.20)$^{a,AB}$ | 31.88 (2.23)$^{a,AB}$ | 27.15 (1.12)$^{a,B}$ |
| 140 | 29.45 (6.66)$^{a,A}$ | 34.36 (5.03)$^{a,A}$ | 34.95 (2.60)$^{a,AB}$ | 30.37 (0.89)$^{a,AB}$ | 30.35 (4.23)$^{a,AB}$ | 33.52 (4.07)$^{a,AB}$ |
| Mean | 31.55 (2.47)$^{a,*}$ | 31.59 (2.01)$^{a}$ | 32.06 (0.90)$^{a}$ | 30.08 (1.03)$^{a}$ | 31.25 (1.32)$^{a}$ | 31.34 (1.41)$^{a}$ |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | 26.30 (1.37)$^{z,Z}$ | 21.81 (0.96)$^{z,Z}$ | 25.60 (2.19)$^{z,Z}$ | 26.72 (1.56)$^{z,Z}$ |
| 35 | 18.11 (2.32)$^{z,Z}$ | 20.81 (2.23)$^{z,Z}$ | 21.96 (0.66)$^{z,Z}$ | 21.59 (1.85)$^{z,Z}$ |
| 70 | 22.10 (1.75)$^{z,Z}$ | 20.77 (4.16)$^{z,Z}$ | 20.60 (2.31)$^{z,Z}$ | 25.46 (1.39)$^{z,Z}$ |
| 105 | 22.85 (0.95)$^{z,Z}$ | 22.70 (0.86)$^{z,Z}$ | 21.66 (0.91)$^{z,Z}$ | 21.68 (1.50)$^{z,Z}$ |
| 140 | 22.83 (2.57)$^{z,Z}$ | 23.05 (2.96)$^{z,Z}$ | 27.47 (1.63)$^{z,Z}$ | 26.68 (1.70)$^{z,Z}$ |
| Mean | 22.44 (1.39)$^{z,Z}$ | 21.83 (1.63)$^{z,Z}$ | 22.93 (1.75)$^{z,Z}$ | 24.43 (1.44)$^{z,Z}$ |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatments. Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 3.3** Soil organic carbon delta $^{13}C$ values (‰) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | -23.62 (0.19)$^{a,*,AB}$ | -23.25 (0.05)$^{a,*,A}$ | -23.32 (0.12)$^{a,*,A}$ | -19.20 (0.12)$^{b,*,A}$ | -19.23 (0.07)$^{b,*,A}$ | -19.81 (0.10)$^{c,*,A}$ |
| 70 | -24.10 (0.34)$^{a,*,A}$ | -23.81 (0.06)$^{a,*,A}$ | -23.58 (0.45)$^{a,*,A}$ | -19.60 (0.11)$^{b,*,A}$ | -19.56 (0.14)$^{b,*,A}$ | -19.63 (0.33)$^{b,*,A}$ |
| 140 | -23.64 (0.55)$^{a,*,B}$ | -23.18 (0.10)$^{a,*,A}$ | -23.32 (0.11)$^{a,*,A}$ | -19.62 (0.25)$^{b,*,A}$ | -19.83 (0.10)$^{b,*,A}$ | -19.64 (0.18)$^{b,*,A}$ |
| Mean | -23.73 (0.25)$^{a,*}$ | -23.41 (0.02)$^{a,*}$ | -23.41 (0.16)$^{a,*}$ | -19.47 (0.10)$^{b,*}$ | -19.51 (0.03)$^{b,*}$ | -19.69 (0.10)$^{b,*}$ |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | -21.63 (0.06)$^{z,Z}$ | -21.73 (0.04)$^{z,Z}$ | -21.62 (0.04)$^{z,Z}$ | -21.66 (0.08)$^{z,Z}$ |
| 70 | -21.61 (0.03)$^{z,Z}$ | -21.88 (0.02)$^{z,Z}$ | -21.81 (0.06)$^{z,Z}$ | -21.64 (0.15)$^{z,Z}$ |
| 140 | -21.61 (0.01)$^{z,Z}$ | -21.83 (0.03)$^{z,Z}$ | -21.71 (0.06)$^{z,Z}$ | -21.80 (0.08)$^{z,Z}$ |
| Mean | -21.62 (0.03)$^{z}$ | -21.82 (0.02)$^{z}$ | -21.71 (0.07)$^{z}$ | -21.70 (0.10)$^{z}$ |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 3.4** Soil organic carbon derived from **a) applied residue** and **b) soil carbon sources** (g C/kg) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil. Overall means (bottom row) were averaged over the 140 day incubation.

a)

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 9.56 (0.33)[a,A] | 8.45 (0.90)[a,A] | 8.43 (1.04)[a,A] | 9.09 (0.35)[a,A] | 9.16 (0.16)[a,A] | 7.14 (0.56)[a,A] |
| 70 | 11.43 (2.31)[a,A] | 8.95 (0.27)[a,A] | 9.57 (3.19)[a,A] | 6.56 (0.15)[a,A] | 6.57 (0.47)[a,A] | 6.48 (0.80)[a,A] |
| 140 | 7.77 (1.13)[a,A] | 7.38 (1.33)[a,A] | 7.76 (0.45)[a,A] | 6.74 (0.64)[a,A] | 5.32 (1.07)[a,A] | 7.44 (1.77)[a,A] |
| Mean | 9.59 (1.06)[a] | 8.26 (0.46)[a] | 8.59 (0.53)[a] | 7.47 (0.82)[a] | 7.02 (1.13)[a] | 7.02 (0.28)[a] |

b)

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 25.21 (4.55)[a,A] | 27.60 (1.35)[a,A] | 26.94 (1.70)[a,A] | 26.31 (1.63)[a,A] | 28.27 (1.48)[a,A] | 30.61 (0.87)[a,A] |
| 70 | 20.12 (0.54)[a,A] | 21.57 (0.42)[a,B] | 23.81 (1.27)[a,A] | 22.35 (1.53)[a,A] | 22.57 (1.84)[a,A] | 25.08 (2.00)[a,A] |
| 140 | 21.68 (6.66)[a,A] | 26.98 (3.79)[a,AB] | 27.19 (2.90)[a,A] | 23.63 (1.23)[a,A] | 21.75 (3.57)[a,A] | 26.09 (3.39)[a,A] |
| Mean | 22.33 (1.51)[a] | 25.38 (1.92)[a] | 25.98 (1.09)[a] | 24.10 (1.17)[a] | 24.19 (2.05)[a] | 27.26 (1.70)[a] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$). Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

*3.4.2 Soil Total Nitrogen*

The interaction effect of treatment by day was not significant [$F(36,80) = 1.28$, $p = 0.18$] for soil TN concentrations (g N/kg).  Main effects showed no significant differences between treatments (Table 3.5).  Although not significant, $C_3$ and $C_4$ TN concentrations were consistently higher than Control TN concentrations.  $C_3$ intercrop treatments were higher than $C_3$-S on day 1, 70 and 140, while $C_4$ intercrop treatments were more consistently higher than $C_4$-M.  The main effect of time was significant in $C_3$-1:2 and $C_4$-2:3.  All treatments except Cont-S and Cont-2:3 decreased in TN concentrations from day 1 to day 140.

The interaction effect of treatment by day was not significant [$F(9,20) = 1.09$, $p = 0.41$] for $\delta^{15}$N-TN.  There were significant main effects of treatment and day (Table 3.6).  On day 1, $C_3$ treatments were significantly more enriched than $C_4$ treatments and significantly more depleted than Control treatments.  On day 140, $C_3$ and $C_4$ treatments were all significantly more depleted than corresponding Control treatments.  There were significant differences between days in $C_3$-S and $C_3$-1:2 treatments only.  All $C_3$ treatments increased (became more enriched in $^{15}$N) and all $C_4$ treatment decreased (became more depleted) over the incubation.  Sole crop Control treatments became more enriched while intercropped Control treatments became more depleted from day 1 to day 140.

44

**Table 3.5** Total nitrogen concentrations (g N/kg) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | $2.34\ (0.37)^{a,A}$ | $2.56\ (0.09)^{a,A}$ | $2.52\ (0.15)^{a,A}$ | $2.37\ (0.13)^{a,A}$ | $2.50\ (0.12)^{a,A}$ | $2.73\ (0.09)^{a,A}$ |
| 35 | $2.34\ (0.21)^{a,A}$ | $1.94\ (0.04)^{a,AB}$ | $1.83\ (0.05)^{a,A}$ | $1.86\ (0.24)^{a,A}$ | $2.08\ (0.07)^{a,A}$ | $2.09\ (0.24)^{a,AB}$ |
| 70 | $1.86\ (0.04)^{a,A}$ | $1.94\ (0.04)^{a,B}$ | $2.22\ (0.12)^{a,A}$ | $2.01\ (0.16)^{a,A}$ | $2.03\ (0.14)^{a,A}$ | $2.19\ (0.19)^{a,AB}$ |
| 105 | $2.53\ (0.29)^{a,A}$ | $2.38\ (0.29)^{a,AB}$ | $2.45\ (0.20)^{a,A}$ | $2.56\ (0.19)^{a,A}$ | $2.71\ (0.21)^{a,A}$ | $2.06\ (0.08)^{a,B}$ |
| 140 | $2.28\ (0.43)^{a,A}$ | $2.47\ (0.28)^{a,AB}$ | $2.48\ (0.20)^{a,A}$ | $2.13\ (0.11)^{a,A}$ | $2.17\ (0.27)^{a,A}$ | $2.33\ (0.19)^{a,AB}$ |
| Mean | $2.27\ (0.19)^{a}$ | $2.26\ (0.14)^{a}$ | $2.30\ (0.13)^{a}$ | $2.19\ (0.10)^{a}$ | $2.30\ (0.07)^{a}$ | $2.28\ (0.11)^{a}$ |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | $1.94\ (0.08)^{z,Z}$ | $2.32\ (0.13)^{z,Z}$ | $2.25\ (0.17)^{z,Z}$ | $2.34\ (0.14)^{z,Z}$ |
| 35 | $1.69\ (0.24)^{z,Z}$ | $1.95\ (0.23)^{z,Z}$ | $2.05\ (0.06)^{z,Z}$ | $2.06\ (0.20)^{z,Z}$ |
| 70 | $1.88\ (0.16)^{z,Z}$ | $1.76\ (0.38)^{z,Z}$ | $1.74\ (0.19)^{z,Z}$ | $2.16\ (0.11)^{z,Z}$ |
| 105 | $2.19\ (0.12)^{z,Z}$ | $2.19\ (0.11)^{z,Z}$ | $2.04\ (0.10)^{z,Z}$ | $2.02\ (0.14)^{z,Z}$ |
| 140 | $2.01\ (0.22)^{z,Z}$ | $2.03\ (0.27)^{z,Z}$ | $2.20\ (0.23)^{z,Z}$ | $2.38\ (0.21)^{z,Z}$ |
| Mean | $1.94\ (0.16)^{z}$ | $2.05\ (0.18)^{z}$ | $2.06\ (0.15)^{z}$ | $2.19\ (0.14)^{z}$ |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 3.6** Total nitrogen delta $^{15}N$ values (‰) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | $6.74\ (0.06)^{a,*,A}$ | $6.53\ (0.01)^{a,*,A}$ | $6.65\ (0.09)^{a,*,A}$ | $6.60\ (0.11)^{a,A}$ | $6.40\ (0.16)^{a,A}$ | $6.61\ (0.10)^{a,A}$ |
| 140 | $6.86\ (0.06)^{a,*,B}$ | $6.72\ (0.09)^{a,*,B}$ | $6.73\ (0.30)^{a,*,A}$ | $6.39\ (0.04)^{b,*,A}$ | $6.27\ (0.07)^{b,*,A}$ | $6.33\ (0.18)^{b,*,A}$ |
| Mean | $6.76\ (0.06)^{a*}$ | $6.63\ (0.05)^{a*}$ | $6.69\ (0.19)^{a*}$ | $6.50\ (0.07)^{a*}$ | $6.34\ (0.10)^{a*}$ | $6.47\ (0.12)^{a*}$ |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | $6.93\ (0.18)^{z,Z}$ | $6.90\ (0.06)^{z,Z}$ | $6.90\ (0.09)^{z,Z}$ | $7.12\ (0.11)^{z,Z}$ |
| 140 | $7.17\ (0.12)^{z,Z}$ | $6.95\ (0.19)^{z,Z}$ | $6.72\ (0.18)^{z,Z}$ | $6.45\ (0.20)^{z,Z}$ |
| Mean | $7.05\ (0.12)^{z}$ | $6.93\ (0.12)^{z}$ | $6.82\ (0.07)^{z}$ | $6.79\ (0.05)^{z}$ |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

The interaction effect of treatment by day for the soil C/N ratio was significant [$F_{(36,68)}$ = 2.56, $p<0.001$)]. Main effects showed significant differences between treatments and days (Table 3.7). The only difference between treatments was on day 70, where $C_3$-2:3 was significantly lower than $C_3$-1:2. Most $C_3$ and $C_4$ treatments, as well as overall means, were significantly higher than all corresponding Control treatments and overall means. Simple effects showed significant differences between times, where C/N ratio was highest on day 70 for $C_3$ and $C_4$ treatments. All Control treatments showed significant differences between days. Overall, $C_3$ and $C_4$ were variable (decreasing between days 1 to 35 and 70 to 105, and increasing between days 35 to 70 and 105 to 140), while Control treatments were more steady except for the decrease from day 1 to 35. From day 1 to 140 $C_3$-2:3, $C_4$-2:3 and Cont-M increased, while the rest decreased.

**Table 3.7** Soil organic carbon/soil total nitrogen ratio (C/N) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 15.00 (0.41)[a,*,AB] | 14.08 (0.38)[a,*,AC] | 14.02 (0.38)[a,*,A] | 14.94 (0.20)[a,*,A] | 14.99 (0.16)[a,*,AB] | 13.84 (0.15)[a,*,AB] |
| 35 | 12.82 (0.55)[a,A] | 14.07 (0.71)[a,*,BC] | 13.45 (0.52)[a,*,A] | 13.44 (0.20)[a,*,AB] | 13.17 (0.28)[a,AD] | 12.90 (0.62)[a,,A] |
| 70 | 15.66 (0.81)[ab,*,B] | 15.74 (0.12)[b,*,A] | 13.56 (0.59)[a,A] | 14.47 (0.43)[ab,*,A] | 14.39 (0.28)[ab,*,B] | 14.47 (0.37)[ab,*,B] |
| 105 | 12.86 (0.76)[a,*,A] | 12.48 (0.15)[a,BC] | 13.11 (0.46)[a,*,A] | 12.04 (0.27)[a,B] | 11.78 (0.34)[a,C] | 13.21 (0.29)[a,*,AB] |
| 140 | 12.78 (0.85)[a,AB] | 13.78 (0.58)[a,*,C] | 14.10 (0.23)[a,*,A] | 14.31 (0.47)[a,*,A] | 13.95 (0.22)[a,*,D] | 14.29 (0.70)[a,*,AB] |
| Mean | 14.08 (0.15)[a,*] | 14.03 (0.11)[a,*] | 13.96 (0.42)[a,*] | 13.84 (0.21)[a,*] | 13.66 (0.10)[a,*] | 13.74 (0.13)[a,*] |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | 13.58 (0.86)[z,Z] | 9.43 (0.63)[y,Z] | 11.36 (0.12)[zy,Z] | 11.41 (0.01)[zy,ZX] |
| 35 | 10.78 (0.19)[z,Y] | 10.72 (0.15)[z,Z] | 10.73 (0.08)[z,Z] | 10.51 (0.17)[z,Y] |
| 70 | 11.75 (0.07)[z,ZY] | 11.86 (0.14)[z,Y] | 11.83 (0.05)[z,Z] | 11.80 (0.06)[z,X] |
| 105 | 10.45 (0.11)[z,Y] | 10.38 (0.16)[z,ZY] | 10.60 (0.05)[z,Z] | 10.73 (0.02)[z,ZY] |
| 140 | 11.36 (0.02)[z,Y] | 11.36 (0.10)[z,YX] | 11.30 (0.27)[z,Z] | 11.26 (0.28)[z,ZX] |
| Mean | 11.59 (0.24)[z] | 10.75 (0.22)[z] | 11.15 (0.06)[z] | 11.14 (0.07)[z] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$) between $C_3$ and $C_4$ treatments (a-d) and between Control treatments (z-x). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

*3.4.3 Soil Light Fraction Carbon*

The interaction effect of treatment by day for $LF_C$ concentration (g C/kg) was not significant [F (36,60)= 1.29, p = 0.19]. Main effects showed that all $C_3$ and $C_4$ treatments, and overall means were significantly higher than corresponding Control treatments (Table 3.8). Although not significantly different, $C_3$ $LF_C$ concentrations were usually higher than $C_4$, as were the overall means. No significant differences were found between sampling days in $C_3$ or $C_4$ treatments, but in all Control treatments except Cont-1:2, day 1 was significantly higher than day 35. Over the 140 day incubation, all treatments except for $C_3$-S and $C_4$-2:3 decreased in $LF_C$ concentration.

The interaction effect of treatment by day was not significant [F(18,40) = 0.92, p = 0.56] for $\delta^{13}C$-LF values (‰). Main effects showed that all $C_3$ and $C_4$ treatments, differed from the corresponding Control treatments (from -22.92‰ to -20.44‰), as did the overall means (Table 3.9). Furthermore, all $C_3$ treatments (from -27.42‰ to -26.98‰) were significantly depleted when compared to $C_4$ treatments (from -15.13‰ to -14.71‰). The main effect of time was not significant. All $C_3$ and $C_4$ treatments became more depleted (sole crops the most) while all Control treatments (except Cont-1:2) became more enriched with time (day 1 to 140).

The interaction effect of treatment by day was not significant for $LF_C$ derived from applied residues (g C/kg) [F(10,18) = 1.67, p = 0.20], however there were significant main effects for treatment and time (Table 3.10a). $C_3$ treatments (on day 70 and 140) and overall means were significantly higher than $C_4$ treatments. $C_3$-S, $C_4$-M and $C_4$-1:2 differed significantly over time. Only $C_3$-S and $C_3$-1:2 increased in amount of $C_3$ derived $LF_C$, while all other treatments decreased. The interaction effect of treatment by day was not significant for $LF_C$ derived from old C sources (g C/kg) [F(10,24) = 0.47, p = 0.89]. Main effects of treatment and time were not significant (Table 3.10b). Over time, all treatments decreased in contributions to LFC from old C sources. Light fraction derived from applied residues was significantly higher (3 to 4 times) than LFC derived from the old C sources (Appendix Table 2).

**Table 3.8** Light fraction carbon concentrations (g C/kg) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 8.73 (0.30)[a,*,A] | 9.70 (1.96)[a,*,A] | 10.28 (0.80)[a,*,A] | 7.89 (0.52)[a,*,A] | 7.88 (0.61)[a,*,A] | 8.66 (0.72)[a,*,A] |
| 35 | 8.31 (0.75)[a,*,A] | 8.45 (0.71)[a,*,A] | 9.46 (0.69)[a,*,A] | 6.54 (0.39)[a,*,A] | 7.48 (0.14)[a,*,A] | 7.28 (0.46)[a,*,A] |
| 70 | 7.48 (0.24)[a,*,A] | 9.39 (0.46)[a,*,A] | 8.75 (0.38)[a,*,A] | 7.35 (0.26)[a,*,A] | 7.68 (0.14)[a,*,A] | 8.07 (0.45)[a,*,A] |
| 105 | 8.92 (0.31)[a,*,A] | 8.57 (0.31)[a,*,A] | 9.39 (0.44)[a,*,A] | 6.87 (0.22)[a,*,A] | 7.40 (0.41)[a,*,A] | 7.36 (0.20)[a,*,A] |
| 140 | 9.11 (0.42)[a,*,A] | 8.49 (2.13)[a,*,A] | 8.07 (0.11)[a,*,A] | 6.30 (0.15)[a,*,A] | 6.76 (0.26)[a,*,A] | 6.94 (1.96)[a,*,A] |
| Mean | 8.51 (0.15)[a,*] | 8.92 (1.08)[a,*] | 9.19 (0.26)[a,*] | 6.99 (0.15)[a,*] | 7.44 (0.13)[a,*] | 7.66 (0.58)[a,*] |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | 2.22 (0.30)[z,Z] | 2.07 (0.23)[z,Z] | 1.30 (0.41)[z,Z] | 2.14 (0.07)[z,Z] |
| 35 | 0.88 (0.05)[z,Y] | 0.94 (0.14)[z,Y] | 0.89 (0.10)[z,Z] | 0.86 (0.12)[z,Y] |
| 70 | 0.95 (0.48)[z,Y] | 0.95 (0.17)[z,ZY] | 0.85 (0.11)[z,Z] | 0.95 (0.15)[z,ZY] |
| 105 | 0.80 (0.06)[z,Y] | 0.84 (0.06)[z,Y] | 0.81 (0.04)[z,Z] | 0.61 (0.23)[z,Y] |
| 140 | 0.78 (0.09)[z,Y] | 0.87 (0.08)[z,Y] | 0.99 (0.14)[z,Z] | 0.97 (0.17)[z,ZY] |
| Mean | 1.13 (0.19)[z] | 1.07 (0.03)[z] | 0.96 (0.13)[z] | 1.01 (0.17)[z] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a) and between Control treatments (z-y). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 3.9** Light fraction delta $^{13}C$ values (‰) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | -26.98 (0.14)[a,*,A] | -27.12 (0.09)[a,*,A] | -27.19 (0.08)[a,*,A] | -14.74 (0.13)[b,*,A] | -15.04 (0.41)[b,*,A] | -14.80 (0.21)[b,*,A] |
| 70 | -27.42 (0.09)[a,*,A] | -27.34 (0.19)[*,A] | -27.30 (0.04)[a,*,A] | -14.71 (0.01)[b,*,A] | -14.85 (0.19)[b,*,A] | -14.79 (0.17)[b,*,A] |
| 140 | -27.33 (0.10)[a,*,A] | -27.29 (0.12)[a,*,A] | -27.20 (0.16)[a,*,A] | -15.13 (0.10)[b,*,A] | -15.09 (0.09)[b,*,A] | -15.02 (0.29)[b,*,A] |
| Mean | -27.24 (0.09)[a,*] | -27.25 (0.09)[a,*] | -27.23 (0.09)[a,*] | -14.86 (0.06)[b,*] | -14.99 (0.14)[b,*] | -14.87 (0.09)[b,*] |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | -21.55 (0.14)[z,Z] | -22.24 (0.25)[z,Z] | -21.88 (0.28)[z,Z] | -21.98 (0.19)[z,Z] |
| 70 | -22.32 (0.48)[z,Z] | -22.60 (0.51)[z,Z] | -22.37 (0.49)[z,Z] | -20.96 (1.22)[z,Z] |
| 140 | -21.46 (0.59)[z,Z] | -22.19 (1.07)[z,Z] | -22.92 (1.16)[z,Z] | -20.44 (0.45)[z,Z] |
| Mean | -21.77 (0.39)[z] | -22.34 (0.28)[z] | -22.39 (0.57)[z] | -21.13 (0.59)[z] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 3.10** Light fraction carbon derived from **a) applied residue** and **b) soil carbon sources** (g C/kg) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil. Overall means (bottom row) were averaged over the 140 day incubation.

a)

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 6.70 (0.27)[a,AB] | 6.29 (1.45)[a,A] | 8.07 (0.65)[a,A] | 5.70 (0.27)[a,AB] | 5.44 (0.78)[a,AB] | 6.15 (0.39)[a,A] |
| 70 | 6.04 (0.16)[ab,A] | 7.43 (0.16)[a,A] | 7.16 (0.47)[a,A] | 5.40 (0.22)[b,A] | 5.52 (0.08)[b,A] | 5.39 (0.04)[b,A] |
| 140 | 7.46 (0.58)[a,B] | 8.51 (1.22)[a,A] | 6.65 (0.11)[a,A] | 4.28 (0.26)[b,B] | 4.75 (0.16)[b,B] | 6.06 (0.01)[ab,A] |
| Mean | 6.73 (0.41)[ac] | 7.41 (0.64)[a] | 7.29 (0.41)[a] | 5.13 (0.43)[b] | 5.23 (0.24)[b] | 5.86 (0.24)[ac] |

b)

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 2.03 (0.19)[a,A] | 2.17 (0.47)[a,A] | 2.21 (0.22)[a,A] | 2.19 (0.27)[a,A] | 2.44 (0.19)[a,A] | 2.51 (0.35)[a,A] |
| 70 | 1.44 (0.15)[a,A] | 1.96 (0.38)[a,A] | 1.59 (0.28)[a,A] | 1.94 (0.12)[a,A] | 2.17 (0.08)[a,A] | 2.69 (0.44)[a,A] |
| 140 | 1.65 (0.16)[a,A] | 1.94 (0.20)[a,A] | 1.42 (0.21)[a,A] | 2.02 (0.19)[a,A] | 2.01 (0.23)[a,A] | 2.37 (0.48)[a,A] |
| Mean | 1.71 (0.17)[a] | 2.03 (0.07)[a] | 1.74 (0.24)[a] | 2.05 (0.07)[a] | 2.21 (0.13)[a] | 2.52 (0.09)[a] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$). Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

*3.4.4 Soil Light Fraction Nitrogen*

The interaction effect of treatment by day was significant for $LF_N$ concentrations (g N/kg) [F (36,68) = 1.83, p = 0.016]. Main effects of treatment and day showed significant differences (Table 3.11). Most $C_3$ and $C_4$ treatments were significantly higher than corresponding Control treatments on most days, which was also observed with the overall means. $C_3$ treatments had higher $LF_N$ concentrations than $C_4$ treatments (significant on day 35, and for overall means). $C_3$-2:3 was consistently higher than $C_3$-S, while $C_4$-1:2 and $C_4$-2:3 were higher than $C_4$-M for days 1, 35 and 70. Simple main effects showed that for most treatments, $LF_N$ concentrations were highest on day 1. Control treatments had a large drop in $LF_N$ from day 1 to 35 and over time showed a decrease in $LF_N$ concentrations. $C_4$ treatments had a relatively smaller drop from day 1 to 35 while $C_3$ treatments were relatively stable over time. All $C_3$ and $C_4$ treatments decreased from day 1 to 140, except for $C_3$-S.

The interaction effect of treatment by day was not significant [F(9,20) = 0.75, p = 0.67] for $\delta^{15}$N-LF (‰). There was a significant main effect of treatment type (Table 3.12). $C_3$-S was significantly higher than all $C_4$ treatments while $C_3$-1:2 and $C_3$-2:3 were significantly higher than $C_4$-1:2 on day 1. On day 140, all $C_3$ treatments were significantly higher than all $C_4$ treatments. All $C_3$ treatment overall means were significantly higher than $C_4$ treatment overall means. All $C_4$ treatments and overall means were significantly lower than corresponding Control treatments and overall means. On day 140, $C_3$-S was significantly higher than $C_4$-1:2 and $C_4$-2:3, while $C_3$-1:2 was higher than all $C_4$ treatments. Overall, all treatments became more depleted in $^{15}$N throughout the incubation (only significant in $C_4$-2:3).

**Table 3.11** Light fraction nitrogen (g N/kg) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 0.17 (0.01)[a,AB] | 0.23 (0.07)[a,A] | 0.20 (0.02)[a,AB] | 0.14 (0.01)[a,A] | 0.16 (0.02)[a,A] | 0.17 (0.03)[a,AB] |
| 35 | 0.17 (0.01)[a,*,A] | 0.16 (0.01)[ab,*,A] | 0.18 (0.01)[a,*,A] | 0.11 (0.01)[b,*,A] | 0.11 (0.00)[b,*,A] | 0.12 (0.01)[b,*,AB] |
| 70 | 0.14 (0.01)[a,B] | 0.18 (0.01)[a,*,A] | 0.17 (0.01)[a,*,AB] | 0.12 (0.00)[a,*,A] | 0.13 (0.00)[a,*,A] | 0.15 (0.01)[a,*,A] |
| 105 | 0.13 (0.01)[a,*,B] | 0.13 (0.00)[a,*,A] | 0.14 (0.01)[a,*,B] | 0.11 (0.01)[a,*,A] | 0.12 (0.01)[a,*,A] | 0.11 (0.01)[a,*,B] |
| 140 | 0.17 (0.01)[a,*,AB] | 0.18 (0.03)[a,*,A] | 0.17 (0.02)[a,*,AB] | 0.13 (0.01)[a,*,A] | 0.14 (0.00)[a,*,A] | 0.13 (0.02)[a,*,AB] |
| Mean | 0.16 (0.01)[ac,*] | 0.18 (0.02)[abc,*] | 0.17 (0.00)[a,*] | 0.12 (0.01)[bc,*] | 0.13 (0.00)[c,*] | 0.14 (0.01)[c,*] |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | 0.14 (0.02)[z,Z] | 0.12 (0.02)[z,Z] | 0.08 (0.02)[z,Z] | 0.12 (0.01)[z,Z] |
| 35 | 0.05 (0.00)[z,Y] | 0.05 (0.01)[z,Y] | 0.04 (0.01)[z,Z] | 0.05 (0.01)[z,Y] |
| 70 | 0.06 (0.03)[z,ZY] | 0.06 (0.01)[z,ZY] | 0.05 (0.01)[z,Z] | 0.05 (0.01)[z,Y] |
| 105 | 0.04 (0.00)[z,Y] | 0.04 (0.00)[z,ZY] | 0.05 (0.00)[z,Z] | 0.03 (0.01)[z,Y] |
| 140 | 0.05 (0.01)[z,Y] | 0.05 (0.00)[z,Y] | 0.06 (0.01)[z,Z] | 0.06 (0.01)[z,Y] |
| Mean | 0.07 (0.01)[z] | 0.06 (0.00)[z] | 0.05 (0.01)[z] | 0.06 (0.01)[z] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$) between $C_3$ and $C_4$ treatments (a-c) and between Control treatments (z-y). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 3.12** Light fraction $\delta^{15}N$ values (‰) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 4.33 (0.20)[a,A] | 4.13 (0.24)[ab,A] | 4.02 (0.14)[ab,A] | 2.99 (0.21)[bc,*,A] | 2.47 (0.31)[c,*,A] | 2.98 (0.16)[bc,*,A] |
| 140 | 3.92 (0.08)[a,A] | 4.06 (0.25)[a,A] | 3.71 (0.29)[a,A] | 2.47 (0.08)[b,*,A] | 2.36 (0.14)[b,*,A] | 2.33 (0.01)[b,*,B] |
| Mean | 4.12 (0.12)[a] | 4.09 (0.24)[a] | 3.86 (0.08)[a] | 2.73 (0.12)[b,*] | 2.42 (0.10)[b,*] | 2.66 (0.14) [b,*] |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | 4.86 (0.20)[z,Z] | 4.73 (0.26)[z,Z] | 5.12 (0.18)[z,Z] | 4.89 (0.37)[z,Z] |
| 140 | 4.14 (0.47)[z,Z] | 4.65 (0.20)[z,Z] | 4.33 (0.50)[z,Z] | 4.47 (0.17)[z,Z] |
| Mean | 4.50 (0.30)[z] | 4.69 (0.18)[z] | 4.72 (0.31)[z] | 4.68 (0.27)[z] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-c) and between Control treatments (z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

The interaction effect of treatment by day for $LF_{C/N}$ ratio was significant [$F(36,52) = 1.67$, $p = 0.045$)]. The main effect of treatment showed that all $C_3$ and $C_4$ treatments on all days were significantly higher than corresponding Control treatments, as were the overall means (Table 3.13). Only day 35 showed significant differences between $C_3$ and $C_4$ treatments where $C_3$-S and $C_3$-1:2 were lower than all $C_4$ treatments, and $C_3$-2:3 lower than $C_4$-M and $C_4$-1:2. From days 1 to 70, $C_4$ had a higher $LF_{C/N}$ ratio than $C_3$ treatments which reversed on day 105 and became even by day 140. Simple effects showed that day 105 for all but $C_4$-1:2 had the highest $LF_{C/N}$. Control treatments were relatively constant over time and showed no significant differences between treatments. Overall, all $C_3$ and $C_4$ treatments decreased in $LF_{C/N}$ ratio (except for $C_3$-S and $C_4$-2:3) and all Control treatments increased over the entire incubation.

**Table 3.13** Light fraction carbon/light fraction nitrogen ratios ($LF_{C/N}$) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 50.68 (2.56)[a,*,A] | 49.35 (~)[a,*,A] | 52.87 (3.27)[a,*,AB] | 57.02 2.89[a,*,AB] | 51.21 (8.81)[a,*,A] | 50.89 (3.80)[a,*,A] |
| 35 | 49.27 (1.11)[a,*,B] | 51.21 (1.14)[ab,*,A] | 52.81 (2.07)[a,*,AB] | 62.16 (3.48)[ab,*,A] | 69.33 (1.42)[b,*,B] | 60.96 (1.80)[ab,*,A] |
| 70 | 54.47 (3.40)[a,*,AB] | 53.17 (0.98)[a,*,A] | 51.39 (1.78)[a,*,B] | 61.19 (3.87)[a,*,A] | 57.67 (0.10)[a,*,AB] | 55.80 (2.13)[a,*,A] |
| 105 | 67.95 (4.99)[a,*,A] | 64.95 (1.51)[a,*,A] | 67.23 (5.22)[a,*,A] | 64.46 (8.63)[a,*,AB] | 63.62 (3.11)[a,*,AB] | 68.43 (4.09)[a,*,A] |
| 140 | 54.06 (0.45)[a,*,AB] | 47.25 (7.27)[a,*,A] | 48.50 (4.61)[a,*,B] | 47.23 (1.05)[a,*,B] | 48.91 (1.77)[a,*,A] | 60.89 (0.95)[a,*,A] |
| Mean | 55.29 (1.67)[a,*] | 53.78 (1.25)[a,*] | 54.56 (2.38)[a,*] | 58.41 (3.17)[a,*] | 58.15 (1.84)[a,*] | 59.20 (1.31)[a,*] |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | 15.95 (0.48)[z,Z] | 16.74 (0.26)[z,Z] | 16.40 (0.45)[z,ZY] | 16.27 (0.49)[z,Z] |
| 35 | 18.40 (0.73)[z,Z] | 20.58 (0.24)[z,Z] | 21.61 (1.65)[z,Z] | 18.81 (0.28)[z,Z] |
| 70 | 16.28 (0.28)[z,Z] | 16.07 (0.08)[z,Z] | 16.45 (0.42)[z,Y] | 17.48 (0.72)[z,Z] |
| 105 | 18.09 (0.12)[z,Z] | 19.45 (1.06)[z,Z] | 17.77 (0.61)[z,ZY] | 19.10 (0.51)[z,Z] |
| 140 | 16.56 (0.65)[z,Z] | 17.84 (0.52)[z,Z] | 17.15 (0.52)[z,Y] | 16.81 (0.83)[z,Z] |
| Mean | 17.10 (0.34)[z] | 17.96 (0.31)[z] | 17.88 (0.07)[z] | 17.79 (0.17)[z] |

Standard error of the means are shown in parentheses (n=3, n=1 if ~). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**3.5 Discussion**

*3.5.1 Soil Organic Carbon*

Values of soil organic carbon concentration in the current study were similar to those in a field study by Studdert and Echeverría (2000) in Balcarce, Argentina. Results from the current study indicated an influence of residue application on SOC concentrations. As expected, SOC concentrations were higher in soils with added soybean and maize residues, than in soils with no added residue. Similar results were observed by Dolan et al. (2006) and Ghimire et al. (2012). SOC concentrations in all treatments decreased after residue and water were added to the soil, likely due to the stimulation of microbes. This could possibly display a positive 'priming effect', which is the acceleration of native SOM decay due to the addition of easily accessible and decomposable residue (Kuzyakov et al. 2000). Li and Feng (2002) reported that up to two-thirds of fresh plant litter C can be lost to $CO_2$, but after that, decomposition slows, which leads to accumulation of more stable and less easily decomposable organic C. Therefore, the subsequent increase in SOC concentrations may have been due to more recalcitrant or stable C being formed from the added residues (Li and Feng 2002).

This study showed little effect of cropping practice on SOC concentrations. In a study by Goidts and van Wesemael (2007) it was estimated that a minimum of 7 years are necessary before seeing a significant change in SOC due to agricultural management practices. Since SOC responds slowly to changes (including the addition of residues), the relatively short incubation time (only 140 d), along with a site which has only been intercropped for four years, may account for the small influence of cropping practice on SOC concentrations. However, higher SOC concentrations in all 2:3 treatments suggested higher sequestration, indicating that intercropping with this combination of maize and legume may be a more efficient climate change mitigation technique than the 1:2 intercrop treatment and sole crop treatments (Ghimire et al. 2012).

Values of $\delta^{13}$C-SOC from the current study were similar to those in a study by Costantini et al. (2007), which compared cultivated and grassland soils in the Argentine Pampas. Differences between C$_3$, C$_4$ and Control treatments showed that residue type strongly affected $\delta^{13}$C of SOC. The $\delta^{13}$C values of the C$_3$ and C$_4$ treatments were similar to those of the added residues, which showed the influence of soybean and maize residue $\delta^{13}$C values on SOC $\delta^{13}$C values (Kayler et al. 2011). However in C$_3$ and C$_4$ treatments, $\delta^{13}$C values of SOC were still 5 to 8‰ away from the residue $\delta^{13}$C values, but only 2‰ away from the Control SOC $\delta^{13}$C value. This has been observed in a study where there was a change from C$_3$ to C$_4$ vegetation, where the older C$_3$ derived C had a large influence on SOC (Blagodatskaya et al. 2011). This corresponded to SOC contributions from residue C and older C. Older C, as expected, contributed more to SOC in both C$_3$ and C$_4$ treatments than freshly added residue. This was due to higher contribution to SOC from C sources such as the previous C$_3$/C$_4$ mixed grasslands and crops, as well as the decay and incorporation of residue into the SOM (Oelbermann and Echarte 2011).

*3.5.2 Total Soil Nitrogen*

Values of TN were similar to those found by Dyer et al. (2012) at the same site. Results from the current study showed higher TN concentrations in C$_3$ and C$_4$ treatments than Control treatments, illustrating that residue application increased N cycling (Kurdali 2009). The amount of N from residues stored in soils before the incubation, likely increased TN concentrations within Cont-S and 2:3 soils. Mazzoncini et al. (2011), found that TN stocks increased yearly due to a winter cover crop, increasing the amount of residue N left on the soil. These results collectively showed only a small influence of cropping practice on TN concentrations.

No significant differences in TN concentrations were observed between treatments, likely due to relatively slow turnover times for SOM, making the incorporation of residue into TN small in a 140 d incubation. For example, in a Mediterranean climate, Mazzoncini et al.

(2011) found soil TN stocks to significantly change after 15 years, and encouraged long-term studies, especially when focusing on the SOM pool.

Values of $\delta^{15}$N-TN were similar to those from Oelbermann and Echarte (2007) in a study on soil from the same site. Soils with soybean residue applied, as well as sole Control soils, were enriched in $^{15}$N over time whereas soils with maize residue, as well as intercropped Control soils, were depleted. An enrichment in $^{15}$N could have been due to more aggregated and recalcitrant soils forming (Högberg 1997). Soils rich in N inputs have more isotopic fractionation from processes such as N turnover by microbial decomposition, mineralization, and $NH_3$ volatilization, which generally leads to an enrichment in soil N (Handley and Raven 1992; Högberg 1997; Lynch et al. 2006). Soils that were depleted showed an integration of residue (depleted compared to TN), and that input of depleted N was likely more than the loss of depleted N (Handley and Raven 1992). The more enriched $C_3$-S treatment could have been due to the absence of N fertilizer. The $\delta^{15}$N value of fertilizer has been shown to be lower than natural sources of N, which could have depleted the initial $\delta^{15}$N values of all but the $C_3$-S treatment due to the absence of fertilization on the soybean sole crops (Vitória et al. 2004). The greatest $^{15}$N depletion was seen in the 2:3 treatments of $C_4$ and Control, which may have shown more incorporation of soybean residue. This has been observed in the field by Oelbermann and Echarte (2011), who also found the most depleted TN was in the 2:3 intercrop when compared to soybean sole, maize sole, and 1:2 intercrops.

As expected, residue amended soil had a higher C/N ratio than Control soils. This was due to large inputs of residues with a high C/N ratio relative to SOC. Although there was an increase in N from residues, it was much smaller than the increase in C with the residue inputs (for example, 44.74%C and 1.40%N in soybean residue). Lower C/N ratios within Control treatments suggested that they had more stable aggregates than soils with added residue, as well as lower microbial processing and therefore less change in the C/N ratio over the incubation (Wright and Hons 2005).

*3.5.3 Soil Light Fraction Carbon*

Similar $LF_C$ results in the current study were obtained in Argentina from Argiudoll soils in a study by Conti et al. (1997). As expected, the current study showed a clear effect of residue addition on $LF_C$. Since the LF is made up mainly of decomposing organic matter, addition of residues is almost a direct addition of LF (Soon et al. 2009). Soil LF also has a faster turnover time than SOC, making it easier to see differences in LF in a short time (Six et al. 2002). Initially and throughout the incubation, higher $LF_C$ concentrations in $C_3$ and $C_4$ intercrop treatments suggested that intercrop soil had a higher $LF_C$ concentration. This may have been due to a higher amount of mineralizable C with the addition of residues (Alvarez and Alvarez 2000) and larger pools of mineralizable C in intercrop soils. However, this was not supported by $LF_C$ in Control soils, where sole soils had higher $LF_C$ concentrations, suggesting that the addition of residue and intercropping together, increased $LF_C$. In a cotton-cowpea intercrop study by Rusinamhodzi et al. (2009), intercrops with applied residue had more than three times the amount of N than intercrops and sole crops alone. The incorporated residues led to a higher release of C, more SOM accumulation, and better soil quality (Rusinamhodzi et al. 2009). The large drop in $LF_C$ concentration after the first day in Control treatments suggested high mineralization rates, due to the addition of water (Coppens et al. 2006). In a study by Alvarez and Alvarez (2000), mineralization rates and LF were seen to be strongly correlated in a long-term incubation, using similar soil to the current study. These results could indicate what may happen to slower C pools in a longer term study when residues remain on intercrops.

In this study, $\delta^{13}C$ values showed strong integration of residues into the $LF_C$ (as did $LF_C$ concentrations), but not a strong difference between sole and intercrops. Differences in $C_3$ and $C_4$ from Control treatments were expected. The $C_4$ treatment $\delta^{13}C$-LF values showed more of a difference from Control treatments than $C_3$ treatments. This was due to the difference between $C_4$ and Controls (approximately 10‰) being more than the difference between $C_3$ and Control

(approximately 6.5‰) $\delta^{13}C$ values. The unexpected depletion over the entire incubation in $C_4$ treatments was explained by influences from relatively depleted SOC, which contributed to $\delta^{13}C$-LF (Oelbermann and Echarte 2011).

Contributions from soybean and maize residues to $LF_C$ corresponded with $\delta^{13}C$-LF results, and showed an effect from crop residue management. As expected, LF in $C_3$ and $C_4$ treatments had a much higher contribution from applied residues, than old C sources suggesting high incorporation of residues into the LF (Gregorich et al. 1994). Higher contributions to LF in intercrops from both new and old sources of C suggested more $LF_C$ available in intercrop soil, and a higher turnover of fresh organic matter compared to sole crops (Gregorich et al. 1994). This could mean that a greater amount of C was being stabilized from residue to more stable SOM pools in intercrop soil than sole crop soil.


### 3.5.4 Soil Light Fraction Nitrogen

Similar $LF_N$ concentrations to Control treatments in the current study were found in a study by Oelbermann and Echarte (2011). $LF_N$ in $C_3$ and $C_4$ treatments is higher than those found in the literature, which was accounted for by the addition of residues. Soon et al. (2009) found that by retaining straw residue, $LF_N$ concentrations increased over time. In the current study, higher $LF_N$ concentrations, and a lower decrease over time in $C_3$ and $C_4$, than Control treatments illustrated the increase in $LF_N$, as a result of residue addition. Higher $C_3$ $LF_N$ concentrations suggested that residue quality also affected $LF_N$ concentrations. A study on C and N losses in agroecosystems by Drinkwater et al. (1998), found that crop residue quality affected soil quality, leading to the recommendation of using a diverse source of residue with a low C/N ratio to maintain soil fertility. The greater change over the incubation in 2:3 intercrop $LF_N$ concentrations in $C_3$ and $C_4$ treatments suggested that the 2:3 intercrop ratio had higher

mineralization rates, which would create more plant available nutrients in intercrops (Compton and Boone 2000).

Similar values of $\delta^{15}$N-LF were found in Oelbermann and Echarte (2011) at the same study site.  In the current study, $C_3$ treatments were enriched compared to soybean residue (3.14‰) and $C_4$ treatments were slightly enriched or depleted (day 140) compared to maize residue (2.47‰), which suggested that $C_3$ treatments processed more N into the LF than $C_4$ treatments.  It has been shown that with more microbial transformations of N, more discrimination occurs, enriching the LF in $^{15}$N (Christensen 1992) which illustrated higher N cycling in the $C_3$ treatments.  Control treatments were the most enriched which suggested that soil LF at the site contained N that had been through microbial transformations, and therefore contained more humified material (Christensen 1992; He et al. 2008).  The depletion over time represented the depleted $\delta^{15}$N value of the organic matter source.  It could have also represented immobilization at the beginning of the incubation, and mineralization later in the incubation (Hadas et al. 2004).

In this study there was an effect of residue addition but not cropping type on $LF_{C/N}$ ratio.  Similar C/N ratios in the LF and soybean and maize showed the high amount of residues contributing to the LF.  Lower $LF_{C/N}$ ratio, and higher $LF_C$ and N concentrations in $C_3$ than $C_4$ treatments, both corresponded to the lower quality of maize residues (Mungai and Motavalli 2006).  High $LF_{C/N}$ ratios might have indicated higher levels of C, but with more N simultaneously being supplied to the soil through residue input, it was still possible to have high decomposition rates (Malhi et al. 2003).  The higher C/N ratios of the LF in $C_4$ treatments could have also suggested less mineralization and possible immobilization of the LF, as was seen in Oelbermann and Echarte (2011).

## 3.6 Conclusion

In a 140 d incubation experiment, this study quantified concentrations and $\delta^{13}C$ and $\delta^{15}N$ of the SOC, TN and LF. It also quantified fresh residue and old C source contributions to SOC and $LF_C$, and ratios of SOC/TN and $LF_{C/N}$. This study was the first known laboratory incubation to quantify and compare these characteristics simultaneously for sole and intercrop soil from the temperate zone. Results showed a clear effect of residue addition on soil fractions, evident from higher SOC, TN, $LF_C$ and $LF_N$ concentrations in treatments with added residues. Results also illustrated higher immobilization in $C_4$ treatments due to lower quality litter, and higher mineralization in $C_3$ treatments because of higher quality litter. In addition, this study identified 2:3 as the more desirable intercrop design. This was seen through higher SOC, TN, $LF_C$ and $LF_N$ concentrations, which all suggested higher C sequestration rates in 2:3 intercrop soil. Therefore, intercropping was seen to benefit soil quality while having climate change mitigation potential through C sequestration. It is recommended that long-term studies be pursued to confirm these findings.

# 4. SOIL MICROBIAL BIOMASS AND SOIL MICROBIAL COMMUNITY STRUCTURE

## 4.1 Introduction

Soil microbial biomass (SMB), the living fraction of the soil, contains a diverse array of microbial species of bacteria, fungi, yeast, algae, and any organism under 500 $\mu m^3$ (Brookes et al. 1990). Although a small fraction, the soil microbial biomass (SMB) accounts for only 1 to 4% of SOC, and 2 to 6% of TN, it is responsible for decomposition of soil organic matter (SOM) constituents and the transformation of nutrients (N, P, S) into plant-available forms (Brookes et al. 1985; Schnürer et al. 1985; Anderson and Domsch 1989). Diversity of this fraction is most affected by chemical conditions such as pH, temperature, moisture and organic matter quantity, but can also be affected by other conditions such as pore size, food sources, habitat variability and disturbances (Giller 1996). Due to its fast response time to disturbances, SMB and soil microbial community structure (SMCS) have been shown to be useful indicators of environmental stress and changes in soil management (Garland 1997; Hargreaves et al. 2003). Principle component analysis (PCA) has also been useful in explaining microbial community structure and variance by grouping similar treatments (Müller-Stöver et al. 2012).

Human activities, such as land use management and change, are factors that can considerably alter microbial communities. For example, Moore et al. (2000) found that soil under crop rotation had higher $SMB_C$ and $SMB_N$ than soil under a continuous crop, due to the diversity and volume of crop residues added to the soil. Specifically, an increase in labile material and change in habitat characteristics were responsible for the larger SMB pool (Moore et al. 2000). One land use change that has been seen to positively affect $SMB_C$ and $SMB_N$, is intercropping (Song et al. 2007). In a study of a subtropical intercropping system which in increased plant residue inputs to the soil, a higher microbial biomass, more soil nutrient cycling

and better overall soil quality were recorded (Suman et al. 2006). Furthermore, in an intercrop study by Oelbermann and Echarte (2011), SMB was strongly effected by agricultural management practice due to multiple residue sources.

A number of methods, such as use of the Biolog Ecoplate™, are available to conveniently characterize and compare microbial community diversity within different soil treatments. For example Gomez et al. (2006) used this method to evaluate the effects of adding organic amendments to soil microbial functional diversity and Song et al. (2010) determined arthropod community structure in intercropping systems. Additionally, in a cucumber onion/ garlic intercrop, microbial diversity as measured by Shannon's Diversity Index (SDI), was higher and stayed stable, whereas it decreased in a sole crop each growing season (Zhou et al. 2011). However, a drawback of the Biolog Ecoplate™ method is that only communities that are able to grow on the specific substrates provided are represented (Stefanowicz 2006).

Another tool to evaluate the SMB, is isotopes and the natural abundance method (Šantrůčková et al. 2000). Coyle et al. (2009) found that $SMB_C$ and $SMB_N$ are enriched relative to soil C and N. It was also found that $^{15}N$ was influenced by changes in plant-available C and N (Coyle et al. 2009). A similar study that used $^{13}C$ to quantify soybean and maize residue contributions to SMB, found that it was enriched with $C_3$ sources and depleted with $C_4$ sources (Dijkstra et al. 2006). In an intercropping study in the Argentine Pampa, Oelbermann and Echarte (2011) found that SMB $\delta^{13}C$ and $\delta^{15}N$ values were not as enriched as previous studies when compared to the LF and SOC. This was due to the SMB reflecting two substrate sources with distinct $\delta^{13}C$ and $\delta^{15}N$ values supplied by the intercrops (soybean and maize) (Oelbermann and Echarte 2011). Hauggaard-Nielsen and Jensen (2005) outline that $^{15}N$ can be used in various ways to explore root interactions between a legume and non-legume crop, such as $^{15}N_2$ labeling for $N_2$-fixing plants, $^{15}N$ stem, shoot or leaflet labeling and split root labeling.

Although the SMB has been studied in intercropping systems these studies were restricted to tree-based intercrops or studies of rhizosphere interactions (Lacombe et al. 2009;

Sun et al. 2009). In the temperate region, a baseline study in the field at the same site as the current study has been done (Oelbermann and Echarte 2011). Furthermore, one known study has assessed microbial community structure characteristics focussing on temperate soybean/ maize intercrop soil in an incubation (Dubois 2008). However, there is a lack of information about changes in SMB and SMCS due to $C_3$ and $C_4$ residue input and decomposition. Changes from $C_3$ to $C_4$ residue input, or vice versa, along with isotopes have been used to evaluate soil microbial preferential utilization of new C sources and discrimination against $^{13}C$ during microbial respiration (Ekblad et al. 2002; Blagodatskaya et al. 2011). A study by Kramer et al. (2012) assessed the flow of C from $C_3$ and $C_4$ root and shoot residue to SOC, SMB and fungal soil C pools, and found increasing amounts of incorporated $C_4$ residue respectively. Nitrogen isotopes of the SMB have also been used to determine that the SMB is always enriched when compared to TN (Dijkstra et al. 2006). However, no known study has used SMB, its $\delta^{13}C$ and $\delta^{15}N$ values, as well as SMCS simultaneously to compare sole and intercrop soil in a long-term, controlled incubation, tracing the decay and stabilization of soybean and maize residues.

This study aimed to use SMB and SMCS characteristics to understand soybean and maize crop residue stabilization in sole crops and intercrops in an incubation study. This was accomplished by the following four objectives:

(a) To quantify changes in $SMB_C$ and $SMB_N$ concentrations, and $\delta^{13}C$ and $\delta^{15}N$ values due to soybean and maize crop residue input.

(b) To quantify the $SMB_C$ concentrations derived from soybean and maize crop residues using $^{13}C$ natural abundance.

(c) To quantify changes in $SMB_N$ concentrations and $\delta^{15}N$-SMB due to incorporation of soybean or maize derived crop residues.

(d) To quantify changes in the metabolic diversity of culturable soil microbial communities due to the incorporation of soybean or maize derived crop residues.

## 4.2 Materials and Methods

### 4.2.1 Soil Microbial Biomass

Soil microbial biomass was measured using chloroform fumigation-extraction (Vance et al. 1987; Voroney et al. 2008). Soil from each treatment was divided into two 20 g sub-samples, placed in 100 ml glass jars and divided into two groups: fumigated or non-fumigated. The fumigated treatment for each sample was placed in a desiccator, lined with moist paper towels, in the fume hood. A beaker containing 50 ml of ethanol-free $CHCl_3$ and boiling chips was placed in the middle of the desiccator. The desiccator was evacuated using a vacuum pump (AC Motor Thermally Protected M100GX) until the $CHCl_3$ boiled vigorously for 2 minutes. The desiccator was sealed and kept in the dark at 20-25°C for 24 h. After 24 h the $CHCl_3$ was removed and the desiccator was evacuated with six short evacuations (5 min) and one longer evacuation (20 min) (AC Motor Thermally Protected M100GX) and 5 minutes between each to ensure complete removal of $CHCl_3$ vapour. Microbial biomass was extracted from the fumigated samples, while non-fumigated samples were extracted immediately after weighing out the soil. SMB was extracted from the fumigated and non-fumigated soils by adding 35 ml of 0.05M $K_2SO_4$ to each sample, shaking the mixture for an hour at 400 rpm (Heidolph Unimax 1010 DT) and filtering each sample through a Whatman GF934-AH filter paper. After filtration, samples were stored in glass vials and frozen. When ready for analysis, the extract was freeze dried (Mandel ModulyOD) and packed into 9 mm x 10 mm tin capsules for C, N and isotope analysis at the University of Saskatchewan Stable Isotopes Laboratory (Costech ECS4010 elemental analyzer coupled to a Delta V mass spectrometer with Conflo IV interface). These results were used to find $SMB_C$ and $SMB_N$ using the following set of equations from Voroney et al. (2008):

$$MS = \frac{wet\ soil\ (g) \times 100}{(100 + WS(\%))}$$

(6)

where MS is oven dry-equivalent weight of each SMB sample (g) and WS is soil water content.

$$VS = \left( \frac{wet\ soil\ (g) - dry\ soil\ (g)}{1g/mL} \right) + V_{ext}$$

(7)

where VS is the total volume of solution of extracted soil (ml) and $V_{ext}$ is volume of extractant.

$$C_f\ or\ C_{nf} = \left[ (\%C_f\ or\ \%C_{nf}) \times \left( \frac{VS}{MS} \right) \right] \times 100$$

(8)

where $C_f$ and $C_{nf}$ are total weights of extractable C in fumigated and non-fumigated samples respectively (mg/kg soil) and $\%C_f$ and $\%C_{nf}$ are percentages of organic C in fumigated and non-fumigated samples respectively (obtained by the Elemental Analyzer).

$$SMB_{Cor} = \frac{(C_f - C_{nf})}{K_{eC}}$$

(9)

where $SMB_{Cor}$ is the final C in the microbial pool and $K_{EC}$ is the efficiency of extraction of $SMB_C$ (0.35). The same calculations were used for N fumigated and non-fumigated samples using 0.5 as $K_{EN}$.

*4.2.2 Soil Microbial Biomass Stable Isotopes*

Results from the University of Saskatchewan also provided $SMB_C$ and $SMB_N$ isotope values. $\delta^{13}C$-$SMB_C$ and $\delta^{15}N$-$SMB_N$ were quantified using the following equation from Coyle et al. (2009):

$$\delta^{13}C_{SMB} = \frac{(\delta C_f \times C_f) - (\delta C_{nf} \times C_{nf})}{(C_f - C_{nf})}$$

(10)

where $\delta_{Cf}$ and $\delta_{Cnf}$ are the $\delta^{13}C$ and $\delta^{15}N$ values from fumigated and non-fumigated samples respectively, $C_f$ and $C_{nf}$ are total extractable weights from extractable C and N in fumigated and non-fumigated samples respectively (mg C/kg).

From $\delta^{13}$C-SMB$_C$ the proportion of SMB$_C$ derived from applied residue C and soil C sources, when soybean or maize residues were added, were quantified using the following two end-member mixing model from Liang et al. (1999):

$$applied \cdot C(\%) = \left(\frac{\delta_{SMB} - \delta_{Cont}}{\delta_{residue} - \delta_{Cont}}\right) \times 100$$

(11)

and

$$soil \cdot C(\%) = 1 - applied \cdot C(\%)$$

(12)

where $\delta_{SMB}$ is $\delta^{13}$C of SMB from soils with added residue, $\delta_{Cont}$ is $\delta^{13}$C from corresponding Control treatments (no residue added) and $\delta_{residue}$ is the mean $\delta^{13}$C value of soybean (-28.62‰) or maize (-11.89‰). Contribution from applied residues and soil C sources to SMB concentrations (mg C/kg) were found by multiplying the new and old proportions by SMB$_C$ concentration. N isotope values were reported and discussed in ‰.

### 4.2.3 Soil Microbial Community Structure

Microbial community structure was measured using Biolog Ecoplates™. In preparation of the measurement, all equipment was washed in a 10% acid bath or autoclaved (Tuttnauer Brinkmann 3870EA) and dried completely. Two grams of soil was placed into 20 ml of 0.85% NaCl and shaken to disperse soil solution. The sample was serial diluted to 1:10,000 with ultrapure water and a 150μl sample of the diluted solution was added to the Ecoplate™ using a multi-channel pipette. An absorption reading was taken prior to the Ecoplates™ being placed into an incubator set at a constant temperature of 25°C. Absorption readings at 590 nm were taken every 24 h for seven days and results were expressed as optical density (OD). Readings after 120 hours were used in subsequent calculations because it was the shortest time that allowed the best resolution among treatments. The AWCD was determined using the following equation from Garland (1996):

$$AWCD = \sum \frac{OD_i}{31}$$

(13)

where $OD_i$ is OD (corrected by subtracting the control well) of each well and 31 is the number of different C substrates used on the Ecoplate™. Richness was also quantified as the number of utilized C substrates, or the number of wells with a corrected OD over 0.25 (Garland 1996). The SDI was quantified using the following equation from Gomez et al. (2006):

$$H = \sum p_i \times \ln (p_i)$$

(14)

where

$$p_i = \frac{OD_i}{\Sigma OD_i}$$

(15)

## 4.3 Statistical Analysis

Data were tested for normal distribution ($p > 0.05$; Shapiro-Wilk) and equal variances ($p > 0.05$; Levene's). When data were not normally distributed ($SMB_C$ concentrations and Richness), the following statistical tests were performed on log transformed data. Although data were taken from separate jars, the same soil and applied residue was measured repeatedly over time (Swanston et al. 2002). Therefore, differences between treatments on each day, between sampling days for each treatment, interaction effects from treatment by time, as well as overall means (averaged over the 140 d incubation), were analyzed using a two-way repeated measures analysis of variance (ANOVA). Sampling day was used as the within subject repeated measure, and treatment type was used as the between subject main factor (Norman and Streiner 2008). When the ANOVA had significant main effects or interactions, a Tukey's post-hoc multiple comparison test with a Bonferroni correction was used to identify where differences were

71

(simple effects).  The Bonferroni correction was used to account for the dependence of samples in the repeated measures analysis (Rice 1989).

Principle component analysis was performed on the OD data [standardized by dividing each OD by AWCD as explained in Garland and Mills (1991)] from hour 120 of the Biolog Ecoplate™ incubation.  These data had a normal distribution and equal variances only when log transformed.  Log transformed data were adequate for PCA analysis (p>0.75 in a Kaiser-Meyer-Olkin measure and p<0.05 in a Bartlett's test for sphericity).  Pearson product-moment correlations were preformed between select characteristics (SMB$_C$ and SOC concentrations; $\delta^{13}$C-SMB$_C$ and $\delta^{13}$C-SOC; AWCD and LF$_C$; AWCD and LF$_N$).  For all statistical analyses the threshold probability level for determining significant differences was a p-value less than 0.05.  All data analysis was carried out in IBM SPSS Statistics (version 21, 2012).

## 4.4 Results

### 4.4.1 Soil Microbial Biomass Carbon

The interaction effect of treatment by day was significant for SMB$_C$ concentrations (mg C/kg) [F (27,42) = 3.57, p<0.0001)].  There was a significant main effect of time and treatment.  Simple effects showed that on days 1 and 35 all C$_3$ treatments were significantly higher than all C$_4$ treatments (Figure 4.1).  Furthermore, on day 1 all C$_3$ and C$_4$ treatments were significantly higher than their corresponding Control treatments while on day 35, only C$_3$ treatments were.  All C$_3$ and C$_4$ treatments (except C$_4$-1:2), on day 1 had significantly higher SMB$_C$ concentrations than all other days, and day 35 was significantly higher than days 70 and 140.  Control treatments showed no significant differences between days or treatment.  Overall means of each C$_3$ treatment were significantly higher than corresponding Control treatments but no differences were found between C$_3$ and C$_4$ overall means (Table 4.1).  Overall, C$_3$ and C$_4$ treatments

decreased from day 1 to 140. A positive correlation was significant between $SMB_C$ and SOC concentrations [$r(111) = 0.42$, $p<0.0001$].

The interaction effect of treatment by day was significant [$F(27,39) = 1.24$, $p<0.001$] for $SMB_C$ as a percentage of SOC. Main effects showed significant differences between treatments and between days. $C_3$ treatments on days 1 and 35 were significantly higher than $C_4$ treatments as were some of the overall means (Table 4.2). In all $C_3$ and $C_4$ treatments the percentage of SOC made of SMB significantly decreased over time. There were no significant differences between treatments or days in Control treatments.

The interaction effect of treatment by day was significant [$F(18,40) = 2.34$, $p = 0.017$] for $\delta^{13}C$-$SMB_C$ (‰). Main effects showed significant differences between treatments and days. All $C_3$ treatments and their overall means were significantly more depleted in $^{13}C$ than all $C_4$ treatments and their overall means (Table 4.3). All $C_3$ and $C_4$ treatments differed significantly from corresponding Control treatments. Cont-M was significantly more depleted than all other Control treatments on days 1 and 70. All $C_3$, $C_4$ and Control treatments became more depleted from day 1 to day 140. All $C_3$, $C_4$ and Control treatments except for $C_4$-2:3 were significantly more enriched on day 1 than day 70. A significant positive correlation was found between $\delta^{13}C$-$SMB_C$ and $\delta^{13}C$-SOC [$r(89) = 0.89$, $p<0.0001$].

The interaction effect of treatment by day was not significant for $C_3$ contributions to $SMB_C$ (mg C/kg) [$F(10,10) = 3.60$, $p=0.08$)]. There were significant main effects of treatments and time (Table 4.4a). Between treatments, the only significant difference was on day 140 where $C_3$-1:2 was higher than $C_4$-2:3. For all $C_3$ and $C_4$ treatments, day 1 was significantly higher than days 70 and 140. The interaction effect of treatment and day for $C_4$ contributions to $SMB_C$ (mg C/kg) was significant [$F(10, 10)=7.08$, $p=0.013$]. Simple effects showed significant differences between sampling days but not treatments (Table 4.4b). All treatments (except for $C_4$-2:3) decreased over time where day 1 was significantly higher than day 140.

**Table 4.1** Overall means (averaged over the 140 day incubation) for soil microbial biomass (mg C/kg), soil microbial biomass nitrogen (mg N/kg), average well colour development, richness and Shannon's Diversity Index for all treatments.

| Sample | $SMB_C$ | $SMB_N$ | AWCD | Richness | SDI |
|---|---|---|---|---|---|
| $C_3$-S | 590.91 (23.01)[a] | 67.38 (4.65)[ab] | 0.28 (0.01)[a] | 10.78 (0.40)[a*] | 2.54 (0.05)[a] |
| $C_3$-1:2 | 686.24 (58.61)[a] | 77.17 (5.65)[a*] | 0.38 (0.03)[bc*] | 14.89 (0.44)[b*] | 2.75 (0.04)[a*] |
| $C_3$-2:3 | 601.40 (41.90)[a] | 70.84 (0.71)[a*] | 0.31 (0.02)[ac] | 11.56 (0.97)[a*] | 2.61 (0.06)[a] |
| $C_4$-M | 489.07 (25.58)[a] | 40.94 (2.61)[b] | 0.47 (0.01)[c*] | 15.89 (0.48)[b*] | 2.73 (0.02)[a*] |
| $C_4$-1:2 | 468.43 (101.50)[a] | 37.91 (1.37)[c] | 0.44 (0.00)[c*] | 15.11 (0.11)[b*] | 2.74 (0.03)[a*] |
| $C_4$-2:3 | 481.85 (27.31)[a] | 41.04 (3.52)[b] | 0.41 (0.06)[c*] | 13.44 (0.48)[ab*] | 2.69 (0.06)[a*] |
| Cont-S | 284.81 (17.20)[z] | 37.79 (1.34)[z] | 0.24 (0.00)[z] | 8.44 (0.29)[z] | 2.41 (0.06)[z] |
| Cont-M | 274.72 (23.05)[z] | 32.89 (7.93)[z] | 0.27 (0.00)[z] | 9.78 (0.11)[z] | 2.46 (0.03)[z] |
| Cont-1:2 | 290.23 (14.45)[z] | 31.37 (5.97)[z] | 0.25 (0.01)[z] | 8.33 (0.51)[z] | 2.40 (0.06)[z] |
| Cont-2:3 | 295.02 (21.57)[z] | 23.13 (5.37)[z] | 0.27 (0.00)[z] | 8.33 0.00[z] | 2.41 (0.04)[z] |
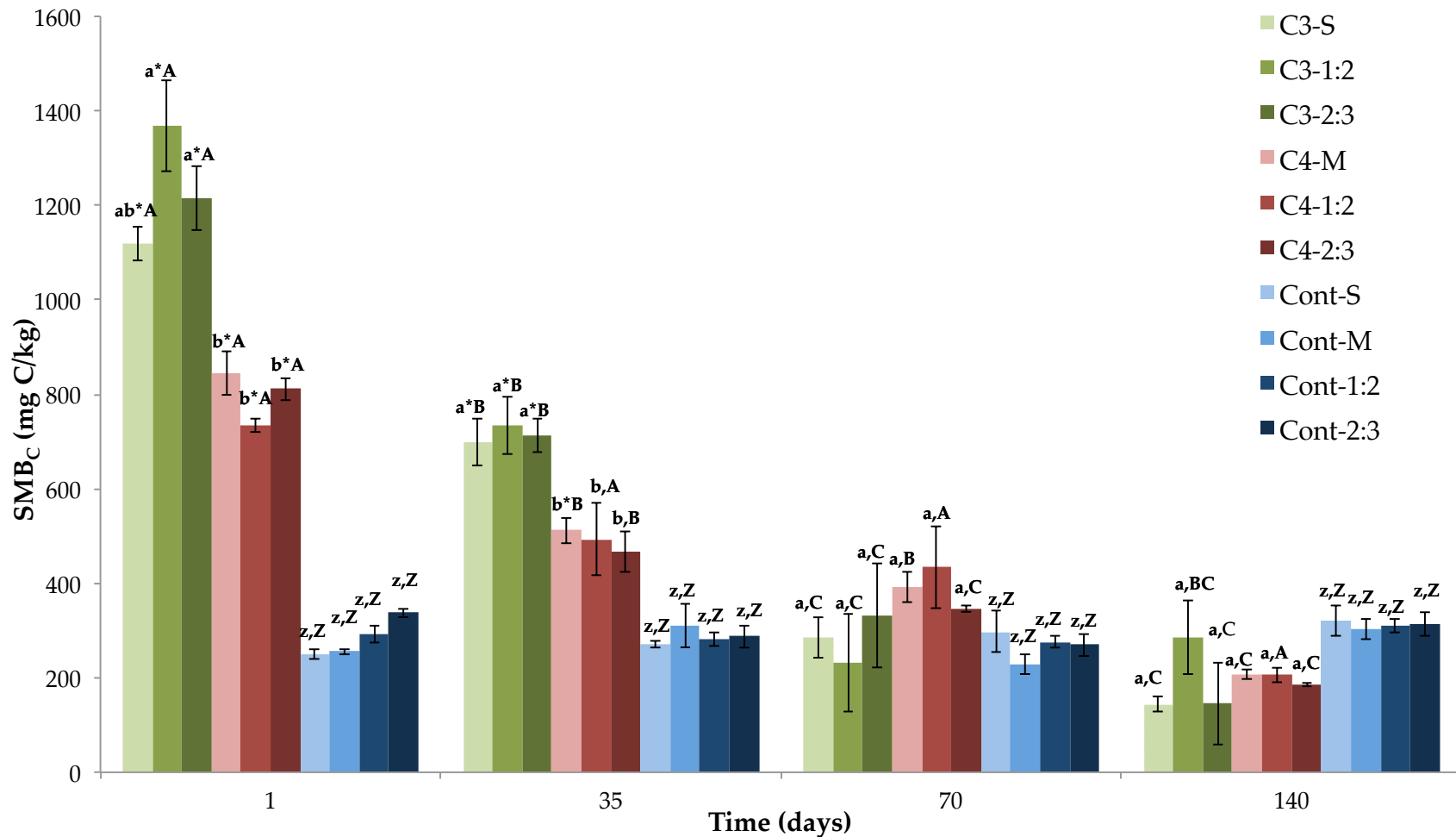
**Figure 4.1** Soil microbial biomass carbon concentration (mg C/kg) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Standard error of the means are shown in parentheses (n=3). Bars with different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-c) and between Control treatments (z). Bars with an asterisk (*) are significantly different from the corresponding Control treatment. Bars with different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 4.2** Soil microbial biomass carbon as a percentage of soil organic carbon (%) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 3.30 (0.32)$^{ab,*,A}$ | 3.79 (0.05)$^{a,*,A}$ | 3.44 (0.06)$^{a,*,A}$ | 2.39 (0.04)$^{b,*,A}$ | 2.04 (0.05)$^{b,A}$ | 2.15 (0.08)$^{c,*,A}$ |
| 35 | 2.34 (0.05)$^{ab,B}$ | 2.71 (0.30)$^{a,*,A}$ | 2.91 (0.15)$^{a,*,A}$ | 2.10 (0.26)$^{ab,AB}$ | 1.84 (0.39)$^{ab,AB}$ | 1.75 (0.11)$^{b,AB}$ |
| 70 | 0.99 (0.24)$^{a,C}$ | 0.75 (0.32)$^{a,B}$ | 0.98 (0.33)$^{a,B}$ | 1.35 (0.05)$^{a,B}$ | 1.52 (0.35)$^{a,AB}$ | 1.10 (0.07)$^{a,B}$ |
| 140 | 0.53 (0.10)$^{a,C}$ | 0.72 (0.17)$^{a,B}$ | 0.46 (0.30)$^{a,B}$ | 0.68 (0.05)$^{a,C}$ | 0.60 (0.01)$^{a,B}$ | 0.50 (0.04)$^{a,C}$ |
| Mean | 1.88 (0.18)$^{ab}$ | 2.11 (0.09)$^{a,*}$ | 1.95 (0.15)$^{a,*}$ | 1.63 (0.09)$^{b}$ | 1.55 (0.38)$^{ab}$ | 1.48 (0.12)$^{ab}$ |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | 0.96 (0.08)$^{z,Z}$ | 1.18 (0.07)$^{z,Z}$ | 1.15 (0.08)$^{z,Z}$ | 1.20 (0.02)$^{z,Z}$ |
| 35 | 1.55 (0.20)$^{z,Z}$ | 1.51 (0.21)$^{z,Z}$ | 1.28 (0.05)$^{z,Z}$ | 1.34 (0.09)$^{z,Z}$ |
| 70 | 1.36 (0.24)$^{z,Z}$ | 1.15 (0.11)$^{z,Z}$ | 1.37 (0.14)$^{z,Z}$ | 1.06 (0.06)$^{z,Z}$ |
| 140 | 1.43 (0.18)$^{z,Z}$ | 1.35 (0.18)$^{z,Z}$ | 1.12 (0.02)$^{z,Z}$ | 1.18 (0.09)$^{z,Z}$ |
| Mean | 1.32 (0.09)$^{z,Z}$ | 1.30 (0.05)$^{z,Z}$ | 1.26 (0.08)$^{z,Z}$ | 1.19 (0.02)$^{z,Z}$ |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$) between $C_3$ and $C_4$ treatments (a) and between Control treatments (z-y). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 4.3** Soil microbial biomass $\delta^{13}C$ (‰) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | -23.83 (0.13)[a,*,A] | -23.74 (0.14)[a,*,A] | -23.64 (0.04)[a,*,A] | -13.30 (0.46)[b,*,A] | -13.05 (0.35)[b,*,A] | -12.68 (0.23)[b,*,A] |
| 70 | -24.02 (0.07)[a,*,B] | -24.11 (0.58)[a,*,B] | -22.92 (0.40)[a,*,B] | -15.61 (0.23)[b,*,B] | -15.03 (0.39)[b,*,B] | -15.73 (0.16)[b,*,A] |
| 140 | -27.73 (0.80)[a,*,AB] | -25.40 (1.13)[a,*,AB] | -27.03 (0.08)[a,*,AB] | -16.58 (0.37)[b,*,A] | -14.30 (0.99)[b,*,AB] | -15.91 (1.85)[b,*,A] |
| Mean | -25.19 (0.29)[a,*] | -24.41 (0.50)[a,*] | -24.10 (0.48)[a,*] | -15.16 (0.31)[b,*] | -14.44 (0.73)[b,*] | -14.77 (0.53)[b,*] |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | -17.81 (0.22)[z,Z] | -19.54 (0.50)[y,Z] | -18.64 (0.52)[z,Z] | -18.98 (0.45)[z,Z] |
| 70 | -20.36 (0.41)[z,Y] | -20.61 (0.13)[y,Y] | -20.44 (0.34)[z,Y] | -20.65 (0.54)[z,Y] |
| 140 | -20.81 (0.50)[z,ZY] | -22.22 (0.18)[z,ZY] | -20.95 (0.49)[z,ZY] | -20.95 (0.11)[z,ZY] |
| Mean | -19.66 (0.26)[z] | -20.79 (0.19)[y] | -20.01 (0.26)[z] | -20.35 (0.04)[z] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (z-y). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 4.4** Soil microbial biomass carbon derived from **a) applied residues** and **b) soil carbon sources** (mg C/kg) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

a)

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 621.31 (21.19)[a,A] | 688.50 (27.88)[a,A] | 615.80 (25.70)[a,A] | 680.73 (59.77)[a,A] | 604.20 (37.58)[a,A] | 739.72 (15.67)[a,A] |
| 70 | 123.04 (1.62)[a,B] | 95.76 (32.10)[a,B] | 87.40 (44.40)[a,B] | 222.26 (10.65)[a,B] | 282.55 (81.46)[a,B] | 191.74 (13.08)[a,B] |
| 140 | 126.19 (9.45)[ab,B] | 198.14 (22.49)[a,C] | 170.74 (53.18)[ab,B] | 113.71 (14.24)[ab,C] | 133.18 (23.41)[ab,B] | 64.90 (7.87)[b,C] |
| Mean | 320.19 (44.72)[a] | 343.71 (8.32)[a] | 261.13 (30.37)[a] | 338.90 (26.61)[a] | 329.50 (105.77)[a] | 336.40 (1.14)[a] |

b)

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 497.87 (35.24)[a,A] | 680.50 (96.56)[a,A] | 667.31 (21.44)[a,A] | 165.11 (63.56)[a,A] | 129.43 (24.37)[a,AB] | 68.53 (25.71)[a,A] |
| 70 | 162.54 (42.36)[a,B] | 135.54 (71.22)[a,B] | 244.19 (98.69)[a,B] | 168.96 (23.71)[a,A] | 151.54 (5.47)[a,B] | 154.16 (18.48)[a,A] |
| 140 | 31.07 (4.99)[a,C] | 87.17 (54.95)[a,B] | 46.40 (21.81)[a,C] | 93.50 (4.83)[a,B] | 73.10 (32.47)[a,A] | 121.38 (12.03)[a,A] |
| Mean | 235.78 (5.71)[a] | 329.37 (61.70)[a] | 298.95 (79.33)[a] | 142.52 (24.51)[a] | 127.52 (18.38)[a] | 109.01 (16.72)[a] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$). Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

## 4.4.2. Soil Microbial Biomass Nitrogen

The interaction effect of treatment by day was significant for $SMB_N$ concentrations (mg N/kg) [$F_{(24,30)} = 2.60$, $p = 0.007$)]. There were significant main effects from treatment and time. Simple effects showed that on day 1, all $C_3$ treatments were significantly higher than all $C_4$ treatments, as was seen with the overall means (Figure 4.2). On day 1, all $C_3$ and $C_4$ treatments except $C_4$-2:3 were significantly higher than the corresponding Control treatment. Significant differences were observed between Control treatments only on day 35. $C_3$ treatments on day 1 were significantly higher than on days 70 and 140. Overall means for $C_3$-1:2 and $C_3$-2:3 were higher than corresponding Control overall means (Table 4.1). Overall, $C_3$ and $C_4$ $SMB_N$ concentrations decreased from day 1 to day 140 ($C_3$ treatments by approximately 70 and $C_4$ from 40 mg N/kg) in $SMB_N$ concentrations.

The interaction effect of treatment by day was not significant [$F_{(9,16)} = 2.05$, $p = 0.10$] for $\delta^{15}N$-$SMB_N$ (‰). Main effects showed no significant differences between treatments and only slight differences between days (Table 4.5). Overall, $C_3$ and $C_4$ treatments except for $C_4$-M became more depleted in $^{15}N$ from day 1 to 140. All Control treatments except Cont-M became more enriched in $^{15}N$ from day 1 to 140, but was only significant in Cont-2:3.
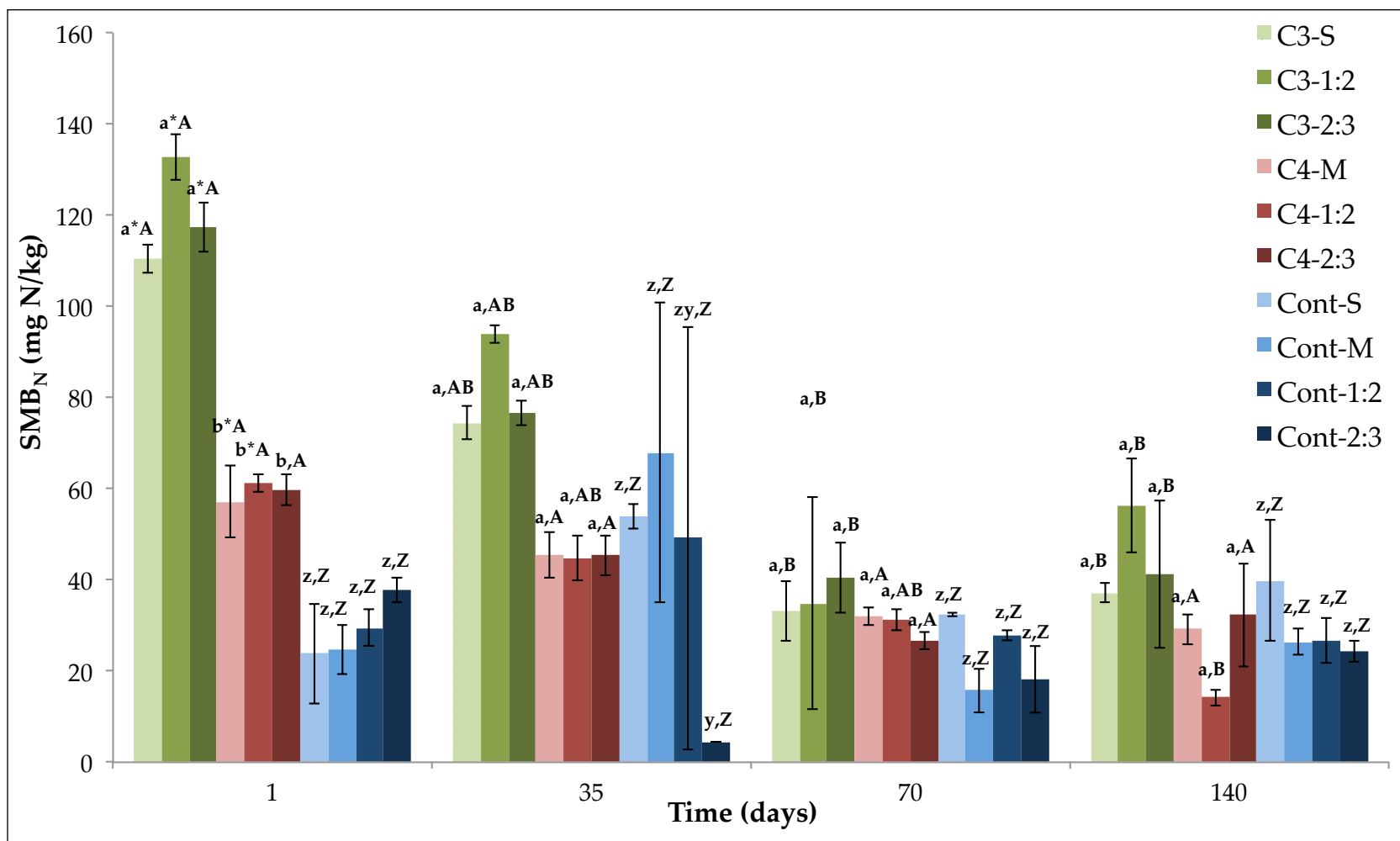
**Figure 4.2** Soil microbial biomass nitrogen concentrations (mg N/kg) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Standard error of the means are shown in parentheses (n=3). Bars with different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (z-y). Bars with an asterisk (*) are significantly different from the corresponding Control treatment. Bars with different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 4.5** Soil microbial biomass nitrogen $\delta^{15}N$ values (‰) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 11.26 (0.44)$^{a,A}$ | 11.49 (0.47)$^{a,A}$ | 11.59 (0.40)$^{a,A}$ | 13.46 (1.96)$^{a,A}$ | 12.02 (0.48)$^{a,A}$ | 12.27 (1.57)$^{a,A}$ |
| 140 | 7.61 (6.49)$^{a,A}$ | 10.48 (1.45)$^{a,A}$ | 7.31 (1.28)$^{a,A}$ | 15.67 (1.33)$^{a,A}$ | 9.62 (2.02)$^{a,A}$ | 11.44 (0.43)$^{a,A}$ |
| Mean | 9.43 (3.19)$^{a}$ | 10.98 (0.57)$^{a}$ | 10.20 (0.85)$^{a,}$ | 14.56 (1.35)$^{a}$ | 10.82 (0.77)$^{a}$ | 11.90 (1.57)$^{a}$ |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | 9.55 (0.15)$^{z,Z}$ | 16.02 (1.43)$^{z,Z}$ | 14.87 (2.38)$^{z,Z}$ | 10.52 (1.26)$^{z,Z}$ |
| 140 | 9.70 (0.17)$^{z,Z}$ | 10.03 (1.68)$^{z,Z}$ | 15.81 (1.87)$^{z,Z}$ | 21.67 (1.21)$^{z,Y}$ |
| Mean | 9.69 (0.12)$^{z}$ | 13.02 (1.48)$^{zy}$ | 15.34 (1.27)$^{zy}$ | 17.56 (0.91)$^{y}$ |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$) between $C_3$ and $C_4$ treatments (a) and between Control treatments (z-y). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

*4.4.3. Soil Microbial Community Structure*

The interaction effect of treatment by day was significant for AWCD [$F_{(18,40)}$ = 13.21, p<0.0001)]. Main effects showed significant differences between treatments and days (Figure 4.3). On day 1, $C_4$-M and $C_4$-2:3 were significantly higher than $C_3$-S and $C_3$-2:3, on day 70, $C_4$-M and $C_4$-1:2 were significantly higher than all $C_3$ treatments, and on day 140, $C_3$-1:2 was significantly higher than all other treatments except for $C_4$-2:3. Simple effects showed that in all $C_3$ treatments, day 1 was significantly higher than day 70 and 140, except for $C_3$-2:3. All three days differed significantly for all $C_4$ treatments. For most Control treatments, day 70 was significantly lower than day 1 and 140. Overall means of $C_3$-S were significantly lower than all other treatments, and all but $C_3$-S were higher than corresponding Control treatments (Table 4.1). There were significant positive correlations found between AWCD, and both $LF_C$ [$r(94)$ = 0.41, p<0.0001] and $LF_N$ [$r(90)$ = 0.30, p = 0.006].

The interaction effect of treatment by day was significant for R [$F_{(18,40)}$ = 9.00, p<0.0001)]. Main effects showed differences between treatments and days (Figure 4.4). On day 70, $C_4$-M and $C_4$-1:2 were significantly higher than all other treatments. Significant simple effects showed that in $C_3$ and $C_4$ treatments, day 1 was significantly higher than all other days except for $C_3$-2:3 and $C_4$-1:2, where day 1 was significantly higher than only day 140. Overall means of $C_3$-1:2, $C_4$-M, and $C_4$-1:2 were significantly higher than overall means of $C_3$-S, and $C_4$-1:2 (Table 4.1). All overall means were significantly higher than corresponding Control overall means. All R values decreased from day 1 to day 140.

The interaction effect of treatment by day was significant for SDI [$F_{(18,40)}$ = 11.43, p<0.0001)]. Main effects showed differences between treatments and days (Table 4.5). Differences between treatments were found on day 1 and 70. On day 1, $C_3$-1:2 was significantly higher than $C_3$-S, and all $C_3$ and $C_4$ treatments were significantly higher than corresponding Control treatments. On day 70, $C_4$-M and $C_4$-1:2 were significantly higher than $C_3$-S, and all but

C$_3$-S and C$_4$-2:3 were higher than corresponding Control treatments. Simple effects showed that in all C$_3$ and C$_4$-2:3 treatments, day 1 was significantly higher than day 70 and 140. Overall means for C$_3$-1:2 and all C$_4$ treatments were significantly higher than the corresponding Control treatment overall means (Table 4.1). No significant differences were found between Control treatments, but all were significantly higher on day 1 than 70. Shannon's diversity index in all C$_3$, C$_4$ and Control treatments decreased from day 1 to 140.
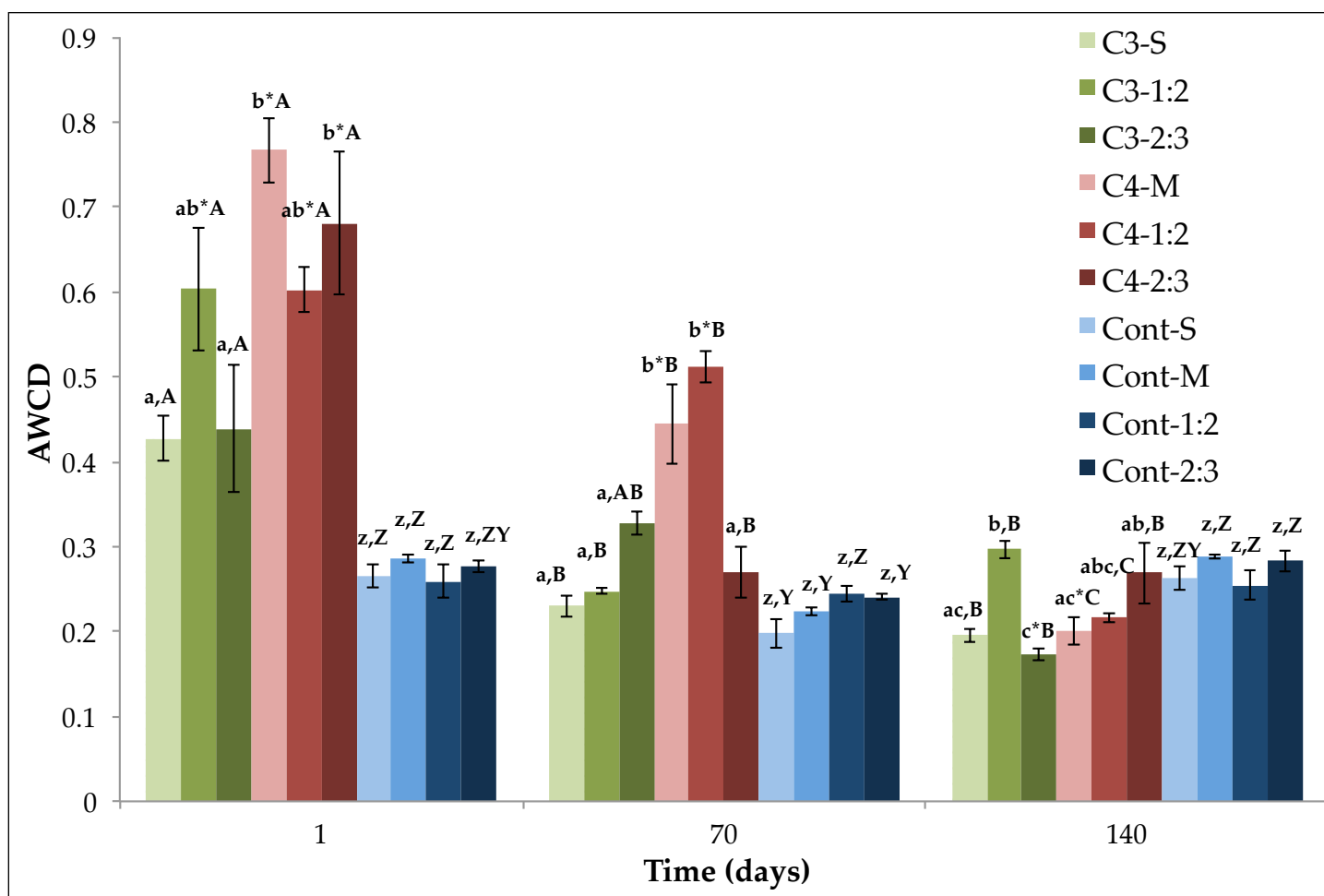
**Figure 4.3** Average well colour development values after 120 hours of incubation in an Ecoplate in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-c) and between Control treatments (z-y). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Figure 4.4** Richness values (number of wells over an OD of 0.25) after 120 hours of incubation in an Ecoplate in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-c) and between Control treatments (z-y). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.
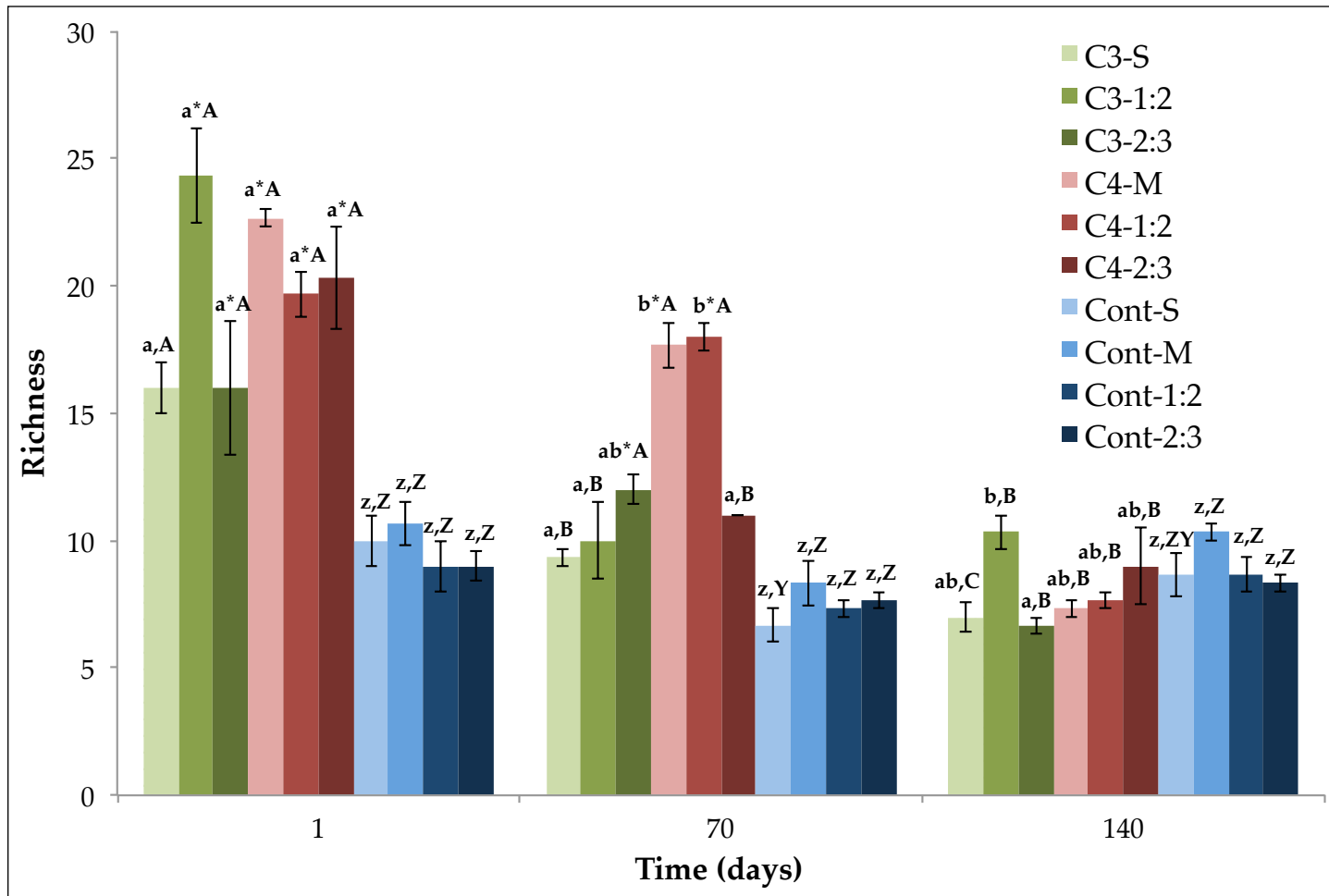
**Figure 4.5** Shannon's Index values after 120 hours of incubation in an Ecoplate in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Standard error of the means are shown in parentheses (n=3). Values ollowed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a-c) and between Control treatments (z-y). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.
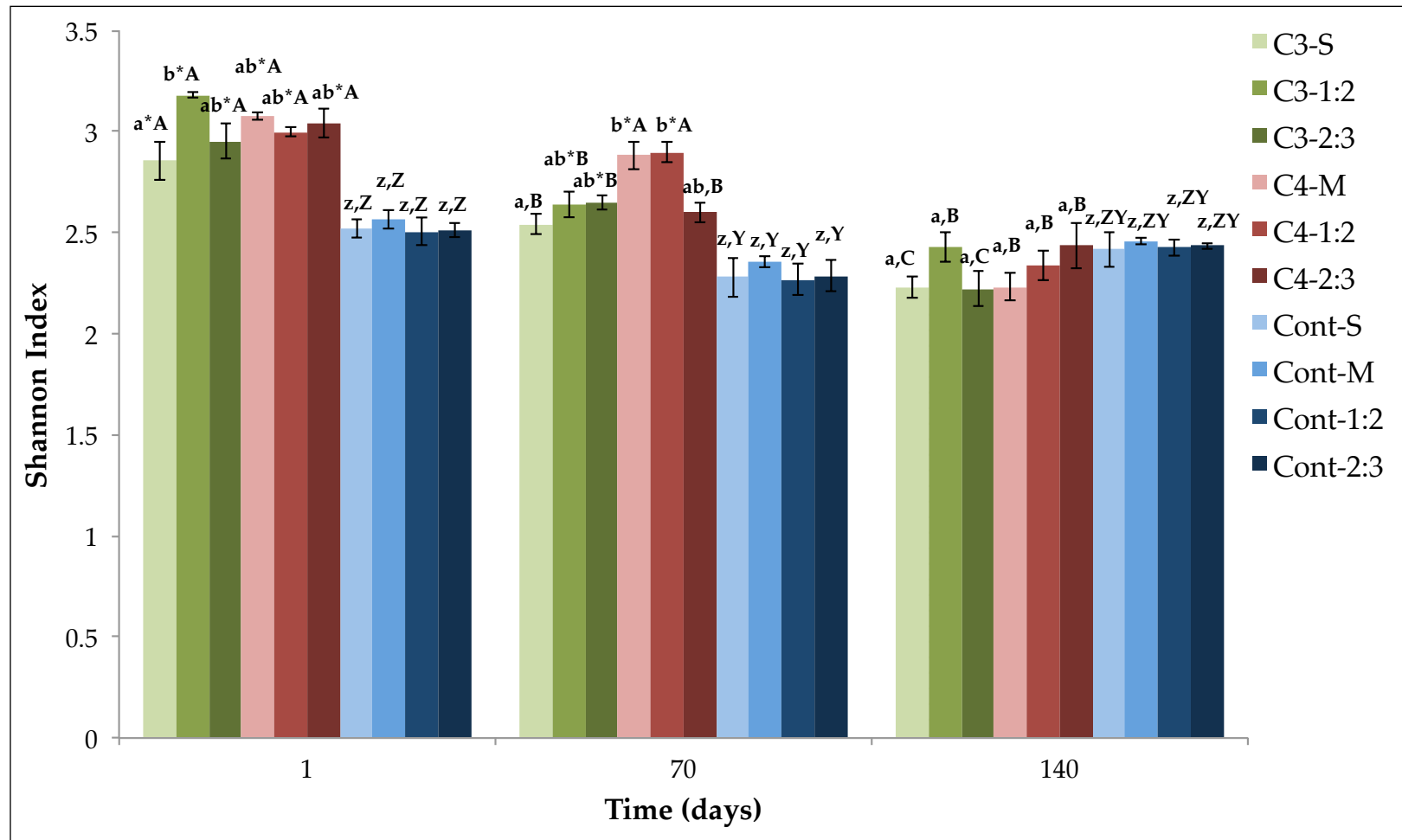
Principal components were significant and PC1 explained 70%, 81% and 88% of the variance, while PC2 explained 15%, 9% and 5% of the variance for days 1, 70 and 140 respectively (Figure 4.6a b and c). On day 1, each treatment type was clustered together, $C_3$ more spread out than Control and $C_4$. $C_3$-1:2 could be observed to be clustered with the $C_4$ treatments on day 1. On day 70, all $C_4$ treatments and $C_3$-2:3 appeared to be clustered, as well as all Control treatments, $C_3$-S and $C_3$-1:2. On day 140, clusters consisting of $C_3$-1:2 and $C_4$-2:3, as well as the remaining $C_3$ and $C_4$ treatments with Cont-M were observed.



**Figure 4.6a** Principle components on soil microbial activity after 120 hours of incubation in Biolog Ecoplates™ of all treatments for day 1.

**Figure 4.6b** Principle components on soil microbial activity after 120 hours of incubation in Biolog Ecoplates™ of all treatments for day 70.



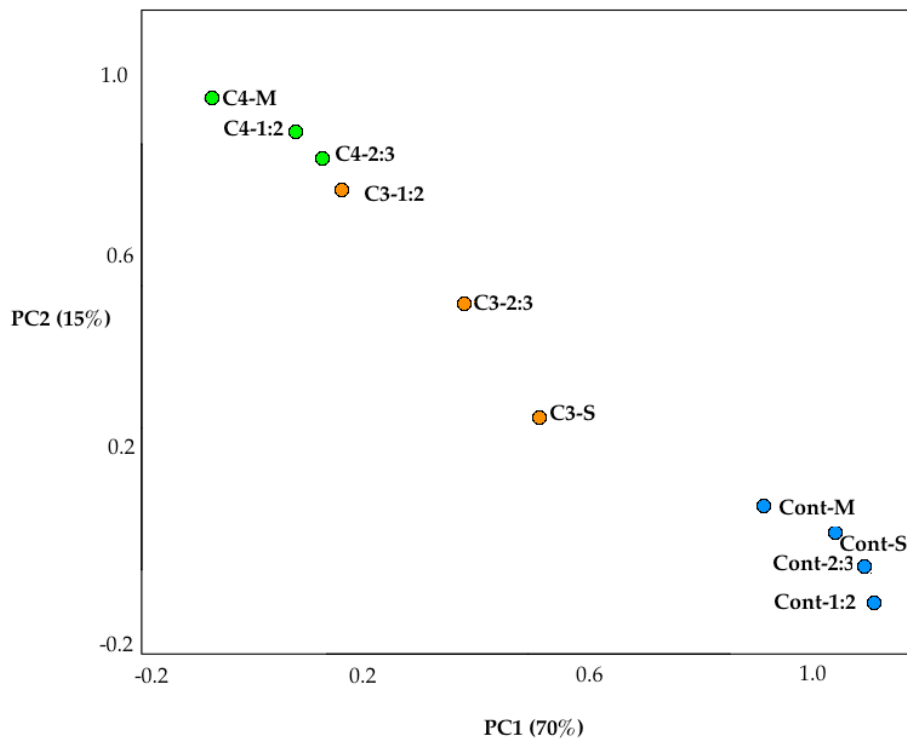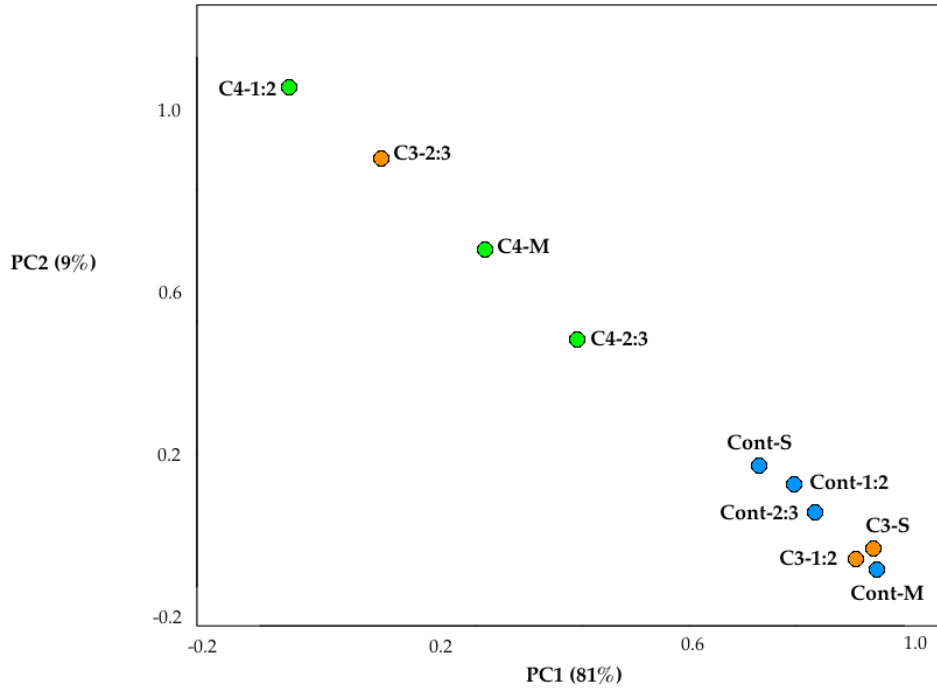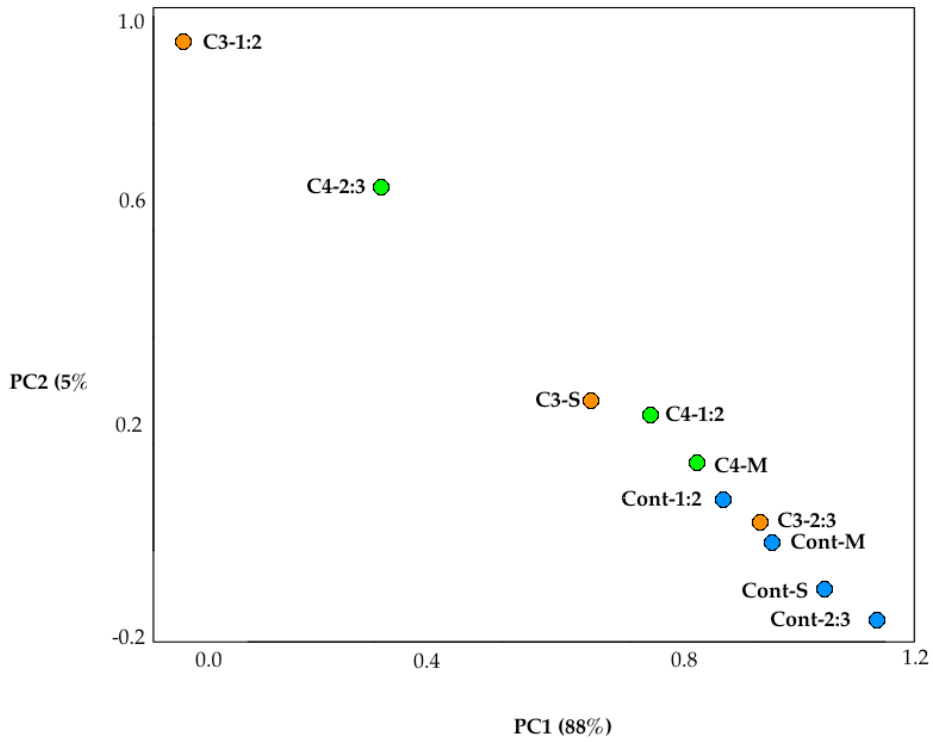**Figure 4.6c** Principle components on soil microbial activity after 120 hours of incubation in Biolog Ecoplates™ of all treatments for day 140.

**4.5 Discussion**

*4.5.1 Soil Microbial Biomass Carbon*

Similar $SMB_C$ concentrations to a similar depth were found in a study by Alvarez et al. (1998) examining the effect of different management practices on SMB. In the current study, there was a clear effect of residue addition on $SMB_C$. High $SMB_C$ concentrations (especially in the $C_3$ treatments) at the beginning of the incubation supported the occurrence of the 'priming effect', which is the increase of native SOM mineralization caused by the addition of easily accessible and decomposable residue (Kuzyakov et al. 2000; Hamer et al. 2009). It has been shown that the priming effect along with the use of high nutrient fresh litter (in the current study from 42 to 45% C and 0.7 to 1.4% N) by microbes, causes more C sequestration (Fontaine et al. 2011). An overestimation of SMB has been observed due to $CHCl_3$ in the fumigation dissolving some of the non-living portion of organic C (Martens 1995). However the fumigation extraction method has been found to be reliable when organic amendments are being included (Vance et al. 1987). Soil microbial biomass C concentrations were considerably higher in $C_3$ than $C_4$ treatments for the first two sampling days, which showed a strong effect of residue on $SMB_C$. Lower C/N ratios and better quality of soybean residue when compared to maize residue, created a more labile organic matter source, causing higher microbial growth (Manjaiah et al. 2000; Suman et al. 2006). This supports the LF results (Chapter 3), illustrating that the LF is closely related to residue lability.

There was also evidence of an effect of cropping type on $SMB_C$. Rivest et al. (2010) found that $SMB_C$ increased in intercrops with soybean, which improved nutrient turnover, highlighting the importance of intercropping with a legume. Results from the current study showed higher $SMB_C$ in $C_3$-1:2 on 3 of 4 sampling days, indicating a more beneficial intercrop treatment for soil microbes. This could have been due to the higher decomposability, less lignin and lower C/N ratio of soybean, compared to maize residues (Uphoff et al. 2006). Furthermore,

SMB$_C$ in both C$_3$ and C$_4$ treatments decreased to be equal or less than Control treatments, likely due to microbes using the newest and most labile material first (Blagodatskaya et al. 2011). Control treatments stayed relatively constant because of microbes degrading lignified or more recalcitrant material throughout the incubation, which did not supply as much energy for microbial population as fresh, labile litters (Fontaine et al. 2011).

Values of SMB$_C$ as a percentage of SOC in the current study were in the range of 1-4%, which is generally found in the literature (Anderson and Domsch 1989; Sparling 1992; Suman et al. 2006). As expected, due to added residue, initial values were higher in C$_3$ and C$_4$ treatments than Control treatments. However, there was a large drop in C$_3$ and C$_4$ treatments after day 35, but not in Control treatments. This was most likely due to the drop in the SMB$_C$ after the same day due to the depletion of available substrate (Müller-Stöver et al. 2012). Moore et al. (2000) found that the percentage of SMB$_C$ of SOC was the highest in rotation crops than in sole soybean and maize crops, due to a higher amount of residue in rotation crops. Similarly, in the current study, C$_3$ intercrop treatments were higher than sole, but not in the C$_4$ treatments, likely illustrating the high quality of soybean residue for microbial biomass (Rivest et al. 2010).

Isotopic values in the SMB$_C$ were differentiated between each treatment. Incorporation of soybean and maize residues into the SMB was observed through the similarity of $\delta^{13}$C-SMB with the $\delta^{13}$C value of the residues. However, results showed that all C$_3$ and C$_4$ treatments became more depleted over time. This may have been due to decomposition of both residue and SOC. When compared to SOC, SMB$_C$ in C$_3$ treatments was depleted in $^{13}$C, and enriched in C$_4$ treatments. This could have been explained by soil microbes using more depleted materials (thereby leaving the more enriched C in the soil), or by fractionation occurring during microbial respiration (Schweizer et al. 1999; Šantrůčková et al. 2000; Dijkstra et al. 2006) since the microbial community became depleted over time even with an enriched source of residue in C$_4$ treatments. However, it may simply have been because of the utilization of each residue type supplied. Microbes in Control treatments with no residue addition also became depleted over

time, most likely because the food source was more recalcitrant, which was supported by the similar isotopic values of $SMB_C$ and SOC at the end of the experiment. These findings agreed with an enrichment of $^{13}C$ of the LF in all treatments (Chapter 3). The relationship between $SMB_C$ and SOC, as well as $\delta^{13}C$-SOC and $\delta^{13}C$-$SMB_C$, observed in this study showed the importance of using SMB as an indicator of soil quality with changes in the soil environment. For example, sequestration rates of SOC were highly and positively correlated with soil microbial enzyme activity in a study by Yuan et al. (2012). Therefore, a change in the soil environment that affects SOC quantity, should also affect SMB, but most likely before SOC due to the faster turnover times of the SMB (Blagodatskaya et al. 2011).

Due to utilization of the applied residue, it was expected that $SMB_C$ in $C_3$ and $C_4$ treatments would have higher contributions from new residues than from old C sources. However, in $C_3$ treatments, contribution from new and old sources was similar. This may have been due to previous contributions, as was seen in Oelbermann and Echarte (2011), or due to the higher biomass of maize (contribution from old C sources is lowest in the soybean sole cropped soil) and from previous $C_3/C_4$ grasslands that dominated the area (Costantini et al. 2007). The large drop in contributions from old C sources in the $C_3$ treatments confirmed this, as the SMB quickly and preferentially incorporated fresh residue sources over older more recalcitrant sources (Blagodatskaya et al. 2011).

*4.5.2 Soil Microbial Biomass Nitrogen*

Similar $SMB_N$ concentrations and turnover times were found at the same site in a baseline study by Oelbermann and Echarte (2011). In the current study, there was a clear effect from residue addition on $SMB_N$, where soybean residues had a greater influence than maize residues. This was expected due to higher N concentration in soybean than maize residues (more than double). In a study by Green and Blackmer (1995), it was seen that with the addition of either soybean and maize residues, immobilization increased, but did so at a faster rate when soybean

was added. This highlights the higher quality of soybean residues in cropping practices and was supported by higher $SMB_N$ in $C_3$ treatments in the current study. Furthermore, in a study using eight lab and field experiments with different soil types and N concentrations, Vigil and Kissel (1991) found that a substrate C/N ratio below 40 will generally cause net mineralization, while above 40 will cause net immobilization. This corresponded to mineralization with soybean residue addition (C/N ratio of 32) and immobilization with maize residue addition (C/N ratio of 64) in the current study. Immobilization, along with an anaerobic environment, can cause conditions for denitrification, especially with the addition of residues with high C/N ratios (Snyder 2011). Although denitrification was not directly measured in this study, conditions for denitrification were very likely to have occurred (soil moisture was 60% filed capacity and C/N ratio of the residues were 32 and 64 for soybean and maize respectively). Both immobilization and denitrification can cause N deficiency in the soil, which limits growth of microbes (Hadas et al. 2004). The higher C/N ratio of the added maize residues explained the lower $SMB_N$ in $C_4$ treatments. The highest $SMB_N$ values for the $C_3$-1:2 treatment suggested this to be a more desirable cropping practice. Control treatments were constant throughout the incubation, suggesting that they were in a relatively steady state between immobilization and mineralization compared to $C_3$ and $C_4$ treatments.

Values of $\delta^{15}N$-SMB in this study were comparable to a study by Dijkstra et al. (2006) where $\delta^{15}N$-SMB was quantified for multiple soil types. The SMB of both $C_3$ and $C_4$ treatments were enriched in $^{15}N$ compared to residue which was expected, due to N transformations after the addition of crop residue. Dijkstra et al. (2008) found that when there is more N mineralization, there is also more enrichment of $SMB_N$. Enrichment over time of the $SMB_N$ may have resulted from decomposition of previously decomposed organic N (Dijkstra et al. 2006). This did not happen in soil amended with soybean residue, since it supplied microbes with more N than the maize residue. In the $C_4$ treatment, only $C_4$-M was enriched, suggesting that intercrop soil also supplied enough labile N for microbes, even without applied soybean

residue.  This could have showed more N cycling in intercrop than sole soils, along with illustrating a potentially better soil quality (Sun et al. 2009).  This is not supported by Control treatments, in which intercropped and soybean sole crop soils became enriched and maize sole crop, depleted.  Since there were no applied residues to Control treatments, this could have been due to the whole soil being more processed than soil with added residue (Dawson et al. 2002).  When compared to TN, the SMB was consistently enriched in $^{15}N$, which has been seen in previous studies (Dijkstra et al. 2006).  This enrichment was due to changes in soil C and N availability and N transformations, more specifically, N dissimilation that preferentially removes lighter N ($^{14}N$) from microbial biomass (Dijkstra et al. 2008, Coyle et al. 2009). Enrichment of $SMB_N$ relative to TN could have also represented decomposition of already decomposed N compounds (Dijkstra et al. 2006).

*4.5.3 Soil Microbial Community Structure*

Soil microbial community structure was quantified to evaluate soybean and maize residue incorporation in sole crops and intercrops.  Average well-colour development represents activity of the microbial community in each Ecoplate™ (Gomez et al. 2006).  In the current study, AWCD showed results in the same range as the study by Gomez et al. (2006).  Richness represents cells in which the microbial community oxidized the C source, measuring how many groups were on each well, or the density of microbial groups living together (Derry et al. 1998). Similar results were found in a study that focused on organic matter amendments to soil (Gomez et al. 2006).  Shannon's diversity index is a measure of community diversity utilizing C in each well (Derry et al. 1998).  A similar range of SDI values were found in a study by Lewis et al. (2002) where SMCS characteristics were measured in rehabilitated soils, and also falls within the normal range of 1.5 to 3.5 for SDI (Magurran 1988).

This study showed that microbial activity, density and diversity were greatest in $C_3$ and $C_4$ treatments immediately after residue was applied (Kuzyakov et al. 2000).  This was most

likely because a relatively young, nutrient-rich food source was made available. In a study by Lupwayi et al. (1998), a greater microbial diversity was found in zero tillage crops, due to more heterogeneous distribution, and greater input of litter on the soil surface than in conventional tillage. The decrease of activity, density and diversity, in $C_3$ and $C_4$ treatments in the current study was likely due to the depletion of resources (Müller-Stöver et al. 2012). This was supported by the positive correlation between LF (both C and N), and AWCD and R; when there was a larger food source for microbes, their activity and density were higher. Lower R over time suggested that fast growing microbes grew in the residue amended soils, supported by the quickly decreasing $SMB_C$ and $SMB_N$ results. As the population of these fast growing microbes declined by day 70, activity, density and diversity also decreased. Another possible reason for the decrease in SMCS parameters is gas accumulation that is seen in closed incubation systems which inhibits microbial growth (as is also seen with SMB values) (Mondini et al. 2010). By day 70 for SMB, and by day 140 for activity, diversity and density, $C_3$ and $C_4$ treatments were approximately equal to Control treatments which could have been due to the accumulation of $CO_2$ in the $C_3$ and $C_4$ treatments.

All three characteristics of activity, density and diversity were the higher in $C_3$-1:2 on 2 of 3 of the sampling days, suggesting a better habitat and food source for microbes in 1:2 intercrops with soybean residue (Giller 1996). In an experiment on the same soil as the current study, it was found that 2:3, and to a lesser extent, 1:2 intercrops had higher microbial activity, density and diversity (Dubois 2008). Furthermore, results from a study by Gomez et al. (2006) show higher activity, density and diversity on native grassland, and when organic matter was applied to soils (with more a more diverse source of food and habitat) than agricultural soils. This corresponds to higher activity, density and diversity in residue amended soils and occasionally in intercrop soils.

Problems of using SDI for quantifying diversity arise due to the difficulty in identifying differences between taxonomic groups, however SDI does take into account the relative

abundances and species richness of the bacterial communities (Watve and Gangal 1996). Furthermore, Stefanowicz (2006) identifies that a drawback of the Ecoplates™ is that some microbes are not identified in analysis, thereby only a portion of the community is represented. Despite drawbacks, these methods are still commonly used in soil microbial community studies (Derry et al. 1998; Gomez et al. 2006; Lewis et al. 2010).

Principle component analysis was performed to determine changes that occur with time in the microbial community as a result of decomposition of soybean and maize residues. Distinct groups, showed how communities of each treatment differed in their C substrate utilization patterns on the Biolog Ecoplate™ (Derry et al. 1998). On day 1, Control, $C_3$, and $C_4$ treatments were distinct from one another, showing differences in C metabolism and distinct microbial communities for each residue treatment. This difference may have resulted from different microbial communities present as a result of residue type, as well as increased utilization of residue C in $C_3$ and $C_4$ treatments than Control treatments (Griffiths et al. 1999). The distinction corresponded to differences in AWCD, R and SDI between each treatment on day 1. Differences in community composition could have also been due to differences in organic matter quality of soybean and maize residues (Bossio et al. 1998).

As soil fractions were further decomposed, treatments became less differentiated, and therefore had more similar microbial communities. However, distinct groups could still be observed. On day 70, a cluster consisting of $C_3$-2:3 and all $C_4$ treatments coincided with the highest TN concentrations, $SMB_C$, $C_4$ derived $SMB_C$, as well as the lowest SOC/TN ratios, $C_3$ derived $CO_2$, and $CO_2$ production rates. These results suggested that this cluster had different metabolic diversities which could have influenced residue stabilization. A temporary enrichment of the $SMB_C$ has been associated with the lability of added residues, which was also observed in the current study on day 70 for $C_3$-2:3 and all $C_4$ treatments (Bossio et al. 1998).

Similarly, the cluster of $C_3$-1:2 and $C_4$-2:3 on day 140 corresponds with the highest $C_3$ contribution to $LF_C$ concentrations, $SMB_C$ derived from $C_3$ sources, SMCS characteristics, as well

as the lowest $CO_2$ production rates. Fierer et al. (2003) suggested that changes to soil microbial diversity can have an influence on soil processes, such as C and N mineralization. For example, a study by Cavigelli and Robertson (2000) comparing agricultural and grasslands under similar conditions, showed that denitrification rates changed with microbial diversity. Therefore, the changes in clusters over time in the current study could have represented differences in residue decomposition processes (Cavigelli and Robertson 2000). This could have illustrated that treatments clustered with Control treatments had less of a C sequestration potential then others, as well as that intercropped treatments especially $C_3$-1:2 and $C_4$-2:3 could have greater C sequestration potential resulting from a more diverse microbial community.

## 4.6 Conclusion

This study quantified $SMB_C$, and $SMB_N$ concentrations (g/kg) and respective $\delta^{13}C$ and $\delta^{15}N$ values (‰), as well as SMCS characteristics from intercropped and sole soils, in an 140 day incubation experiment. Results from this study indicated a clear affect of residue addition on soil microbial communities and soil processing by microbial communities. A possible difference between quality of residue was also seen in this chapter, indicating that soybean could have been a higher quality residue regarding characteristics of the microbial community structure. It is probable that more immobilization occurred in soils with maize residue and mineralization occurred in soils with soybean residue. Higher values for $SMB_C$, $SMB_N$, microbial diversity, density and activity suggested that the 1:2 intercrop treatment with soybean residue added was a more desirable intercrop treatment with respect to microbial biomass and community structure. PCA data showed differentiation of treatments on the basis of residue incorporation and C stabilization, showing the most beneficial at the end of the incubation for two intercrop treatments. These results showed 1:2 as the most desirable intercrop and contradict with results that found 2:3 as the most desirable intercrop (Chapter 3). However, this chapter still confirms the importance of including a legume in complex agroecosystems.

# 5. GREENHOUSE GASES

## 5.1 Introduction

Global carbon dioxide ($CO_2$) and nitrous oxide ($N_2O$) concentrations have increased by approximately 100 ppm and 60 ppb, respectively, in the last 150 years, contributing considerably to climate change (IPCC 2007). This is due in part to modern, industrialized agriculture which emits greenhouse gases (GHGs) through deforestation, burning fossil fuels, as well as through management practices that contribute to the rapid decomposition of soil organic matter (SOM) and the application of nitrogen (N) fertilizers (Janzen 2004; Johnson 2009). Agriculture may be responsible for 10 to 12% of all GHG emissions, equal to 5.1 to 6.1 Gt $CO_2$-eq/year (Niggli et al. 2009), and is expected to increase by 30 to 65% for $N_2O$ by 2030 and remain stable for $CO_2$ (Smith et al. 2007).

While agriculture is a significant source of GHGs, it has also been demonstrated to be a C sink (Marland et al. 2003). If best management practices are implemented, it is estimated that agriculture could potentially mitigate 5,500 to 6,000 Mt $CO_2$-eq/year by 2030 (Smith et al. 2008). Some specific techniques to increase SOC and reduce atmospheric $CO_2$ include rotation or cover crops with perennials or legumes, nutrient, tillage and residue management, and agroforestry (Smith et al. 2008). More broadly, increased vegetation growth removes more $CO_2$ from the atmosphere, and if plant residue is retained on the soil, more SOC is sequestered, specifically, if the amount of organic matter input is greater than the rate of decomposition (Post et al. 1982; Wang et al. 2010). In Argentina, practices such as no-tillage agriculture have been adopted to decelerate soil degradation, and have helped remove approximately 20 Mt of $CO_2$ eq/year from the atmosphere (Smith et al. 2007). Along with GHGs being removed from the atmosphere, better crop management aids in higher overall soil quality and more sustainable agriculture

(Wang et al. 2010). Mitigation of $N_2O$ emissions is also important, as $N_2O$ is 200 times more efficient at radiative forcing than $CO_2$ (Hillel 1998). The main agricultural emissions of $N_2O$ are from the use of fertilizers and animal manures. Therefore the reduction in the use of these are important in mitigating $N_2O$ emissions (Johnson et al. 2007). For example the management and reduction of synthetic N fertilizers can reduce the amount of $N_2O$ emitted (cite). One way to achieve this is a land use change from sole crops to other forms of land use. For example rotations, and intercrops have both been shown to reduce dependence on N fertilizers and lower $N_2O$ emissions (Drury et al. 2008; Dyer et al. 2012). However, adding a legume species to intercropping systems has also shown an increase in $N_2O$ production (Guo et al. 2009).

Intercropping has been shown to increase C sequestration. For example one study found that a *Gliricidia sepium* (a leguminous tree) and maize intercropping system in southern Malawi, sequestered more C than a maize sole crop (Makumba et al. 2007). A study in the Canadian temperate region found $CO_2$ and $N_2O$ emissions were lower from a tree-based intercropping system than from sole crops (Peichl et al. 2006; Beaudette et al. 2010). Evers et al. (2010) suggested that lower $CO_2$ emissions in intercrops were due to higher C sequestration in above and below-ground biomass, and lower $N_2O$ emissions were due to trees utilizing extra N fertilizer. Similarly, barley/pea intercropping was found to lose less N through $N_2O$ than sole crops (Pappa et al. 2011). These studies collectively demonstrate the GHG and climate change mitigation potential of complex agroecosystems such as intercropping.

Although GHG emissions have been studied in intercrops, it has primarily been in tree-based intercrops (Makumba et al. 2007; Evers et al. 2010). Furthermore, only one known field study has been carried out measuring GHG from a soybean/maize intercropping system in a temperate region (Dyer et al. 2012). The effect of $C_3$ or $C_4$ residue application and decomposition on $CO_2$ has been thoroughly studied, for example in Manitoba Glenn et al. (2011) evaluated the amount of maize residue lost to respiration after harvest on a previously $C_3$ dominated soil. Furthermore, decomposition of $C_3$ legumes has been found to be correlated

with $CO_2$ production rates in a field experiment in India (Arunachalam et al. 2003). It has also been found that decomposition will not necessarily decline with higher $CO_2$ concentrations as previously thought (Ross et al. 2002). Potthoff et al. (2005) analyzed how a mixture of residues and the addition of N affected decomposition and $CO_2$ production rates. However, there have been no known controlled long-term incubations that have focused on $CO_2$ and $N_2O$ emissions from intercrops and sole crops with added residues. A useful tool used to evaluate C and N transformations in complex agroecosystems is isotopes. [13]C from respired $CO_2$ has been used to determine whether ^{13}C discrimination occurs during decomposition, contributing to SOM enrichment (Boström et al. 2007). Furthermore, using ^{13}C-labeled lignin, and ^{13}C from respired $CO_2$, an incubation study by Bahri et al. (2008) confirmed that lignin originating from plant matter is more recalcitrant than other fresh material (Bahri et al. 2008). Carbon isotopes from respired $CO_2$ have also been used as an informative tool to study C transformations when changing from a $C_3$ to a $C_4$ dominated ecosystem or vice versa. For example Ekblad et al. (2002) determined that microbial discrimination of ^{13}C was negligible during respiration, by measuring $\delta$[13]C from respired $CO_2$ after changing microbial substrates from $C_3$ to $C_4$. Furthermore, ^{13}C from respired $CO_2$ has been used to distinguish between contributions from $C_3$ and $C_4$ crops throughout the season in a rotation or between different soil substrates (Liang et al. 1999; Griffis et al. 2005). So far, little knowledge exists about contributions from soybean and maize residues to $CO_2$ from legume/cereal intercrops and respective sole crops. No know incubation study has been carried out on soil from a temperate zone soybean/maize intercropping system that combine the use of GHG measurements and $\delta$[13]C from respired $CO_2$.

This study evaluated the influence of soybean and maize residue stabilization upon soil respired $CO_2$ and $N_2O$ between sole crops and intercrops, in an incubation study. This was accomplished through two objectives:

(a) To quantify changes in $CO_2$ and $N_2O$ production rates due to soybean and maize crop residue input.

(b) To quantify C contributions to respired $CO_2$ from soybean and maize residues, as well as the fractionation from SOC to $CO_2$ due to residue incorporation, using $\delta^{13}C$ natural abundance.


## 5.2 Materials and Methods

### 5.2.1 Greenhouse Production Rates

Septa on jars were sealed with silicone which made the experiment a closed system and allowed for gas samples to be taken without removing the lid. Concentrations of $CO_2$ and $N_2O$ (ppm) from the headspace of each incubation jar was measured every four days for the first 14 days to capture the initial peak that commonly occurs in incubation experiments (Kristiansen et al. 2004; Crow et al. 2006). After the first 14 days, one sample was taken every seven days for 126 days. Greenhouse gas samples were taken with a syringe, through the septum and injecting 4 ml of gas from the headspace into evacuated 3 ml Exetainer vials (Labco Limited, Ceredigion, UK). Samples were analyzed using a gas chromatograph (G.C., Agilent Technologies 6890N Network GC System, California, USA) at the University of Waterloo. Standards of 10,000 ppm, 20,000 ppm, 40,000 ppm $CO_2$ and 0.04 ppm, 0.01 ppm, and 1 ppm $N_2O$ were used to calibrate the GC. The following equation, adapted from Hogg et al. (1992) was used to determine daily production rates (R) of both $CO_2$ and $N_2O$:

$$R = \left(\frac{D \times V}{t}\right) \times \left[\frac{(C_s - C_a)}{M}\right]$$

(16)

where D is density of C or N in the jar adjusted for temperature (g/l), V is volume of effective headspace in the jar (0.962 l), t is the time interval between samples (days), $C_s$ and $C_a$ are concentrations of the GHG in the sample, air jar respectively (ppm), and M is dry mass of the soil sample (g).

*5.2.2 $\delta^{13}C$ of Respired Carbon Dioxide*

Gas samples to be analyzed for stable C isotopes were taken by injecting 15 ml of air from the headspace into evacuated 12 ml Exetainer vials (Labco Limited, Ceredigion, UK). Vials were sent to the University of California Davis Stable Isotope Facility and analyzed for $\delta^{13}C$-$CO_2$ using a ThermoScientific PreCon-GasBench system interfaced to a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, Germany). Results from UC Davis were reported in per mill (‰). Proportions of $CO_2$ derived from applied residues and soil C sources when soybean or maize residues were added to the soil, was quantified using the following two end-member mixing model from Liang et al. (1999):

$$C_3 \cdot C(\%) = \left( \frac{\delta_{CO_2} - \delta_{Cont}}{\delta_{Soy} - \delta_{Cont}} \right) \times 100$$

(17)

and

$$C_4 \cdot C(\%) = 1 - C_3 \cdot C(\%)$$

(18)

where $\delta_{CO_2}$ is $\delta^{13}C$ from $CO_2$ respired from soils with added residue, $\delta_{Cont}$ is $\delta^{13}C$ from $CO_2$ from Control treatments soil (no residue added) and $\delta_{residue}$ is the mean $\delta^{13}C$ value of soybean (-28.62‰) or maize (11.89‰) residue. Contributions from $C_3$ and $C_4$ to respired $CO_2$ were quantified by multiplying proportions of applied residues and old C sources and production rates (µg C/g/day). Fractionation factor ($\Delta$) was also quantified by using the following equation from Fry (2006):

$$\Delta = \delta_{product} - \delta_{source}$$

(19)

where $\delta_{product}$ is the $\delta^{13}C$ of the respired $CO_2$ and $\delta_{source}$ is $\delta^{13}C$ of the SOC.

## 5.3 Statistical Analysis

Data were tested for normal distribution ($p > 0.05$; Shapiro-Wilk) and equal variances ($p > 0.05$; Levene's). Although data were taken from separate jars, the same soil and applied residue was measured repeatedly over time (Swanston et al. 2002). Therefore, differences between treatments on each day, between sampling days for each treatment, interaction effects from treatment by time, as well as overall means (averaged over the 140 day incubation), were analyzed using a two-way repeated measures analysis of variance (ANOVA). Sampling day was used as the within subject repeated measure, and treatment type was used as the between subject main factor (Norman and Streiner 2008). When the ANOVA had significant main effects or interactions, a Tukey's post-hoc multiple comparison test with a Bonferroni correction was used to identify where differences were (simple effects). The Bonferroni correction was used to account for the dependence of samples in the repeated measures analysis (Rice 1989). Pearson product-moment correlations were preformed between select characteristics ($CO_2$ and $LF_C$ concentrations) from Chapter 3 and 5. For all statistical analyses the threshold probability level for determining significant differences was a p-value less than 0.05. All data analysis was carried out in IBM SPSS Statistics (version 21, 2012).

## 5.4 Results

### 5.4.1 Greenhouse Gas Production Rates

The interaction effect of treatment by day for $CO_2$ production rates (ug $CO_2$-C/g/day) was significant [$F_{(176,308)} = 3.72$, $p < 0.0001$]. Main effects showed significant differences between treatments, and days. Treatments amended with soybean and maize residue were significantly higher than corresponding Control treatments on all sampling days. Significant differences between $C_3$ (Figure 5.1a) and $C_4$ (Figure 5.1b) treatments were found on days 10 and 21. Overall means showed significant differences between soybean and maize amended treatments, and

102

corresponding Control treatments (Table 5.1). $C_3$ treatments were generally higher than corresponding $C_4$ treatments ($C_3$-1:2 and $C_3$-2:3 were higher than $C_4$-1:2 and $C_4$-2:3 respectively).

All treatments except for $C_3$-S and $C_4$-M had significant differences between days. In the remaining treatments, all showed significant differences between days 7 and 10 (except $C_4$-2:3 and Cont-S) and days 14 and 21 (except $C_3$-1:2 and all Control treatments). Furthermore, $C_3$-2:3 showed a significant difference from day 10 to 14. From day 29 to 35, $C_3$-1:2, Cont-M and Cont-2:3 increased significantly. Another peak was observed on day 63 from treatments $C_3$-1:2, $C_4$-1:2, $C_4$-2:3 and Cont-S which decreased significantly on day 70. The highest peak was seen at day 10 for all $C_3$ treatments and $C_4$-M, while the highest peak for $C_4$-1:2 and 2:3 were on day 14. All Control treatments increased steadily from day 1 to 140. A positive correlation was significant between $CO_2$ and $LF_C$ concentrations [$r(144) = 0.85$, $p<0.0001$]. In $C_3$ and $C_4$ treatments the amount of residue C lost as $CO_2$-C was calculated. By day 42, 14.2 to 15.4% in $C_3$ treatments, and 12.3 to 13.5% of residue C in $C_4$ treatments was lost as $CO_2$-C. By the end of the incubation, 37.6 to 38.7% in $C_3$ treatments, and 34.8 to 37.6% in $C_4$ treatments of residue C was lost as $CO_2$-C. There were no significant differences between $C_3$ and $C_4$ treatments by day 42, or by the end of the incubation.
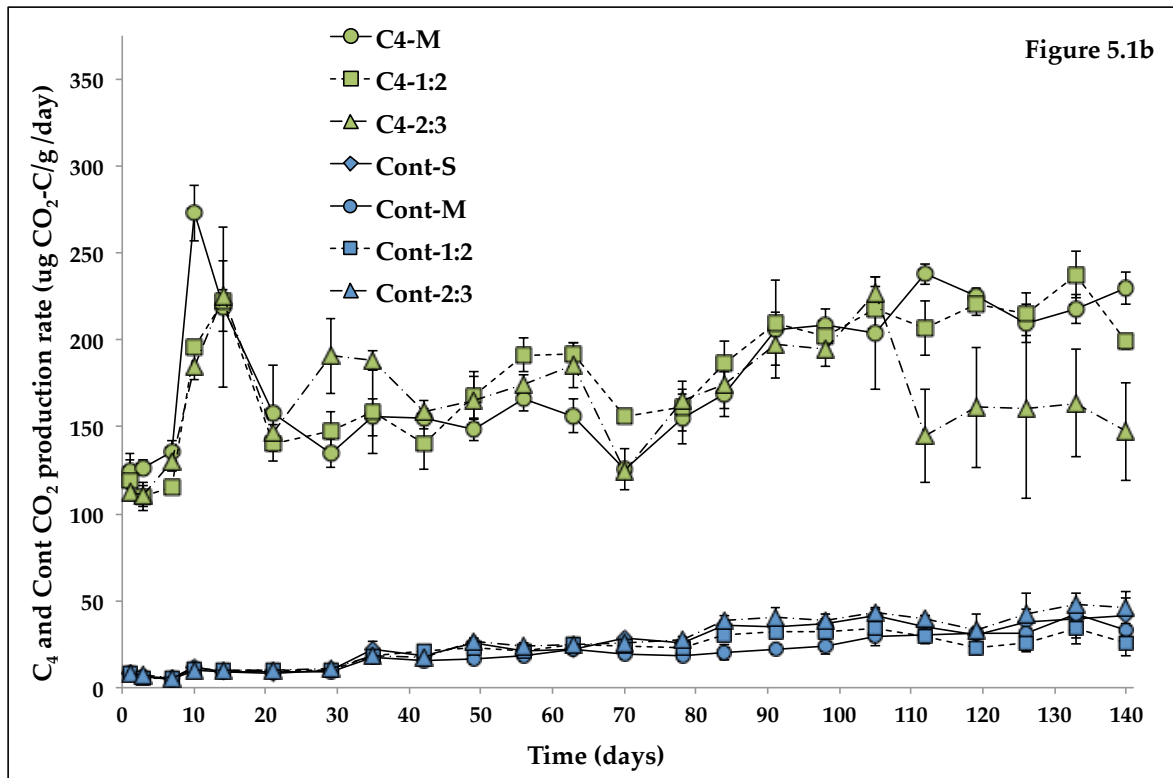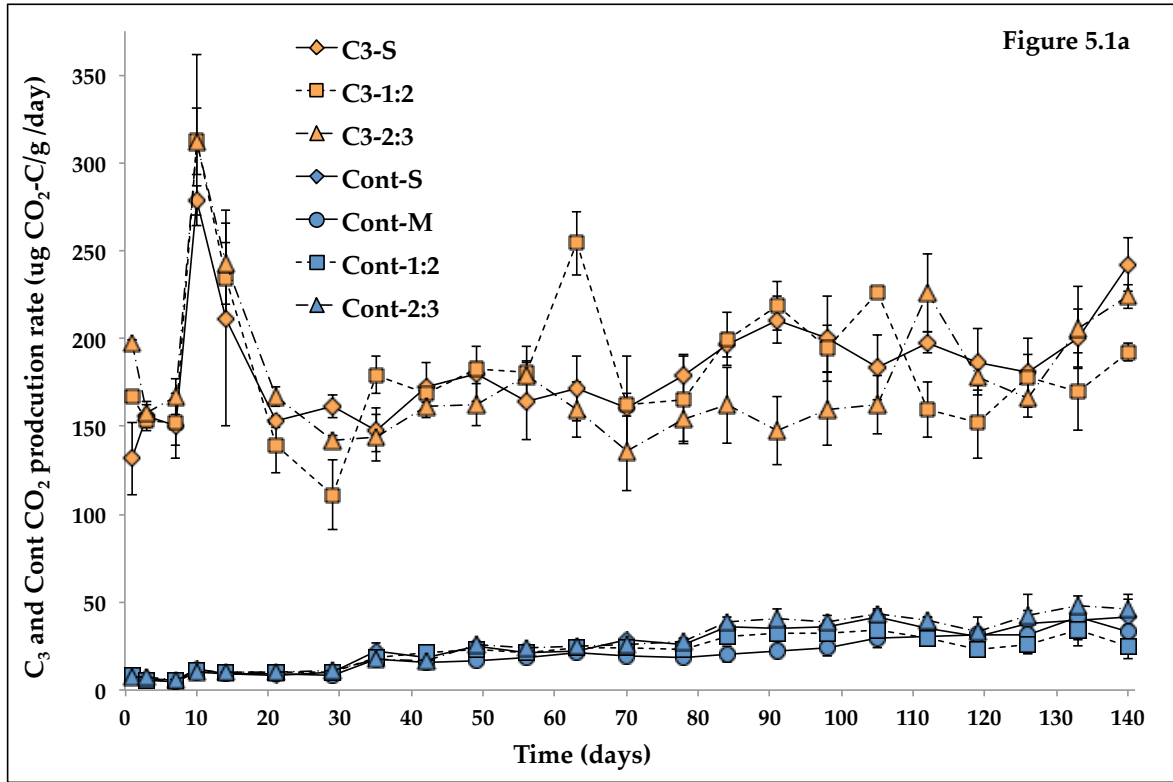
**Figure 5.1** Mean $CO_2$ production rates (ug $CO_2$-C/g/day) from soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. **a**) $C_3$ (soybean) and **b**) $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added.

The interaction effect of treatment by day was significant for $N_2O$ production rates (ng $N_2O$-N/g/day) [$F(176,286) = 5.46$, $p<0.0001$]. There were significant main effects from treatments, and days. The only significant differences between $C_3$ and $C_4$ treatments ($p<0.01$) were on day 10 (where $C_3$-S was significantly higher than $C_4$-2:3) and on day 21 (where $C_4$-1:2 and $C_4$-2:3 were significantly higher than $C_3$-2:3) (Figure 5.2a and b). $C_3$ and $C_4$ treatments were consistently lower than corresponding Control treatments after day 29 (except for $C_4$-M and Cont-M). Overall means showed significant differences between all $C_3$ and $C_4$ treatments, and corresponding Control treatments (Table 5.2).

Production rates of $N_2O$ also showed significant differences between days within treatments. From day 1 to 7, $N_2O$ production rates decreased significantly for all treatments. From day 7 to 10, $C_3$-S and Cont-S significantly increased while $C_4$-2:3 significantly decreased. From day 10 to 35 all $C_3$ and $C_4$ treatments had a steady but slow decrease in $N_2O$ (significant for all but $C_4$-M). Control treatments were consistently above zero after day 7 and steadily increased until day 42 (significant for Cont-S and Cont-M), but no significant differences between days were seen for Control treatments after day 42. In addition peaks were observed on day 63, day 78 and again on 133 for all Control treatments. Day 42 was significantly higher than day 35 in all $C_3$ and $C_4$ treatments (except for $C_4$-1:2), showing another peak in $N_2O$ production rates. A significant drop from day 42 to 56 was observed for all $C_3$ and $C_4$ treatments, excluding $C_4$-1:2. All $C_4$ treatments and $C_3$-2:3 had a significant decrease from day 63 to 70 and a significant increase from day 70 to 78. All $C_3$ and $C_4$ treatments significantly decreased from day 78 to 84 and increased from day 84 to 91. From day 112 to 119, $C_3$-S and $C_3$-1:2 increased significantly. Finally, from day 133 to 140, all $C_3$ treatments, $C_4$-M and $C_4$-1:2 significantly decreased. After day 63, all $C_3$ and $C_4$ treatments were below 0 except on day 133 where $C_4$-2:3 was slightly higher.
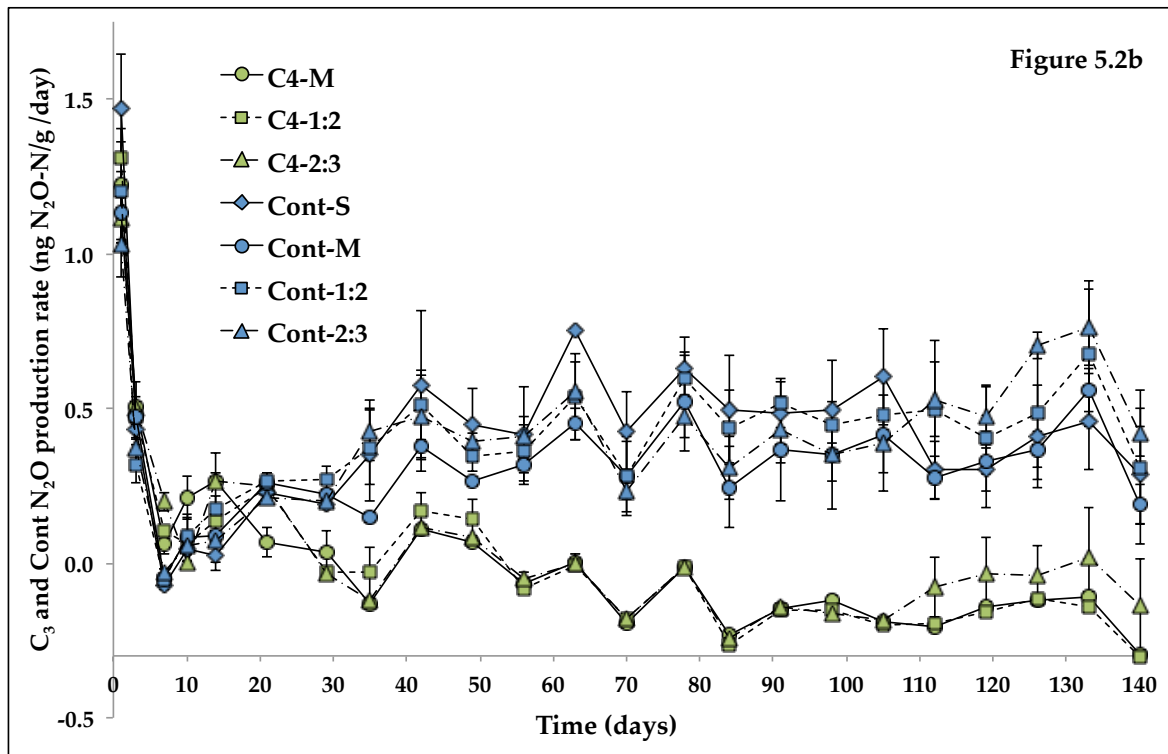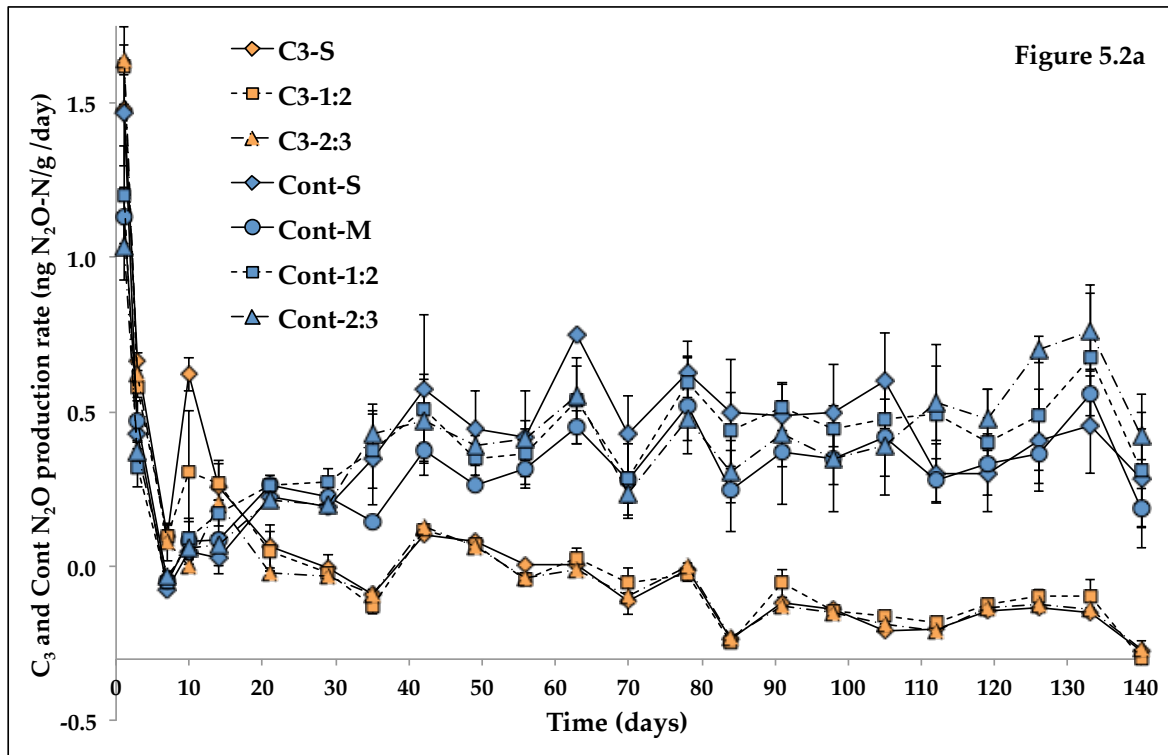
**Figure 5.2** Mean N₂O production rates (ng $N_2O$-N/g/day) from soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. **a)** $C_3$ (soybean) and **b)** $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added

**Table 5.1** Overall mean $CO_2$ (ug C/g/day) and $N_2O$ (ng N/g/day) production rates (averaged over the 140 day incubation) from soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops, using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added.

| Treatment | $CO_2$ (ug C/g/day) | $N_2O$ (ng N/g/day) |
|-----------|---------------------|---------------------|
| $C_3$-S | 184.32 (5.06)[a,*] | 0.07 (0.00)[a,*] |
| $C_3$-1:2 | 185.02 (5.74)[a,*] | 0.06 (0.01)[a,*] |
| $C_3$-12:3 | 178.96 (7.74)[a,*] | 0.05 (0.02)[a,*] |
| $C_4$-M | 179.93 (6.14)[a,*] | 0.02 (0.00)[a,*] |
| $C_4$-1:2 | 178.88 (1.87)[a,*] | 0.03 (0.02)[a,*] |
| $C_4$-2:3 | 166.64 (9.76)[a,*] | 0.06 (0.09)[a,*] |
| Cont-S | 23.06 (1.59)[z] | 0.42 (0.03)[z] |
| Cont-M | 19.44 (1.40)[z] | 0.33 (0.04)[z] |
| Cont-1:2 | 21.13 (2.24)[z] | 0.41 (0.08)[z] |
| Cont-2:3 | 25.90 (1.31)[z] | 0.40 (0.02)[z] |

Standard error of the overall means (averaged over the 140 day incubation) are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05) between $C_3$ and $C_4$ treatments (a) and between Control treatments (z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment.

*5.4.2 $\delta^{13}C$ of Respired Carbon Dioxide*

The interaction effect between treatments and days was significant for $\delta^{13}C$ values from respired $CO_2$ (‰) [$F(18,38) = 14.96$, $p < 0.0001$)]. There was a significant main effect of treatment and day (Table 5.2). On all days and in overall means, $C_4$ treatments were significantly more enriched in $^{13}C$ than all $C_3$ treatments, and both treatments differed significantly from corresponding Control treatments, except for on day 140. There were no significant differences found between any Control treatments. Simple effects showed that all $C_3$, $C_4$, and Control treatments were significantly more enriched on day 1 than on day 70. Control treatments became more depleted, approximately 7 times more than all other treatments over the incubation.

The interaction effect of treatment by day was significant for respired $CO_2$ derived from applied residues [$F(10,24) = 5.38$, $p = 0.001$)]. Simple effects showed significant differences between treatments and days (Table 5.3a). On day 140, $C_4$-M was significantly higher than $C_3$-1:2 and $C_3$-2:3. Only $C_3$-2:3 and $C_4$-M changed significantly over time. Overall mean of $C_4$-M was significantly higher than $C_3$-2:3. The interaction effect between treatment and day was not significant for respired $CO_2$ derived from old C sources [$F(10,24) = 1.71$, $p = 0.20$]. Main effects showed significant differences between treatments all days (Table 5.3b). Differences between sampling day occurred in $C_3$-2:3 only, where day 140 was significantly higher than day 1 and 70. Overall means of all $C_3$ treatments were significantly higher than all $C_4$ treatments. $CO_2$-C derived from old C sources increased over the incubation for all treatments.

The interaction effect of treatment by day for C fractionation factor ($\Delta$, ‰) was significant [$F(18,36) = 12.90$, $p<0.0001$)]. Main effects showed significant differences between treatments and days (Table 5.4). For all times, all $C_3$ treatments were significantly higher than all $C_4$ treatments, and overall means. No significant differences were found between Control treatments, however, simple effects showed that in all $C_3$, $C_4$, and Control treatments except for $C_3$-S and $C_3$-2:3, day 1 was significantly lower than day 70. For all treatments, fractionation factor increased from day 1 to 140, and Control treatments did so 6 to 7 times more.

**Table 5.2** $\delta^{13}C$ values from respired $CO_2$ (‰) from soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | -24.41 (0.06)$^{a,*,A}$ | -24.36 (0.24)$^{a,*,A}$ | -24.33 (0.10)$^{a,*,A}$ | -11.31 (0.40)$^{b,*,A}$ | -12.01 (0.34)$^{b,*,A}$ | -11.72 (0.08)$^{b,*,A}$ |
| 70 | -26.01 (0.62)$^{a,*,B}$ | -26.37 (0.24)$^{a,*,B}$ | -25.74 (0.58)$^{a,*,B}$ | -13.66 (0.50)$^{b,*,B}$ | -14.57 (0.38)$^{b,*,B}$ | -13.76 (0.31)$^{b,*,B}$ |
| 140 | -25.67 (0.11)$^{a,AB}$ | -24.99 (0.30)$^{a,AB}$ | -24.94 (1.16)$^{a,AB}$ | -13.27 (0.22)$^{b,*,AB}$ | -13.63 (0.18)$^{b,*,AB}$ | -12.89 (1.29)$^{b,*,AB}$ |
| Mean | -25.36 (0.10)$^{a,*}$ | -25.24 (0.11)$^{a,*}$ | -25.00 (0.29)$^{a,*}$ | -12.75 (0.14)$^{b,*}$ | -13.40 (0.04)$^{b,*}$ | -12.79 (0.45)$^{b,*}$ |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|---|---|---|---|---|
| 1 | -16.59 (0.73)$^{z,Z}$ | -16.86 (0.58)$^{z,Z}$ | -15.78 (1.66)$^{z,Z}$ | -16.87 (0.23)$^{z,Z}$ |
| 70 | -22.89 (0.48)$^{z,Y}$ | -24.02 (1.30)$^{z,Y}$ | -23.44 (0.96)$^{z,Y}$ | -23.40 (0.41)$^{z,Y}$ |
| 140 | -23.58 (1.49)$^{z,Y}$ | -24.17 (0.99)$^{z,Y}$ | -22.51 (2.05)$^{z,Y}$ | -23.52 (0.55)$^{z,Y}$ |
| Mean | -20.59 (0.47)$^{z}$ | -21.68 (0.30)$^{z}$ | -20.58 (0.76)$^{z}$ | -21.26 (0.12)$^{z}$ |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (z-y). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 5.3** Proportion of respired $CO_2$-C derived from **a) applied residue** and **b) soil carbon sources** (ug C/g/day) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 103.44 (19.77)[a,A] | 110.49 (4.63)[a,A] | 125.43 (1.03)[a,A] | 124.74 (10.14)[a,A] | 114.83 (11.95)[a,A] | 112.77 (2.67)[a,A] |
| 70 | 87.25 (15.65)[a,A] | 89.89 (12.95)[a,A] | 63.24 (17.50)[a,B] | 107.15 (8.56)[a,A] | 119.40 (6.26)[a,A] | 103.97 (1.10)[a,A] |
| 140 | 123.12 (32.51)[ab,A] | 67.54 (22.63)[a,A] | 61.78 (28.65)[a,B] | 203.73 (11.35)[b,B] | 166.04 (1.78)[ab,A] | 130.37 (17.42)[ab,A] |
| Mean | 104.60 (20.62)[ab] | 89.31 (11.31)[ab] | 83.48 (13.04)[a] | 145.21 (6.24)[b] | 133.42 (2.62)[ab] | 115.70 (5.60)[ab] |

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|---|---|---|---|---|---|---|
| 1 | 55.86 (11.64)[a,A] | 56.22 (5.92)[a,A] | 72.26 (1.06)[a,A] | 0.00[b,A] | 4.61 (4.61)[b,A] | 0.00[b,A] |
| 70 | 73.80 (16.82)[ab,A] | 72.23 (9.71)[a,A] | 72.80 (7.43)[a,A] | 18.45 (3.72)[b,A] | 36.48 (4.49)[ab,A] | 20.27 (2.45)[b,A] |
| 140 | 118.92 (37.73)[ab,A] | 124.68 (27.44)[ab,A] | 162.32 (30.58)[a,B] | 25.87 (2.24)[b,A] | 33.01 (2.70)[b,A] | 17.07 (10.90)[b,A] |
| Mean | 82.86 (11.66)[a] | 84.38 (13.26)[a] | 102.46 (7.60)[a] | 14.77 (0.82)[b] | 24.70 (0.53)[b] | 12.45 (3.97)[b] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different (p<0.05). Values followed by different upper case letters are significantly different between days (p<0.05). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**Table 5.4** $^{13}C$ fractionation factor ($\Delta$, ‰) in soybean sole crop (S), maize sole crop (M), 1:2 and 2:3 (maize:soybean) intercrops over a 140 day incubation study using the top 20 cm of soil from Balcarce, Argentina. $C_3$ (soybean) and $C_4$ (maize) indicate the type of residue added to the soil, Control treatments have no residue added. Positive values denote an enrichment and negative values denote a depletion in $^{13}C$. Overall means (bottom row) were averaged over the 140 day incubation.

| Day | $C_3$-S | $C_3$-1:2 | $C_3$-2:3 | $C_4$-M | $C_4$-1:2 | $C_4$-2:3 |
|-----|---------|-----------|-----------|---------|-----------|-----------|
| 1 | -0.79 (0.28)[a,*,A] | -1.11 (0.31)[a,*,A] | -1.01 (0.14)[a,*,A] | 7.89 (0.60)[b,*,A] | 7.22 (0.44)[b,A] | 8.09 (0.13)[b,*,A] |
| 70 | -1.91 (0.27)[a,A] | -2.56 (0.27)[a,B] | -2.16 (0.93)[a,A] | 5.95 (0.41)[b,*,B] | 4.99 (0.21)[b,*,B] | 5.88 (0.74)[b,*,B] |
| 140 | -2.03 (1.03)[a,A] | -1.81 (0.31)[a,AB] | -1.62 (0.97)[a,A] | 6.35 (0.39)[b,*,AB] | 6.19 (0.13)[b,*,AB] | 6.74 (1.54)[b,*,AB] |
| Mean | -1.58 (0.21)[a,*] | -1.83 (0.09)[a,*] | -1.60 (0.26)[a,*] | 6.73 (0.24)[b,*] | 6.15 (0.11)[b,*] | 6.90 (0.16)[b,*] |

| Day | Cont-S | Cont-M | Cont-1:2 | Cont-2:3 |
|-----|--------|--------|----------|----------|
| 1 | 5.05 (0.65)[z,Z] | 4.87 (0.52)[z,Z] | 5.84 (1.69)[z,Z] | 4.79 (0.32)[z,Z] |
| 70 | -1.28 (0.48)[z,Y] | -2.14 (1.27)[z,Y] | -1.63 (0.92)[z,Y] | -1.76 (0.38)[z,Y] |
| 140 | -1.98 (1.50)[z,Y] | -2.34 (1.04)[z,Y] | -0.81 (1.97)[z,Y] | -1.72 (0.43)[z,Y] |
| Mean | 1.04 (0.50)[z] | 0.13 (0.32)[z] | 1.14 (0.74)[z] | 0.44 (0.02)[z] |

Standard error of the means are shown in parentheses (n=3). Values followed by different lower case letters are significantly different ($p<0.05$) between $C_3$ and $C_4$ treatments (a-b) and between Control treatments (y-z). Values followed by an asterisk (*) are significantly different from the corresponding Control treatment. Values followed by different upper case letters are significantly different between days ($p<0.05$). Statistical tests done in SPSS using repeated measure ANOVA and Tukey's with a Bonferroni correction if ANOVA was significant.

**5.5 Discussion**

*5.5.1 Greenhouse Gas Production Rates*

The $CO_2$ production rates (μg $CO_2$-C/g/day) in the current study were similar to those in a study on intercropping by Oelbermann et al. (2009). As expected, results from the current study indicated that there was an effect of residue application on $CO_2$ emissions. This was expected because it has been observed that $LF_C$ and $CO_2$, or mineralization rates, are strongly related (Alvarez and Alvarez 2000). However, because the experiment was a closed system, there is a possibility of overestimating $CO_2$ due to the accumulation of $CO_2$ stored in the soil solution with no aeration, and the release of that $CO_2$ during measurement (Mondini et al. 2010).

Three phases caused by decomposition of organic material in the current study were observed. This has been observed before in a study by Oelbermann et al. (2008) who carried out an incubation study on undisturbed Arctic soils, as well as Potthoff et al. (2005) who studied the mineralization of maize residue with N additions. In the current study the phases were seen only in $C_3$ and $C_4$ treatments. The first phase was a rapid increase in respiration (seen from days 7 to 10), the second phase a peak in $CO_2$ (day 10 or 14), and the third phase, a decrease in respiration to a more stabile state (after day 35). These phases occurred because of the high availability of labile C at the beginning of the incubation, the decrease in labile material and subsequent use of more recalcitrant C by microbes (Oelbermann et al. 2008). Furthermore, in a study by Poll et al. (2008) it was found that the phases corresponded to decomposition by bacteria in the beginning and decomposition by fungi in the later phase of the incubation, corresponding to high SMB at the beginning of the current incubation. These phases were not seen in the Control treatments (no added residue) due to slower decomposition of more recalcitrant material for the entire incubation (Potthoff et al. 2005).

Higher peaks and a higher mean over the entire incubation period in $C_3$ treatments, showed an effect of residue type on $CO_2$ production. In field studies it has been observed that

maize sole crops have higher $CO_2$ emissions than maize/soybean rotations, due to the higher C inputs from maize sole crops (Omonode et al. 2007). In the current study, higher $CO_2$ production rates in $C_3$ treatments may have been due to equal amounts of added soybean and maize residues, which was not consistent with study site conditions, where maize had higher C residue additions than soybean (Oelbermann and Echarte 2011). However, higher $CO_2$ emissions from alfalfa-wheat-barley than from corn-wheat-barley systems have been observed in southern Alberta, due to the incorporation of a legume (Ellert and Janzen 2008).

Lower $CO_2$ production rates, along with higher SOC concentrations (Chapter 3) in the 2:3 intercrop in both $C_3$ and $C_4$ treatments suggested a more desirable intercrop. Little research exists on GHG emissions from maize/soybean intercropping systems (Dyer et al. 2012), however, GHG emissions from crop rotations of maize and soybean are similar. In a study by Drury et al. (2008), $CO_2$ production from maize sole crops was at least double maize and soybean rotations, due to the quality of current and previous crop residue additions. Contrasting results were in the Control treatments of the current study, where the 2:3 intercrop had the highest overall $CO_2$ production rate, suggesting the combination of residue and intercropping may have had a complimentary affect which reduced $CO_2$ production rates.

The amount of C lost from residue to $CO_2$-C in the current study is lower than field (Arunachalam et al. 2003; Vachon and Oelbermann 2011) and incubation studies (Liang et al. 1999; Poll et al. 2008) that focus on decay of residues. For example Poll et al. (2008) found that by day 42 approximately 45% and by day 140 approximately 60% of residue C had been mineralized to $CO_2$-C in an incubation to determine how soil moisture affects C during the decay of residues. The reason for the low percent of residues mineralized is the amount of residues applied. Liu et al. (2009) found that as more residue was applied to the incubation, less of a percentage would be mineralized to $CO_2$-C. In their study, residue application rate was from approximately 6 to 22 g C/kg soil, which resulted in 10 to 11% by day 42 and 19 to 25% by day 140 of residue C being lost as $CO_2$-C (Liu et al. 2009). Similarly, in the current study, rate of

113

application of residues was approximately 11 mg C/g of soil and mineralization of residue C to $CO_2$-C was low.

Production rates of $N_2O$ (ng $N_2O$-N/g/day) in the current study were in the same range as a study by Millar and Baggs (2004) where $N_2O$ emissions were quantified after sole crop and agroforestry residue inputs in Kenyan soils. In the current study, there was a clear effect of residue addition on $N_2O$ production rates, where high initial $N_2O$ production in all treatments was likely caused by the stimulation of microbes from residue addition. More specifically, residues could have provide conditions in favour of $N_2O$ production, such as anaerobic micro-sites, allowing denitrification (Millar and Baggs 2004; Lang et al. 2011). Large increases in $N_2O$ production have been seen before 24 h which is why the peak on day 1 might have been missed in the current study (Frimpong and Baggs 2010). The subsequent decrease in $C_3$ and $C_4$ treatments may have been due to the degraded source of C and N for microbes. Swerts et al. (1996) observed that $N_2O$ production was influenced by $O_2$, C and $NO_3^-$ quantities. High microbial activity in the first 35 to 70 days may have depleted labile C and $NO_3^-$, subsequently producing less $N_2O$ (Swerts et al. 1996; Grandy and Robertson 2006).

Results from the current study indicated that this particular soil acted as a sink for N when residues were applied and a N source when no residues were applied. Lower $N_2O$ emissions when crop residues were retained, was also observed by Patiño-Zúñiga et al. (2009) in a field experiment focusing on conservation agriculture in the semi-arid, subtropical region in Central Mexico. However the opposite has also been observed in a similar study at the same site (Dendooven et al. 2012). Soils acting as a sink for $N_2O$ in incubation experiments (Swerts et al. 1996) and in the field (Chen et al. 1997) have also been recorded, but reasons for the sinks are rarely discussed in the literature due to the complexity of the N cycle including $N_2O$ consumption (Chapuis-Lardy et al. 2006). Often, $N_2O$ sinks are treated as measurement errors which could possibly be the case in the current study (Chapuis-Lardy et al. 2006). Low soil pH has been found to increase $N_2O$ emissions, possibly indicating that addition of residues

114

influenced soil pH, and decreased $N_2O$ production (Lang et al. 2011). Generally, soil $N_2O$ production increases with soil moisture, which may provide another explanation for the positive $N_2O$ production rates in Control treatments, but negative in $C_3$ and $C_4$ treatments (Ball et al. 1999). Although the same amount of water was added to Control and residue amended treatments, it is possible that residue in $C_3$ and $C_4$ treatments absorbed some of the water, lowering the soil water content. Although not directly measured, residues amended in the Control treatments could have allowed for better denitrification conditions and a higher $N_2O$ production rate (Millar and Baggs 2004). However, $N_2O$ measurements have been observed to be different in incubation experiments than in the field due to incubations causing semi-anaerobic conditions (Nagano et al. 2012). Therefore, lowered microbial activity in the $C_3$ and $C_4$ treatments due to high $CO_2$ concentrations could have caused lower $N_2O$ rates, whereas, better conditions for $N_2O$ production (low $O_2$) could have caused higher $N_2O$ production rates (Mondini et al. 2010; Nagano et al. 2012).

Treatments with soybean residues had higher overall $N_2O$ production rates than maize residues, possibly due to a higher N concentration in soybean residue. Baggs et al. (2000) observed that soils with a high N concentration and residues with a low C/N ratio, increased $N_2O$ emissions. In the current study, soybean residues had a C/N ratio that was approximately half that of the maize residues which could have accounted for the higher $N_2O$ production rates from soybean residue amended soils. However, it has also been observed that maize crops emitted more than three times the $N_2O$ than that of soybean crops under numerous cropping practices in central Iowa due to higher fertilizer applications (Parkin and Kaspar 2006). Gregorich et al. (2008) also observed that the addition of fertilizer had a greater affect on $N_2O$ production rate than the type of residue applied to the soil. In the current study the higher application of fertilizer on treatments with maize could have affected the $N_2O$ production rate from the soil.

Intercrops in the $C_3$ treatment emitted less $N_2O$ than $C_3$-S which was due to multiple residue sources. Niklaus et al. (2006) found that as plant diversity of a pasture increased, $N_2O$ production decreased, but increased with the addition of legume species. Soybean and maize residues decompose at different rates and have different N concentrations, which can change amounts of C and N in the respective cycles (Novoa and Tejeda 2006). In the current study, the higher N concentration of the soybean residues with the lower concentration of the maize residues, lowered $N_2O$ production rates in intercrop treatments. Furthermore, applied residues with low lignin concentrations (such as legumes) have been seen to increase $N_2O$ emission rates in the southeastern part of England (Garcia-Ruiz and Baggs 2007). In a study by Pappa et al. (2011) near Edinburgh, it was observed that if the previous crop was a legume the amount of $N_2O$ emitted was higher than when the previous crop was a cereal, showing that even older legume residues can influence $N_2O$ production. This was seen in the current study in $C_4$ intercrop treatments that had a higher $N_2O$ production rate than $C_4$-M due to the influence of previously incorporated soybean residues.

## 5.5.2 $\delta^{13}C$ of Respired Carbon Dioxide

Similar $\delta^{13}C$ values from respired $CO_2$ were found in a study measuring $C_3$ and $C_4$ substrate induced respiration from microbes (Ekblad and Högberg 2000). In the current study, $\delta^{13}C$ of respired $CO_2$ showed effects from residue addition, including the type of residue added and crop management practice. As expected, $^{13}C$ from respired $CO_2$ in $C_3$ and $C_4$ treatments were similar to the biological soil sources (residue type and SMB), $C_3$-$CO_2$ being more depleted and $C_4$-$CO_2$ more enriched in $^{13}C$ (Amundson et al. 1997). Although Control treatments appeared to have changed more than $C_3$ and $C_4$ treatments throughout the incubation, all treatments began at a $\delta^{13}C$ value of ambient air. Rapid changes in $\delta^{13}C$ from respired $CO_2$ as a result of residue addition, within the first 10 minutes of an incubation have been noted, and most likely occurred

in the $C_3$ and $C_4$ treatments (Ekblad et al. 2002). Slower changes in Control treatments were due to less microbial stimulation than in residue amended soils (Frimpong and Baggs 2010). As expected, $CO_2$ in $C_3$ and $C_4$ treatments became depleted and enriched in $^{13}C$ respectively, relative to Control treatments, shifting towards the $\delta^{13}C$ value of the added residue. However, an influence on the $\delta^{13}C$ values from respired $CO_2$ from a small amount of microbial utilization of depleted C sources, such as lignin (maize residues have a higher lignin concentration than soybean) could have caused $C_4$ and Control treatments to become depleted from day 1 to 70 (Uphoff et al. 2006; Schwendenmann and Pendall 2008). This was observed by Ekblad et al. (2002) where $CO_2$ in incubations with $C_3$, $C_4$, or no residues amended all became depleted in $^{13}C$ with time. This was further supported by the increase in contribution from older C sources to $CO_2$ in $C_4$ treatments. At the beginning of the incubation, there was sufficient maize residue to support microbial decomposition and growth, but as the source was mineralized, microbes utilized older C sources that were more depleted in $^{13}C$ (Kristiansen et al. 2004). Enrichment in all treatments from day 70 to 140 was not expected, but was likely due to more processed, older sources of C being utilized by microbes (Pendall and King 2007). Furthermore, a change in microbial community, or an increase in the use of dead microbes as a food source, could have reversed $\delta^{13}C$ values from being depleted to being enriched (Crow et al. 2006). In $C_3$, $C_4$ and Control treatments, 1:2 and 2:3 intercrop treatment $CO_2$ was less depleted in $^{13}C$ than sole crops, suggesting different or increased microbial processes in intercrop soil (Crow et al. 2006).

As expected, contributions from fresh residue to $CO_2$ in $C_3$ and $C_4$ treatments were higher than contributions from old soil C sources, especially at the beginning of the incubation. All $C_3$ treatments decreased in contributions from residue except for $C_3$-S, which was most likely due to the higher amount of soybean residues at the beginning of the incubation from the soybean sole crop soil. The decrease in soybean residue contribution was due to availability of soybean residues during the incubation in $C_3$ intercrop treatments (Liang et al. 1999). This initial flush of contribution from the residue source applied was also observed by Kristiansen et

117

al. (2004). Increasing contributions of old C sources in $C_3$ and $C_4$ treatments suggested increased use of older, more recalcitrant sources as a substrate for microbial decomposition (Robinson and Scrimgeour 1995; Pendall and King 2007). The priming effect is the mineralization of older C sources due to the addition of fresh C sources (Hamer et al. 2009). In the current study, the higher contribution of old C sources to $CO_2$ in $C_3$ treatments than in $C_4$ treatments, could have indicated that positive priming effect occurred only in the $C_3$ treatments (Brookes et al. 1990). This also corresponds to the contribution from old and new C sources being equal in $C_3$ treatments, but higher for new C sources than old in the $C_4$ treatments.

In the current study, fractionation factor illustrated the change in $\delta^{13}C$ values in SOC (the source) to $CO_2$ (the product) from microbial decomposition. $CO_2$ in $C_3$ treatments was depleted when compared to SOC, whereas $CO_2$ in $C_4$ treatments was enriched, due to the utilization of each residue type. The decrease from day 1 to 70, then the increase from day 70 to 140 in fractionation factor for almost all treatments suggests that microbes initially utilized more depleted, fresh sources, whereas later they utilized more enriched, processed material (Oelbermann et al. 2008). Similar results for the Control treatments were seen in a study by Kristiensen et al. (2004), where there was an initial strong enrichment relive to the SOC, and later a depletion. This initial flush was due to the decomposition of previous microbial populations and fractionation during decomposition (Kristiensen et al. 2004). This was further supported by $\delta^{13}C$ results and contributions from residue and old soil C to $CO_2$. For $C_3$-S, Cont-S and Cont-M treatments, a continued, but slower depletion from day 70 to 140 suggested utilization of lignin, as it is more depleted than the labile portion of the residue (Melillo et al. 1989; Preston et al. 2006).

### 5.5.3 Overview of Isotopes

Overall, $\delta^{13}C$ and $\delta^{15}N$ values from this study showed a strong influence of the applied residue type (Figure 5.3), illustrating that isotopes can be a useful tool when studying C and N dynamics. Carbon isotopes from both SMB and $LF_C$ rapidly and strongly incorporated $\delta^{13}C$ values from each residue type, while the lack of a fresh C source in the Control treatments created $\delta^{13}C$-$SMB_C$ values more similar to SOC with more variable $CO_2$ emissions. $\delta^{13}C$ from respired $CO_2$, as expected was similar to the $\delta^{13}C$ value of the added residues, illustrating the importance of the substrate $\delta^{13}C$ values to isotope studies. In addition, SOC incorporated $\delta^{13}C$ values from residue, but at a much slower rate than SMB and LF, due to turnover times, which illustrated the usefulness of SMB and LF as indicators of long-term SOC levels.

The results from the current study demonstrated the complexity of the soil N cycle. Soil microbial biomass N was enriched compared to all other parameters, which was expected because of the enrichment that results from microbial processes. The LF was depleted in $^{15}N$ compared to SMB and TN, but slightly enriched compared to residues, suggesting the presence of microbial transformations. In addition, these results highlighted the close link between the residue and the LF component of soil. The similarity in $\delta^{15}N$ values of TN between $C_3$, $C_4$, and the Control treatments, and the differences in $\delta^{15}N$ values of TN and LF, suggested a small influence of residues on soil TN.
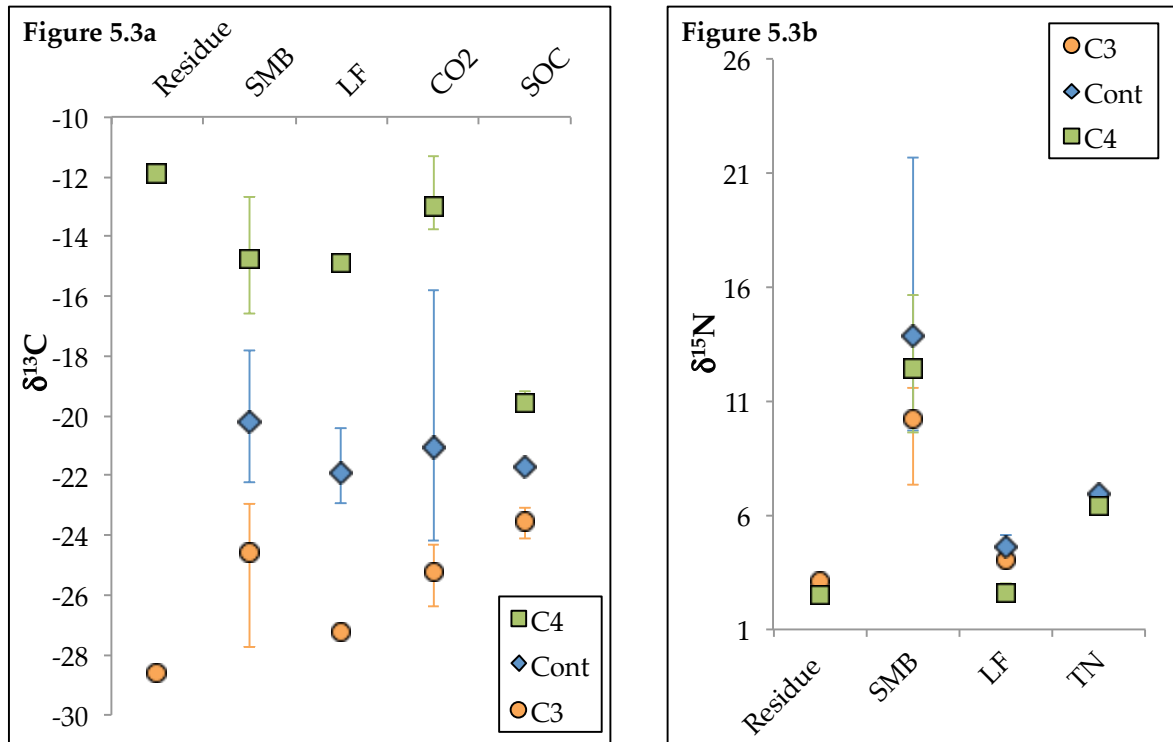
**Figure 5.3** Overall mean values of **a)** $\delta^{13}C$ and **b)** $\delta^{15}N$ for residue, soil microbial biomass (SMB), light fraction (LF), carbon dioxide ($CO_2$), soil organic carbon (SOC), and total nitrogen (TN) for all treatment types ($C_3$, $C_4$ and Control). The bars show the range throughout the incubation for each characteristic.

## 5.6 Conclusion

In this study, GHGs and $\delta^{13}C$ from respired $CO_2$ were quantified, and provided information on $CO_2$ derived from freshly amended and old C sources, and fractionation factor. A clear effect of residue addition was observed by lower $CO_2$ production rates, higher $N_2O$ production rates and a slower change in the $\delta^{13}C$ of $CO_2$ in $C_3$ and $C_4$ treatments. Three phases in microbial respiration were observed over the incubation period in $C_3$ and $C_4$ treatments due to preferential utilization of labile sources at the beginning, and use of more recalcitrant C sources at the end of the incubation. This was supported by the early depletion and later enrichment of $CO_2$ in most treatments, labile sources being more depleted than recalcitrant sources. Furthermore, it was found that soils with applied residue may have provided a possible sink of $N_2O$. The 2:3 intercrop treatment had the lowest $CO_2$ production rate in both $C_3$ and $C_4$ treatments, and therefore may be a more desirable intercrop design.

# 6.   SUMMARY, CONCLUSIONS AND FUTURE RESEARCH

## 6.1 Summary and Conclusions

Mitigation of climate change is an issue generating much attention through scientific research, resulting in suggestions and recommendations to adhere to stricter GHG emission regulations. This study focused on potential mitigation of climate change through intercropping, a sustainable form of agriculture. In a controlled environment, C and N dynamics were compared between soybean sole crop, maize sole crop and intercrop soil when soybean and maize residues were applied. More specifically, this research evaluated transformations of C and N following the amendment of soybean and maize residues. This study illustrated the usefulness of C and N isotopes in understanding the complexities of C and N cycles in agroecosystems. SOC, TN, $LF_C$, $LF_N$, turnover times (Chapter 3), $SMB_C$, $SMB_N$, turnover times, SMCS characteristics (Chapter 4), $CO_2$, $N_2O$ (Chapter 5) and respective isotopes were all quantified.

The integration of residue into soil fractions was consistently observed throughout the experiment. The effect of residue addition on soil characteristics was seen through higher amounts of SOC, TN and SMB concentrations, along with differences in GHG emissions, which were observed from distinct $\delta^{13}C$ values from respired $CO_2$. This study also illustrated that soybean residue is of higher quality which could allow for more mineralization than maize, which tends to immobilize nutrients to a greater extent. These results emphasized the importance of including $N_2$-fixing legumes in complex agroecosystems such as intercrops.

Throughout the incubation, it was observed that microbes preferentially utilize easily accessible components of crop residues at the beginning of the incubation. However, as the labile material decays over time, the more recalcitrant residue constituents (such as lignin) were

likely utilized to sustain microbial growth. For instance, $C_3$ and $C_4$ treatments had increased SOC, $\delta^{15}$N-TN and decreased $SMB_C$, all at the end of the incubation, showing a change in substrate utilization. Furthermore, changes to the $\delta^{13}$C values of both $SMB_C$ and $CO_2$ illustrated the influence of residue incorporation into the SMB to respired $CO_2$.

Results from this study also provided insight into the most advantageous intercrop design. In Chapters 3 and 5, the most desirable intercrop design appeared to be 2:3, while 1:2 appeared to be more desirable in Chapter 4. This discrepancy can be explained by the difference in each characteristic as a result of intercropping. Higher SOC, $LF_C$ and $LF_N$, and lower $CO_2$ production rates indicated more C sequestration potential in the 2:3 intercrop treatment; while higher $SMB_C$, $SMB_N$ and some SMCS characteristics indicated a positive affect on the microbial community from the 1:2 intercrop treatment. This illustrated the importance of choosing more than one characteristic to determine overall benefits from different cropping practices. These results also indicated that the decision of which intercrop design to implement, should be dependent upon the overall objective and long-term goal of the land manager. For instance, if the overall goal was C sequestration, 2:3 would be the recommended intercrop design. At the very least, this study indicated that intercropping is a more sustainable land management practice than sole cropping, resulting in better overall soil quality and more C and N sequestration.


## 6.2 Future Research and Recommendations

This study compared C and N dynamics between sole crops and intercrops. However, some areas may benefit from further research. Specifically, it would be beneficial to execute an incubation experiment where both soybean and maize residues are added to represent field conditions more closely. For example, adding the correct proportions of soybean and maize residues onto each soil type (different amounts of residue could be based on previous

agronomic data taken from the site) may be more realistic than using a single residue on each soil type. This would more closely represent field conditions, allowing isotopic data to better represent environmental conditions. Furthermore, to better simulate field-like conditions, root residue could be added since up to a quarter of the below-ground biomass can come from roots. This is especially true with intercropping systems which contain two sources of root biomass, one of which is a legume that has root nodules to aid in $N_2$-fixation.

The issue of using a laboratory experiment to simulate field conditions is the difference in time scales. Longer incubation times would allow the system to reach some sort of equilibrium after the addition of residue and water, as changes were still observed at the end of the experiment. Adding measurements of $\delta^{15}N$ from $N_2O$, as well as increased N parameters ($NH_4^+$ or $NO_3^-$) may allow for a better understanding of the N dynamics in an agroecosystem with $N_2$-fixing plants. Alternatively, a long term field study at the study site to monitor changes in SOC, TN and GHG dynamics over time would allow for a comparison between changes in the laboratory and field. Monitoring field conditions would account for soil-plant-atmospheric interactions in the agroecosystem, allowing for a better understanding and feasibility for future laboratory experiments.

As climate change begins to affect agriculture, the demand for sustainable practices will grow. This study highlights the importance of intercropping as a more sustainable form of agriculture, proving to be both beneficial for soil quality, as well as a mitigation strategy to climate change. Although there will be obstacles, the necessary implementation and adaptation of such sustainable practices will provide multiple benefits for the social and physical environment.

# LITERATURE CITED

Abah, J., Ishaq, M.N., and Wada, A.C. (2010). The role of biotechnology in ensuring food security and sustainable agriculture. *African Journal of Biotechnology* 9:8896-8900.

Accoe, F., Boeckx, P., Busschaert, J., Hofman, G., and Van Cleemput, O. (2004). Gross N transformation rates and net N mineralisation rates related to the C and N contents of soil organic matter fractions in grassland soils of different age. *Soil Biology & Biochemistry* 36:2075-2087.

Alvarez, C.R., Alvarez, R., Grigera, M.S., and Lavado, R.S. (1998). Associations between organic matter fractions and the active soil microbial biomass. *Soil Biology and Biochemistry* 30:767-773.

Alvarez, C.R., Taboada, M.A., Gutierrez Boem, F.H., Bono, A., Fernandez, P., and Prystupa, P. (2009). Topsoil Properties as Affected by Tillage Systems in the Rolling Pampa Region of Argentina. *Soil and Water Management and Conservation* 73:1242-1250.

Alvarez, R. (2001). Estimation of carbon losses by cultivation from soils of the Argentine Pampa using the Century Model. *Soil Use and Management* 17:62-66.

Alvarez, R., and Alvarez, C.R. (2000). Soil Organic Matter Pools and Their Association with Carbon Mineralization Kinetics. *Soil Science Society of America* 64:184-189.

Alvarez, R., and Grigera, S. (2005). Analysis of Soil Fertility and Management Effects on Yields of Wheat and Corn in the Rolling Pampa of Argentina. *Journal of Agronomy & Crop Science* 191:321-329.

Amundson, R., Stern, L., Baisden, T., and Wang, Y. (1998). The isotopic composition of soil and soil-respired $CO_2$. *Geoderma* 82:83-114.

Anderson, R.L. (2010). A rotation design to reduce weed density in organic farming. *Renewable Agriculture and Food Systems* 25:189-195.

Anderson, T.H., and Domsch, K.H. (1989). Ratios of microbial biomass carbon to total organic carbon in arable soils. *Soil Biology and Biochemistry* 21:471-479.

Andrade, F.H. (1995). Analysis of growth and yield of maize, sunflower and soybean grown at Balcarce, Argentina. *Field Crops Research* 41:1-12.

Arunachalam,K., Singh, N.D., and Arunachalam, A. (2003). Decomposition of leguminous crop residues in a 'jhum' cultivation system in Arunachal Pradesh, India. *Journal of Plant Nutrition and Soil Science* 166:731-736.

Baggs, E.M., Rees, R.M., Smith, K.A., and Vinten, J.A. (2000). Nitrous oxide emission from soils after incorporating crop residues. *Soil Use and Management* 16:82-87.

Bahri, H., Rasse, D.P., Rumpel, C., Dignac, M.-F., Bardoux, G., and Mariotti, A. (2008). Lignin degradation during a laboratory incubation followed by 13C isotope analysis. *Soil Biology & Biochemistry* 40:1916-1922.

Balesdent, J., and Mariotti, A. (1987). Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. *Soil Biology and Biochemistry* 19:25-30.

Ball, B.C., Scott, A., and Parker, J.P. (1999). Field $N_2O$, $CO_2$ and $CH_4$ fluxes in relation to tillage, compaction and soil quality in Scotland. *Soil & Tillage Research* 53:29-39.

Banik, P., Midya, A., Sarkar, B.K., and Ghose, S.S. (2006). Wheat and chickpea intercropping systems in an additive series experiment: Advantages and weed smothering. *European Journal of Agronomy* 24:325-332.

Barajas-Aceves, M. (2005). Comparison of different microbial biomass and activity measurement methods in metal-contaminated soils. *Bioresource Technology* 96:1405-1414.

Barajas-Aceves, M., Ansorena, G.J., Dendooven, L., and Brookes, P.C. (1999). Soil microbial biomass and organic C in a gradient of zinc concentrations in soils around a mine spoil tip. *Soil Biology and Biochemistry* 31:867-876.

Beaudette, C., Bradley, R.L., Whalen, J.K., McVetty, P.B.E., Vessy, K., and Smith, D.L. (2010). Tree-based intercropping does not compromise canola (*Brassica napus* L.) seed oil yield and reduces soil nitrous oxide emissions. *Agriculture, Ecosystems and Environment* 139:33-39.

Bedoussac, L. and Justes, E. (2010). The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. *Plant and Soil* 330:19-35.

Beedy, T.L., Snapp, S.S., Akinnifesi, F.K., and Sileshi, G.W. (2010). Impact of *Gliricidia sepium* intercropping on soil organic matter fractions in a maize-based cropping system. *Agriculture, Ecosystems and Environment* 138:139-146.

Bender, M.M. (1968). Mass spectrometric studies of carbon 13 variations in corn and other grasses. *Radiocarbon* 10:468-472.

Bernoux, M., Cerri, C.C., Neill, C., and de Moraes, J.F.L. (1998). The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma* 83:43-58.

Bilenca, D., and Miñarro, F. (2004). *Identificación de áreas valiosas de pastizal (AVPs) en las pampas y campos de Argentina, Uruguay y sur de Brasil.* (1st ed.). Buenos Aires, Argentina: Fundación Vida Silvestre.

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., and Kuzyakov, Y. (2011). Turnover of soil organic matter and of microbial biomass under $C_3$-$C_4$ vegetation change: Consideration of $^{13}C$ fractionation and preferential substrate utilization. *Soil Biology & Biochemistry* 43:159-166.

Bolinder, M.A., Angers, D.A., Gregorich, E.G., and Carter, M.R. (1999). The response of soil quality indicators to conservation management. *Canadian Journal of Soil Science* 79:37-45.

Bono, A., Alvarez, R., Buschiazzo, D.E., and Cantet, R.J.C. (2008). Tillage Effects on Soil Carbon Balance in a Semiarid Agroecosystem. *Soil Science Society of America* 72:1140-1149.

Borlaug, N. (2004). Biotech Can Feed 8 Billion. *New Perspectives Quarterly* 24:97-102.

Bossio, D.A., Scow, K.M., Gunapala, N., and Graham, K.J. (1998). Determinants of Soil Microbial Communities: Effects of Agricultural Management, Season, and Soil Type on Phospholipid Fatty Acid Profiles. *Microbial Ecology* 36:1-12.

Boström, B., Comstedt, D., and Ekblad, A. (2007). Isotope fractionation and $^{13}C$ enrichment in soil profiles during the decomposition of soil organic matter. *Oecologia* 153:89-98.

Boutton, T.W., Archer, S.R., and Midwood, A.J. (1999). Stable Isotopes in Ecosystem Science: Structure, Function and Dynamics of a Subtropical Savanna. *Rapid Communications in Mass Spectrometry* 13:1263-1277.

Boutton, T.W., Archer, S.R., Midwood, A.J., Zitzer, S.F., and Bol, R. (1998). $\delta^{13}C$ values of soil organic carbon and their use in documenting vegetation change in a subtropical savanna ecosystem. *Geoderma* 82:5-41.

Brookes, P.C., Landman, A., Pruden, G., and Jenkinson, D.S. (1985). Chloroform fumigation and the release of soil nitrogen: A rapid dirext extraction method to measure microbial biomass nitrogen in soil *Soil Biology and Biochemistry* 17:837-842.

Brookes, P.C., Ocio, J.A., and Wu, J. (1990). The Soil Microbial Biomass: Its Measurement, Properties and Role in Soil Nitrogen and Carbon Dynamics Following Substrate Incorporation. *Soil Microorganisms* 35:39-51.

Cao, M., and Woodward, F.I. (1998). Net primary and ecosystem production and carbon stocks of terrestrial ecosystems and their responses to climate change. *Global Change Biology* 4:185-198.

Caride, C., Piñeiro, G., and Paruelo, J.M. (2012). How does agricultural management modify ecosystem services in the argentine Pampas? The effects on soil C dynamics. *Agriculture, Ecosystems and Environment* 154:23-33.

Cavigelli, M.A., and Robertson, G.P. (2000). The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecological Society of America* 81:1402-1414.

Chapin, F.S., Matson, P.A., and Mooney, H.A. (2002). *Principles of Terrestrial Ecosystem Ecology.* NewYork, NY: Springer Science+Business Media, LLC.

Chapuis-Lardy, L., Wrage, N., Metay, A., Chotte, J-L., and Bernoux, M. (2007). Soils, a sink for N2O? A review. *Global Change Biology* 13:1-17.

Chen, G.X., Huang, G.H., Huang, B., You, K.W., Wu, J., and Xu, H. (1997). Nitrous oxide and methane emissions from soil-plant systems. *Nutrient Cycling in Agroecosystems* 49:41-45.

Christensen, B.T., 1992. Physical fractionation of soil and organic matter in primary particle size and density separates. *Advances in Soil Science* 20:1-90.

Compton, J.E., and Boone, R.D. (2000). Long-term impacts of agriculture on soil carbon and nitrogen in New England forests. *Ecology* 81:2314-2330.

Conner, A.J., Glare, T.R., and Nap, J.P. (2003). The release of genetically modified crops into the environment: Part II. Overview of ecological risk assessment. *The Plant Journal* 33:19-46.

Conti, M.E., Arrigo, N.M., and Marelli, H.J. Relationship of soil carbon light fraction, microbial activity, humic acid production and nitrogen fertilization in the decaying process of corn stubble. *Biology and Fertility of Soils* 25:75-78.

Coppens, F., Garnier, P., De Gryze, S., Merckx, R., and Recous, S. (2006). Soil moisture, carbon and nitrogen dynamics following incorporation and surface application of labelled crop residues in soil columns. *European Journal of Soil Science* 57:894-905.

Costantini, A., De-Polli, H., Rossiello, R.P., and Plencovich, M.C. (2007). Organic Carbon Origin and Stock in Cultivated and Grassland Soil of the Argentine Pampa. *Communications in Soil Science and Plant Analysis* 38:2767-2778.

Coyle, J.S., Dijkstra, P., Doucett, R.R., Schwartz, E., Hart, S.C., and Hungate, B.A. (2009). Relationships between C and N availability, substrate age, and natural abundance 13C and 15N signatures of soil microbial biomass in a semiarid climate. *Soil Biology & Biochemistry* 41:1605-1611.

Creamer, C.A., Filley, T.R., Boutton, T.W., Oleynik, S., and Kantola, I.B. (2011). Controls on soil carbon accumulation during woody plant encroachment: Evidence from physical fractionation, soil respiration, and $\delta^{13}C$ of respired $CO_2$. *Soil Biology & Biochemistry* 43:1678-1687.

Crow, S.E., Sulzman, E.W., Rugh, W.D., Bowden, R.D., and Lajtha, K. (2006). Isotopic analysis of respired $CO_2$ during decomposition of separated soil organic matter pools. *Soil Biology & Biochemistry* 38:3279-3291.

Cure, J.D., Rufty, T.W., Israel Jr., D.W. (1989). Alterations in soybean leaf development and photosynthesis in a $CO_2$-enriched atmosphere. *Botanical Gazette* 150:337-345.

Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., and Tu, K.P. (2002). Stable Isotopes in Plant Ecology. *Annual Review of Ecology and Systematics* 33:507-559.

Dendooven, L., Patiño-Zúñiga, L., Verhulst, N., Luna-Guido, M., Marsh, R., and Govaerts, B. (2012). Global warming potential of agricultural systems with contrasting tillage and residue management in the central highlands of Mexico. *Agriculture, Ecosystems and Environment* 152:50-58.

Derry, A.M., Staddon, W.J., and Trevors, J.T. (1998). Functional diversity and community structure of microorganisms in uncontaminated and creosote-contaminated soils as determined by sole-carbon-source-utilization. *World Journal of Microbiology & Biotechnology* 14:571-578.

Diels, J. Vanlauwe, B. Van der Meersch, M.K. Sanginga, N. and Merckx, R. (2004). Long-term soil organic carbon dynamics in a subhumid tropical climate: 13C data in mixed C3/C4 cropping and modeling with ROTHC. *Soil Biology & Biochemistry* 36:1739-1750.

Dijkstra, P., Ishizu, A., Doucett, R., Hart, S.C., Schwartz, E., Menyailo, O.V., and Hungate, B.A. (2006). $^{13}$C and $^{15}$N natural abundance of the soil microbial biomass. *Soil Biology & Biochemistry* 38:3257-3266.

Dijkstra, P., LaViolette, C.M., Coyle, J.S., Doucett, R.R., Schwartz, E., Hart, S.C., and Hungate, B.A. (2008). $^{15}$N enrichment as an integrator of the effects of C and N on microbial metabolism and ecosystem function. *Ecology Letters* 11:389-397.

Dolan, M.S., Clapp, C.E., Allmaras, R.R., Baker. J.M., and Molina, J.A.E. (2006). Soil organic carbon and nitrogen in a Minnesota soil as related to tillage, residue and nitrogen management. *Soil & Tillage Research* 89:221-231.

Domínguez, G.F., Diovisalvi, N.V., Studdert, G.A., and Monterubbianesi, M.G. (2009). Soil organic C and N fractions under continuous cropping with contrasting tillage systems on Mollisols of the southeastern Pampas. *Soil and Tillage* 102:93-100.

Drijber, R.A., Doran, J.W., Parkhurst, A.M., and Lyon, D.J. (2000). Changes in soil microbial community structure with tillage under long-term wheat-fallow management. *Soil Biology & Biochemistry* 32:1419-1430.

Drinkwater, L.E., Wagoner, P., and Sarrantonio, M. (1998). Legume-based cropping systems have reduced carbon and nitrogen losses. *Nature* 396:262-265.

Drury, C.F., Yang X.M., Reynolds, W.D., and McLaughlin, N.B. (2008). Nitrous oxide and carbon dioxide emissions from monoculture and rotational cropping of corn, soybean and winter wheat. *Canadian Journal of Soil Science* 88:163-174.

Dubois, C.A. (2008). Evaluating soil respiration under laboratory conditions in complex and sole crop agroecosystems. (Undergraduate Thesis, University of Waterloo).

Dyer, L., Oelbermann, M., and Echarte, L. (2012). Soil carbon dioxide and nitrous oxide emissions during the growing season from temperate maize-soybean intercrops. *Journal of Plant Nutrition and Soil Science* 175:394-400.

Ehleringer, J.R., and Vogel, J.C. (1993). Historical Aspects of Stable Isotopes in Plant and Carbon and Water Relations. In Ehleringer, J.R., Hall, A.E., and Farquhar, G.D. (Ed), *Stable Isotopes and Plant Carbon-Water Relation* (pp. 9-18). San Diego, California: Academic Press, Inc.

Ekblad, A., and Högberg, P. (2000). Analysis of $\delta^{13}$C of $CO_2$ distinguishes between microbial respiration of added $C_4$-sucrose and other soil respiration in a $C_3$-ecosystem. *Plant and Soil* 219:197-209.

Ekblad, A., Nyberg, G., and Högberg, P. (2002). $^{13}$C-discrimination during microbial respiration of added $C_3$-, $C_4$- and $^{13}$C-labelled sugars to a $C_3$-forest soil. *Ecosystems Ecology* 131:245-249.

Ellert, B.H., and Janzen, H.H. (2008). Nitrous oxide, carbon dioxide and methane emissions from irrigated cropping systems as influenced by legumes, manure and fertilizer. *Canadian Journal of Soil Science* 88:207-217.

Evers, A., Bambrick, A., Lacombe, S., Dougherty, M.C., Peichl, M., Gordon, A.M., Thevathasan, N.V., Whalen, J., and Bradley, R.L. (2010). Potential Greenhouse Gas Mitigation through Temperate Tree-Based Intercropping Systems. *The Open Agriculture Journal* 4:49-57.

Feng, X. (2002). A theoretical analysis of carbon isotope evolution of decomposing plant litters and soil organic matter. *Global Biogeochemical Cycles* 16:66.1-66.11.

Ferreras, L.A. Costa, J.L. Garcia, F.O. and Pecorari, C. (2000). Effect of no-tillage on some soil physical properties of a structural degraded Petrocalcic Paleudoll of the southern "Pampa" of Argentina. Soil & Tillage Research 54: 31-39.

Field, C.B., and Raupach, M.R. (Ed.). (2004). *The Global Carbon Cycle: Integrating Humans, Climate, and the Natural World.* Washington, D.C.: Island Press.

Fierer, N., Schimel, J.P., and Holden, P.A. (2003). Influence of Drying–Rewetting Frequency on Soil Bacterial Community Structure. *Microbial Biology* 45:63-71.

Filloy, J., and Bellocq, M.I. (2007). Patterns of bird abundance along the agricultural gradient of the Pampean region. *Agriculture, Ecosystems and Environment* 120:291-298.

Flanagan, L.B., and Ehleringer, J.R. (1998). Ecosystem-atmosphere $CO_2$ exchange: interpreting signals of change using stable isotope ratios. *TREE* 13:10-14.

Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revaillot, S., and Maron, P.A. (2011). Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biology & Biochemistry* 43:86-96.

Food and Agriculture Organization (FAO) of the United Nations. (2005). *Cattle ranching is encroaching on forests in Latin America.* Retrieved April 3rd, 2011 from http://www.fao.org/newsroom/EN/news/2005/102924/index.html

Food and Agriculture Organization (FAO) of the United Nations. (2011). *FAOSTAT*. Retrieved July, 2012. http://faostat.fao.org/site/291/default.aspx

Francey, R.J., Allison, C.E., Etheridge, D.M., Trudinger, C.M., Enting, I.G., Leuenberger, M., Langenfelds, R.L., Michel, E., and Steele, L.P. (1999). A 1000-year precision record of δ13C in atmospheric $CO_2$. *Tellus* 51:170-193.

Freiberg, C., Fellay, R., Bairoch, A., Broughton, W.J., Rosenthal, A., and Perret, X. (1997). Molecular basis of symbiosis between *Rhizobium* and legumes. *Nature* 387:394-401.

Frimpong, K.A., and Baggs, E.M. (2010). Do combined applications of crop residues and inorganic fertilizer lower emission of N2O from soil? *Soil Use and Management* 26:412-424.

Fry, B. (2006). *Stable Isotope Ecology.* Baton Rouge, LA:Springer Science

Garcia-Ruiz, R., and Baggs, E.M. (2007). N2O emission from soil following combined application of fertiliser-N and ground weed residues. *Plant Soil* 299:263-274.

Gardiner, D.T. and Miller, R.W. (2008). *Soils in Our Environment* (11th ed.). Upper Saddle River, New Jersey:Pearson Prentice Hall.

Garland, J.L. (1996). Analytical approaches to the characterization of samples of microbial communities using patterns of potential C source utilization. *Soil Biology & Biochemistry* 28:213-221.

Garland, J.L. (1997). Analysis and interpretation of community-level physiological profiles in microbial ecology. *FEMS Microbiology Ecology* 24:289-300.

Garland, J.L., and Mills, A.L. (1991). Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilization. *Applied and Environmental Microbiology* 57:2351-2359.

Ghaley, B.B., Hauggaard-Nielsen, H., Høgh-Jensen, H. And Jensen, E.S. (2005). Intercropping of wheat and pea as influenced by nitrogen fertilization. *Nutrient Cycling in Agroecosystems* 73:201-212.

Ghimire, R., Adhikari, K.R., Chen, Z.-S., Shah, S.C., and Kahal, K.R. (2012). Soil organic carbon sequestration as affected by tillage, crop residue, and nitrogen application in rice–wheat rotation system. *Paddy Water Environment* 10:95-102.

Giller, K.E., Ormesher, J., and Awah, F.M. (1991). Nitrogen transfer from *phaseolus* bean to intercropped maize measured using ^{15}N-enrichment and ^{15}N-isotope dilution methods. *Soil Biology and Biochemistry* 23:339-346.

Giller, P.S. (1996). The diversity of soil communities, the 'poor man's tropical rainforest'. *Biodiversity and Conservation* 5:135-168.

Glaser, B. (2005). Compound-specific stable-isotope (δ13C) analysis in soil science. *Journal of Plant Nutrition and Soil Science* 168:633-648.

Glenn, A.J., Amiro, B.D., Tenuta, M., Wagner-Riddle, C., Drewitt, G., and Warland, J. (2011). Contribution of crop residue carbon to soil respiration at a northern Prairie site using stable isotope flux measurements. *Agriculture and Forest Meteorology* 151:1045-1054.

Goidts, E., and van Wesemael, B. (2007). Regional assessment of soil organic carbon changes under agriculture in Southern Belgium (1955–2005). *Geoderma* 141:341-354.

Gomez, E., Ferreras, L., and Toresani, S. (2006). Soil bacterial functional diversity as influenced by organic amendment application. *Bioresource Technology* 97:1484-1489.

Gomez, K.A., and Gomez, A.A. (1984). *Statistical Procedures for Agricultural Research* (2nd Ed). Philippines: John Wiley & Sons, Inc.

Grandy, A.S., and Robertson, P. (2006). Initial cultivation of a temperate-region soil immediately accelerates aggregate turnover and $CO_2$ and $N_2O$ fluxes. *Global Change Biology* 12:1507-1520.

Green, C.J., and Blackmer, A.M. (1995). Residue decomposition effects on nitrogen availability to corn following corn or soybean. *Soil Science Society of America* 59:1065–1070.

Gregorich, E.G., and Beare, M.H. (2006). Physically Uncomplexed Organic Matter. In Carter, M.R., and Gregorich, E.G. (Eds.), *Soil Sampling and Methods of Analysis.* Boca Raton, FL:CRC Press.

Gregorich, E.G. Carter, M.R. Angers, D.A. Monreal, C.M. and Ellert, B.H. (1994). Towards a minimum data set to assess soil organic matter quality in agricultural soils. *Canadian Journal of Soil Science* 74:367-385.

Gregorich, E.G., Rochette, P., St-Georges, P., McKim, U.F., and Chan, C. (2008). Tillage effects on N2O emission from soils under corn and soybeans in Eastern Canada. *Canadian Journal of Soil Science* 88:153-161.

Griffis, T.J., Baker, J.M., and Zhang, J. (2005). Seasonal dynamics and partitioning of isotopic CO2 exchange in a C3/C4 managed ecosystem. *Agricultural and Forest Meteorology* 132:1-19.

Griffiths, B.S., Ritz, K., Ebblewhite, N., and Dobson, G. (1999). Soil microbial community structure: Effects of substrate loading rates. *Soil Biology and Biochemistry* 31:145-153.

Guo, Z.L., Cai, C.F., Li, Z.X., Wang, T.W., and Zheng., M.J. (2009). Crop residue effect on crop performance, soil $N_2O$ and $CO_2$ emissions in alley cropping systems in subtropical China. *Agroforestry Systems* 76:67-80.

Hadas, A., Kautsky, L., Goek, M., and Kara, E.E. (2004). Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. *Soil Biology & Biochemistry* 36:255-266.

Haile-Mariam, S., Collins, H.P., Wright, S., and Paul, E.A. (2008). Fractionation and Long-Term Laboratory Incubation to Measure Soil Organic Matter Dynamics. *Soil Science Society of America* 72:370-378.

Hall, A. J., Rebella, C.M., Ghersa, C.M., and Culot, J.P. (1992). Field-crop systems of the Pampas. In Pearson, C.J. (Ed.) *Field Crop Systems. Ecosystems of the World* (413-450). Elseivier: Amsterdam.

Hamer, U., Potthast, K., and Makeschin, F. (2009). Urea fertilisation affected soil organic matter dynamics and microbial community structure in pasture soils of Southern Ecuador. *Applied Soil Ecology* 43:226-233.

Handley, L.L., and Raven, J.A. (1992). The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant, Cell and Environment* 15:965-985.

Hargreaves, P.R., Brookes, P.C., Ross, G.J.S., and Poulton, P.R. (2003). Evaluating soil microbial biomass carbon as an indicator of long-term environmental change. *Soil Biology & Biochemistry* 35:401-407.

Harrera, L.P., Laterra, P., Maceira, N.O., Zelaya, K.D., and Martínez, G.A. (2009). Fragmentation Status of Tall-Tussock Grassland Relicts in the Flooding Pampa, Argentina. *Rangeland Ecological Management* 62:73-82.

Hassink, J. (1995). Density fractions of soil macroorganic matter and microbial biomass as predictors of C and N mineralization. *Soil Biology & Biochemistry* 27:1099-1108.

Hauggaard-Nielsen, H., Ambus, P., and Jensen, E.S. (2001). Interspecific competition, N use and interference with weeds in pea-barley intercropping. *Field Crops Research* 70:101-109.

Hauggaard-Nielsen, H., Gooding, M., Ambus, P., Corre-Hellou, G., Crozat, Y., Dahlmann, C., Dibet, A., von Fragstein, P., Pristeri, A., Monti, M., and Jensen, E.S. (2009). Pea-barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. *Field Crops Research* 113:64-71.

Hauggaard-Nielsen, H., and Jensen, E.S. (2005). Facilitative root interactions in intercrops. *Plant and Soil* 274:237-250.

He, Y., Xu, Z., Chen, C., Burton, J., Ma, Q., Ge., Y., and Xe, J. (2008). Using light fraction and macroaggregate associated organic matters as early indicators for management-induced changes in soil chemical and biological properties in adjacent native and plantation forests of subtropical Australia. *Geoderma* 147:116-125.

Herencia, J.F., Ruiz, J.C., Melero, S., Garcia Galavís, P.A., and Maqueda, C. (2008). A short-term comparison of organic *v.* conventional agriculture in a silty loam soil using two organic amendments. *Journal of Agricultural Science* 146:677-687.

Hillel, D. (1998). *Environmental Soil Physics*. San Diego, CA: Academic Press.

Högberg, P. (1997). [15]N natural abundance in soil-plant systems. *New Phytologist* 137:179-203.

Hogg, E.H., Lieffers, V.J., and Wein, R.W. (1992). Potential Carbon Losses From Peat Profiles: Effects of Temperature, Drought Cycles, and Fire. *Ecological Applications* 2:298-306.

Horrigan, L., Lawrence, R.S., Walker, P. (2002). How Sustainable Agriculture Can Address the Environmental and Human Health Harms of Industrial Agriculture. *Environmental Health Perspectives* 110:445-456.

Iijima, M., Izumi, Y., Yuliadi, E., Sunyoto, and Ardjasa, S.W. (2004). Cassava-Based Intercropping Systems on Sumatra Island in Indonesia: Productivity, Soil Erosion and Rooting Zone. *Plant Production Science* 7:347-355.

Ikerd, J.E. (1993). The need for a systems approach to sustainable agriculture. *Agriculture, Ecosystems and Environment* 46:147-160.

Instituto Nacional de Tecnología Agropecuaria (INTA). (2012). Registro de datos agrometeorológicos. Retrieved April 2012 from http://anterior.inta.gov.ar/balcarce/info/meteorologia/meteoro2.htm.

International Panel on Climate Change (IPCC). (2007). Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge, United Kingdom:Cambridge University Press.

Janzen, H.H. (2004). Carbon cycling in earth systems - a soil science perspective. *Agriculture Ecosystems & Environment* 104:399-417.

Johnson, J.M.-F., Franzluebbers, A.J., Lachnicht Weyers, S., and Reicosky, D.C. (2007). Agricultural opportunities to mitigate greenhouse gas emissions. *Environmental Pollution* 150:107-124.

Johnson, R. (2009). *Climate Change: The Role of the U.S. Agriculture Sector and Congressional Action.* U.S.A.: Congressional Research Service, CRS Report for Congress: Prepared for Members and Committees of Congress (RL33898).

Kayler, Z.E., Kaiser, M., Gessler, A., Ellerbrock, R.H., and Sommer, M. (2011). Application of $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of organic matter fractions sequentially separated from adjacent arable and forest soils to identify carbon stabilization mechanisms. *Biogeosciences* 8:2895-2906.

Kim, H.Y., Lieffering, M., Kobayashi, K., Okada, M., Mitchell, M.W., and Gumpertz, M. (2003). Effects of free-air $CO_2$ enrichment and nitrogen supply on the yield of temperate paddy rice crops. *Field Crops Research* 83:261-270.

Kimble, J.M., Everett, L.R., Follett, R.R., and Lal, R. (2002). Carbon Sequestration and the Integration of Science, Farming, and Policy. In Kimble, J.M., Lal, R., and Follett, R.F. (Ed), Agricultural Practices and Policies for Carbon Sequestration in Soil (3-12). Boca Raton, Florida: Lewis Publishers.

Kimbrell, A. (2002). Corporate Lies: Busting the Myths of Industrial Agriculture. In A. Kimbrell's *The Fatal Harvest Reader: The Tragedy of Industrial Agriculture* (pp. 3-32). Sausalito, CA: Foundation for Deep Ecology.

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmera, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmalwasserh, A., Totsche, K.U., Walker, F., Scheu, S., and Kandeler, E. (2012). Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. *Pedobiologia-International Journal of Soil Biology* 55:111-119.

Kristiansen, S.M., Brandt, M., Hansen, E.M., Magid, J., and Christensen, B.T. (2004). $^{13}C$ signature of $CO_2$ evolved from incubated maize residues and maize-derived sheep faeces. *Soil Biology & Biochemistry* 36:99-105.

Kurdali, F. (2009). Growth and Nitrogen Fixation in Dhaincha/Sorghum and Dhaincha/ Sunflower Intercropping Systems Using 15Nitrogen and 13Carbon Natural Abundance Techniques. *Communications in Soil Science and Plant Analysis* 40:2995-2014.

Kuzyakov, Y., and Bol, R. (2005). Three sources of $CO_2$ efflux from soil partitioned by 13C natural abundance in an incubation study. *Rapid Communications in Mass Spectrometry* 19:1417-1423.

Kuzyakov, Y., Friedel, J.K., and Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Biology & Biochemistry* 32:1485-1498.

Lacombe, S. Bradley, R.L. Hamel, C. and Beaulieu, C. (2009). Do tree-based intercropping systems increase the diversity and stability of soil microbial communities? Agriculture, Ecosystems and Environment 131:25-31.

Lal, R., Follett, R.F., Stewart, B.A., and Kimble, J.M. (2007). Soil carbon sequestration to mitigate climate change and advance food security. *Soil Science* 172:943-956.

LaMondia, J.A., Elmer, W.H., Mervosh, T.L., and Cowles, R.S. (2002). Integrated management of strawberry pests by rotation and intercropping. *Crop Protection* 21:837-846.

Lang, M., Cia, Z., and Chang, S.X. (2011). Effects of land use type and incubation temperature on greenhouse gas emissions from Chinese and Canadian soils. *Journal of Soils and Sediments* 11:15-24.

Leite, L.F.C., Mendonça, E.S., and Machado, P.L.O.A. (2007). Influence of organic and mineral fertilsation on organic matter fractions of a Brazilian Acrisol under maize/ common bean intercrop. *Australian Journal of Soil Research* 45:25-32.

Lewis, D.E., White, J.R., Wafula, D., Athar, R., Dickerson, T., Williams, H.N., and Chauhan, A. (2010). Soil Functional Diversity Analysis of a Bauxite-Mined Restoration Chronosequence. *Microbial Ecology* 59:710-723.

Li, L., Sun, J., Zhang, F., Li, X., Yang, S., and Rengel, Z. (2001). Wheat/maize or wheat/soybean strip intercropping I. Yield advantage and interspecific interactions on nutrients. *Field Crops Research* 71:123-137.

Li, X, and Feng, Y. (2002). *Carbon Sequestration Potentials in Agricultural Soils.* Alberta Research Council:Edmonton, Alberta.

Liang B.C., Gregorich, E.G., and MacKenzie, A.F. (1999). Short-term mineralization of maize residues in soils as determined by carbon-13 natural abundance. *Plant and Soil* 208:227-232.

Lindstrom, P., Mondshine, M., Calopedis, S., and Checklick, N., and Billups, S. (2001). *Emissions of Greenhouse Gases in the United States 2000.* Washington, D.C.: Energy Information Administration

Liu, L., King, J.S., Booker, F.L., Giardina, C.P., Allen, H.L., and Hu, S. (2009). Enhanced litter input rather than changes in litter chemistry drive soil carbon and nitrogen cycles under elevated $CO_2$: a microcosm study. *Global Change Biology* 15:441-453.

Lupwayi, N.Z., Rice, W.A., and Clayton, G.W. (1998). Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. *Soil Biology & Biochemistry* 30:1733-1741.

Lynch, D.H., Voroney, R.P., and Warman, P.R. (2006). Use of 13C and 15N natural abundance techniques to characterize carbon and nitrogen dynamics in composting and in compost-amended soils. *Soil Biology & Biochemistry* 38:103-114.

Magurran, A.E. (1988). *Ecological Diversity and It's Measurement*. Croom Helm, Australia: University Press, Cambridge.

Mahaney, W.M., Smemo, K.A., and Gross, K.L. (2008). Impacts of $C_4$ grass introductions on soil carbon and nitrogen cycling in $C_3$-dominated successional systems. *Oecologia* 157:295-305.

Makoi, J.H.J.R., Chimphango, S.M.B., and Dakora, F.D. (2010). Photosynthesis, water-use efficiency and δ13C of five cowpea genotypes grown in mixed culture and at different densities with sorghum. *Photosynthetica* 48:143-155.

Makumba, W. Akinnifesi, F.K. Janssen, B. and Oenema, O. (2007). Long-term impact of a gliricidia-maize intercropping system on carbon sequestration in southern Malawi. Agriculture, Ecosystems and Environment 118:237-243.

Malhi, S., Brandt, S., and Gill, K.S. (2003). Cultivation and grassland type effects on light fraction and total organic C and N in a Dark Brown Chernozemic soil. *Canadian Journal of Soil Science* 83:145-153.

Manjaiah, K.M., Voroney, R.P., and Sen, Utpal. (2000). Soil organic carbon stocks, storage profile and microbial biomass under different crop management systems in a tropical agriculture ecosystem. *Biology and Fertility of Soils* 31:273-278.

Marland, G., West, T.O., Schlamadinger, B., and Canella, L. (2003). Managing soil organic carbon in agriculture: the net effect on greenhouse gas emissions. *Tellus* 55:613-621.

Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P.M., Hamer, U., Heim, A., Jandl, G., Ji, R., Kaiser, K., Kalvitz, K., Kramer, C., Leinweber, P., Rethemeyer, J., Schäffer, A., Schmidt, M.W.I., Schwark, L., and Wiesenberg, G.L.B. (2008). How relevant is recalcitrance for the stabilization of organic matter in soils? *Journal of Plant Nutrition and Soil Science* 171:91-110.

Martens, R. (1995). Current methods for measuring microbial biomass C in soil: Potentials and limitations. *Biology and Fertility of Soils* 19:87-99.

Martin, A., Mariotti, A., Balesdent, J., Lavelle, P., and Vuattoux, R. (1990). Estimate of organic matter turnover rate in a savanna soil by $^{13}$C natural abundance measurements. *Soil Biology and Biochemistry* 22:517-523.

Mazoyer, M., and Roudart., L. (2006). *A History of world agriculture: from the neolithic age to the current crisis.* New York, NY: Monthly Review Press.

Mazzoncini, M., Sapkota, T.B., Bárberi, P., Antichi, D., and Risaliti, R. (2011). Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content. *Soil & Tillage Research* 114:165-174.

Melillo, J.M. Aber, J.D. Linkins, A.E. Ricca, A. Fry, B. and Nadelhoffer, K.J. (1989). Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. Plant and Soil 115:189-198.

Mendelsohn, R., and Dinar, A. (2009). *Climate Change and Agriculture: an Economic Analysis of Global Impacts, Adaption and Distributional Effects.* Cheltenham, UK: Edward Elgar Publishing Limited.

Merino, A., Pérez-Batallón, P., and Macías, F. (2004). Responses of soil organic matter and greenhouse gas fluxes to soil management and land use changes in a humid temperate region of southern Europe. *Soil Biology & Biochemistry* 36:917-925.

Midwood, A.J., and Boutton, T.W. (1998). Soil carbonate decomposition by acid has little effect on δ13C of organic matter. *Soil Biology & Biochemistry* 30:1301-1307.

Millar, N., and Baggs, E.M. (2004). Chemical composition, or quality, of agroforestry residues influences $N_2O$ emissions after their addition to soil. *Soil Biology & Biochemistry* 36:935-943.

Mondini, C., Sinicco, T., Cayuela, M.L., and Sanchez-Monedero, M.A. (2010). A simple automated system for measuring soil respiration by gas chromatography. *Talanta* 81:849-855.

Moore, J.M., Klose, S., and Tabatabai, M.A. (2000). Soil microbial biomass carbon and nitrogen as affected by cropping systems. *Biology and Fertility of Soils* 31:200-210.

Müller-Stöver, D., Hauggaard-Nielsen, H., Eriksen, J., Ambus, P., and Johansen, A. (2012). Microbial biomass, microbial diversity, soil carbon storage, and stability after incubation of soil from grass–clover pastures of different age. *Biology and Fertility of Soils* 48:371-383.

Mungai, N.W., and Motavalli, P.P. (2006). Litter quality effects on soil carbon and nitrogen dynamics in temperate alley cropping systems. *Applied Soil Ecology* 31:32-42.

Nagano, H., Kato, S., Ohkubo, S., and Inubushi, K. (2012). Emissions of carbon dioxide, methane, and nitrous oxide from short- and long-term organic farming Andosols in central Japan. *Soil Science and Plant Nutrition* 58:793-801.

Nap, J.P., Metz, P.L.J., Escaler, M., and Conner, A.J. (2003). The release of genetically modified crops into the environment. Part I. Overview of current status and regulations. *The Plant Journal* 33:1-18.

Natarajan, M., and Willey, R.W. (1986). The effects of water stress on yield advantages of intercropping systems. *Field Crops Research* 13:117-131.

National Research Council. (1993). *Soil and Water Quality: An Agenda for Agriculture.* Washington, D.C.: National Academy Press.

Nier, A.O., and Gulbransen, E.A. (1939). Variations in relative abundance of carbon isotopes. *Journal of the American Chemical Society* 61:697-698.

Niggli, U., Fließbach, A., Hepperly, P., and Scialabba, N. (2009). *Low Greenhouse Gas Agriculture: Mitigation and Adaptation Potential of Sustainable Farming Systems.* Rome: Food and Agriculture Organization.

Niklaus, P.A., Wardle, D.A., and Tate, K.R. (2006). Effects of plant species diversity and composition on nitrogen cycling and the trace gas balance of soils. *Plant and Soil* 282:83-98.

Norman, G.R., and Streiner, D.L. (2008). *Biostatistics: The Bare Essentials* (3rd ed.). Hamilton, Ontario: BC Decker Inc.

Nosetto, M.D., Jobbágy, E.G., Brizuela, A.B., and Jackson, R.B. (2012). The hydrologic consequences of land cover change in central Argentina. *Agriculture, Ecosystems and Environment* 154:2-11.

Novoa, R.S.A., and Tejeda, H.R. (2006). Evaluation of the $N_2O$ emissions from N in plant residues as affected by environmental and management factors. *Nutrient Cycling in Agroecosystems.* 75:29-46.

Oelbermann, M., and Echarte, L. (2011). Evaluating soil carbon and nitrogen dynamics in recently established maize-soybean inter-cropping systems. *European Journal of Soil Science* 62:35-41.

Oelbermann, M., Echarte, L., Vachon, K., and Dubios, C. (2009). The role of complex agroecosystems in sequestering carbon and mitigating global warming. *Earth and Environmental Science* 6:1-3.

Oelbermann, M., English, M., and Schiff, S.L. (2008). Evaluating carbon dynamics and microbial activity in arctic soils under warmer temperatures. *Canadian Journal of Soil Science* 88:31-44.

Oelbermann, M., and Voroney, R.P. (2007). Carbon and nitrogen in a temperate agroforestry system: Using stable isotopes as a tool to understand soil dynamics. *Ecological Engineering* 29:342-349.

Oelbermann, M., Voroney, R.P., and Gordon, A.M. (2004). Carbon sequestration in tropical and temperate agroforestry systems: a review with examples from Costa Rica and souther Canada. *Agriculture, Ecosystems and Environment* 104:359-377.

Oelbermann, M., Voroney, R.P., Kass D.C.L., and Schlönvoigt, A.M. (2006b). Soil carbon and nitrogen dynamics using stable isotopes in 19- and 10-year-old tropical agroforestry systems. *Geoderma* 130:356-367.

Oelbermann, M., Voroney, R.P., Thevathasan, N.V., Gordon, A.M., Kadd, D.C.L. and Schlönvoigt, A.M. (2006a). Soil carbon dynamics and reside stabilization in a Costa Rican and souther Canadian alley cropping system. *Agroforestry Systems* 68:27-36.

O'Leary, M.H. (1988). Carbon Isotopes in Photosynthesis. *BioScience* 38:328-336.

Omonode, R.A., Vyn, T.J., Smith, D.R., Hegymegi, P., and Gál, A. (2007). Soil carbon dioxide and methane fluxes from long-term tillage systems in continuous corn and corn–soybean rotations. *Soil &Tillage Research* 95:182-195.

Organization for Economic Co-operation and Development. (2001). *Environmental Indicators for Agriculture, Methods and Results.* Paris, France: OECD Publications Service.

Oyedele, D.J., Schjønning, P., Sibbesen, E., and Debosz, K. (1999). Aggregation and organic matter fractions of three Nigerian soils as affected by soil disturbance and incorporation of plant material. *Soil & Tillage Research* 50:105-114.

Pappa, V.A., Rees, R.M., Walker, R.L., Baddeley, J.A., and Watson, C.A. (2011). Nitrous oxide emissions and nitrate leaching in an arable rotation resulting from the presence of an intercrop. *Agriculture, Ecosystems and Environment* 141:153-161.

Parayil, G. (2003). Mapping technological trajectories of the Green Revolution and the Gene Revolution from modernization to globalization. *Research Policy* 32:971-990.

Parkin, T.B., and Kaspar, T.C. (2006). Nitrous Oxide Emissions from Corn-Soybean Systems in the Midwest. *Journal of Environmental Quality* 35:1496-1506.

Patiño-Zúñiga, L., Ceja-Navarro, J.A., Govaerts, B., Luna-Guido, M., Sayre, K.D., and Dendooven, L. (2009). The effect of different tillage and residue management practices on soil characteristics, inorganic N dynamics and emissions of N2O, CO2 and CH4 in the central highlands of Mexico: a laboratory study. *Plant Soil* 314:231-241.

Patterson, D.T., Westbrook, J.K., Joyce, R.J.V., Lingren, P.D., and Rogasik, J. (1999). Weeds, insects, and diseases. *Climatic Change* 43:711-727.

Peichl, M. Thevathasan, N.V. Gordon, A.M. Huss, J. and Abohassan, R.A. (2006). Carbon sequestration potentials in temperate tree-based intercropping systems, southern Ontario, Canada. Agroforestry Systems 66:243-257.

Pendall, E. and King, J.Y. (2007). Soil organic matter dynamics in grassland soils under elevated $CO_2$: Insights from long-term incubations and stable isotopes. Soil Biology & Biochemistry 39:2628-2639.

Pennock, D., Yates, T., and Braidek, J. (2008). Soil Sampling Designs. In Carter, M.R., and Gregorich, E.G. (Eds.), *Soil Sampling and Methods of Analysis.* Boca Raton, FL:CRC Press.

Peri, P.L., Ladd, B., Pepper, D.A., Bonser, S.P., Laffan, S.W., and Amelung, W. (2012). Carbon ($\delta$13C) and nitrogen ($\delta$15N) stable isotope composition in plant ans oil in Souther Patagonia's native forests. *Global Change Biology* 18:311-321.

Poll, C., Marhan, S., Ingwersen, J., and Kandeler, E. (2008). Dynamics of litter carbon turnover and microbial abundance in a rye detritusphere. *Soil Biology & Biochemistry* 40:1306-1321.

Post, W.M., Emanuel, W.R., Zinke, P.J., and Stangenberger, A.G. (1982). Soil carbon pools and world life zones. *Nature* 298:156-159.

Potthoff, M, Dyckmans, J., Flessa, H., Muhs, A., Beese, F., and Joergensen, R.G. (2005). Dynamics of maize (*Zea mays* L.) leaf straw mineralization as affected by the presence of soil and the availability of nitrogen. *Soil Biology & Biochemistry* 37:1259-1266.

Preseton, C.M., Trofymow, J.A., and Flanagan, L.B. (2006). Decomposition, $\delta$13C, and the "lignin paradox". *Canadian Journal of Soil Science* 86:235-245.

Qiu, L., Wei, X., Zhang, X., Cheng, J., Gale, W., Guo, C., and Long, T. (2012). Soil organic carbon losses due to land use change in a semiarid grassland. *Plant Soil* 355:299-309.

Raji, J.A. (2007). Intercropping soybean and maize in a derived savanna ecology. *African Journal of Biotechnology* 6:1885-1887.

Reynolds, S.G. (1970). The gravimetric method of soil moisture determination. Part I: A study of equipment, and methodological problems. *Journal of Hydrology* 11:258-273.

Reza Miri, H., Rasteegar, A., and Reza Bagheri, A. (2012). The impact of elevated $CO_2$ on growth and competitiveness of $C_3$ and $C_4$ crops and weeds. *European Journal of Experimental Biology* 2:1144-1150.

Rice, W.R. (1989). Analyzing Tables of Statistical Tests. *Evolution* 43:223-225.

Rivest, D. Cogliastro, A. Bradley, R.L. and Olivier, A. (2010). Intercropping hybrid poplar with soybean increases soil microbial biomass, mineral N supply and tree growth. *Agroforestry Systems* 80:33-40.

Robinson, D., and Scrimgeour, C.M. (1995). The contribution of plant C to soil $CO_2$ measured using $\delta$13C. *Soil Biology and Biochemistry* 27:1653-1656.

Ross, D.J., Tate, K.R., Newton, P.C.D., and Clark, H. (2002). Decomposability of C3 and C4 grass litter sampled under different concentrations of atmospheric carbon dioxide at a natural $CO_2$ spring. *Plant and Soil* 240:275-286.

Rusinamhodzi, L., Murwira, H.K., and Nyamangara, J. (2009). Effect of cotton-cowpea intercropping on C and N mineralisation patterns of residue mixtures and soil. *Australian Journal of Soil Research* 47:190-197.

Samahadthai, P., Vityakon, P., and Saenjan, P. (2010). Effects of different quality plant residues on soil carbon accumulation and aggregate formation in a tropical sandy soil in Northeast Thailand as revealed by a 10-year field experiment. *Land Degradation & Development* 21:463-473.

Šantrůčková, H., Bird, M.I., and Lloyd, J. (2000). Microbial processes and carbon- isotope fractionation in tropical and temperate grassland soils. *Functional Ecology* 14: 108-114.

Schimel, D.S. Braswell, B.H. Holland, E.A. McKeown, R. Ojima, D.S. Painter, T.H. Parton, W.J. and Townsend, A.R. (1994). Climatic, edaphic, and biotic controls over storage and turnover of carbon in soils. *Global Biogeochemical Cycles* 8:279-293.

Schnürer, J., Clarholm, M., and Rosswall, T. (1985). Microbial biomass and activity in an agricultural soil with different organic matter contents. *Soil Biology and Biochemistry* 17:611-618.

Schrumpf, M., Schulze, E.D., Kaiser, K., and Schumacher, J. (2011). How accurately can soil organic carbon stocks and stock changes be quantified by soil inventories? *Biogeosciences* 8:1193-1212.

Schweizer, M., Fear, J., and Cadisch, G. (1999). Isotopic ($^{13}$C) Fractionation During Plant Residue Decomposition and its Implications for Soil Organic Matter Studies. *Rapid Communications in Mass Spectrometry* 13:1284-1290.

Schwendenmann, L., and Pendall, E. (2008). Response of soil organic matter dynamics to conversion from tropical forest to grassland as determined by long-term incubation. *Biology and Fertility of Soils* 44:1053-1062.

Six, J., Feller, C., Denef, K., Ogle, S.M., de Moraes Sa, J.C., and Albrecht, A. (2002). Soil organic matter, biota and aggregation in temperate and tropical soils - Effects of no-tillage. *Agronomie* 22:755-775.

Smith, K. (Eds.). (2010). *Nitrous Oxide and Climate Change.* Washington, D.C.: Earthscan LLC.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S. (2007). Policy and technological constraints to implementation of greenhouse gas mitigation options in agriculture. *Agriculture, Ecosystems and Environment* 118:6-28.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., Ogle, S., O'Mara, F., Rice, C., Scholes, B., Sirotenko, O., Howden, M., McAllister, T., Pan, G., Romanenkov, V., Schneider, U., Towprayoon, S., Wattenback, M., and Smith, J. (2008). Greenhouse gas mitigation in agriculture. *Philosophical Transactions of the Royal Society B* 363:789-813.

Snyder, D.M. (2011). A characterization of the controls of the nitrogen and oxygen isotope ratios of biologically-produced nitrous oxide and nitrate in soils. (Ph.D. Thesis, University of Waterloo).

Song, B.Z., Wu, H.Y., Kong, Y., Zhang, J., Du, Y.L., Hu, J.H., and Yao, Y.C. (2010). Effects of intercropping with aromatic plants on the diversity and structure of an arthropod community in a pear orchard. *BioControl* 55:741-751.

Song, Y.N., Zhang, F.S., Marschner, P., Fan, F.L., Gao, H.M., Bao, X.G., Sun, J.H, and Li, L. (2007). Effect of intercropping on crop yield and chemical and microbiological properties in rhizosphere of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and faba bean (*Vicia faba* L.). *Biology and Fertility of Soils* 43:565-574.

Soon, Y.K., Haq, A., and Arshad, M.A. (2009). Carbon and nitrogen contents of different-sized light fraction organic matter as influenced by tillage and residue management. *Canadian Journal of Soil Science* 89:281-286.

Sparling, G.P. (1992). Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. *Australian Journal of Soil Research* 30:195–207.

Spedding, T.A., Hamel, C., Mehuys, G.R., and Madramootoo, C.A. (2004). Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. *Soil Biology & Biochemistry* 36:499-512.

Stefanowicz, A. (2006). The Biolog Plates Technique as a Tool in Ecological Studies of Microbial Communities. *Polish Journal of Environmental Studies* 15:669-676.

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., and Six, J. (2009). Soil carbon saturation: Implications for measurable carbon pool dynamics in long-term incubations. *Soil Biology & Biochemistry* 41:357-366.

Studdert, G.A., and Echeverría, H.E. (2000). Crop Rotations and Nitrogen Fertilization to Manage Soil Organic Carbon Dynamics. *Soil Science Society of America* 64:1496-1503.

Suman, A., Lal, M., Singh, A.K., and Gaur, A. (2006). Microbial Biomass Turnover in Indian Subtropical Soils under Different Sugarcane Intercropping Systems. *Agronomy Journal* 98:698-704.

Sun, Y.M. Zhang, N.N. Wang, E.T. Yuan, H.L. Yang, J.S. and Chen, W.X. (2009). Influence of intercropping and intercropping plus rhizobial inoculation on microbial activity and community composition in rhizosphere of alfalfa (Medicago sativa L.) and Siberian wild rye (Elymus sibiricus L.). *Federation of Microbiological Societies Microbiology Ecology* 70:218-226.

Swanston, C.W., Caldwell, B.A., Homann, P.S., Ganio, L., and Sollins, P. Carbon dynamics during a long-term incubation of separate and recombined density fractions from seven forest soils. *Soil Biology and Biochemistry* 34:1121-1130.

Swerts, M., Merckx, R., and Vlassak, K. (1996). Influence of carbon availability on the production of NO, $N_2O$, $N_2$ and $CO_2$ by soil cores during anaerobic incubation. *Plant and Soil.* 181:145-151.

Taboada, M.A., Micucci, F.G., Cosentino, D.J., and Lavado, R.S. (1998). Comparison of compaction induced by conventional and zero tillage in two soils of the Rolling Pampa of Argentina. *Soil & Tillage Research* 49:57-63.

Tan, Z., Lal, R., Owens, L., and Izaurralde, R.C. (2007). Distribution of light and heavy fractions of soil organic carbon as related to land use and tillage practice.

Tilman, D. (1998). The greening of the green revolution. *Nature* 398:211-212.

Unkovich, M., Herridge, D., Peoples, M., Cadisch, G., Boddey, R., Giller, K., Alves, B., and Chalk, P. (2008). *Measuring plant-associated nitrogen fixation in agricultural systems.* Canberra, Australia: Australian Centre for International Agricultural Research.

Uphoff, N., Ball, A.S., Fernandes, E., Herren, H., Husson, O., Laing, M., Palm, C., Pretty, J., Sanchez, P., Sanginga, N., and Thies, J. (2006). *Biological Approaches to Sustainable Soil Systems.* Boca Raton, FL, USA: CRC Press.

Vachon, K. (2008). *Soil carbon and nitrogen dynamics and greenhouse gas mitigation in intercrop agroecosystems in Balcarce, Argentina.* MES thesis, Department of Environment and Resource Studies, University of Waterloo, Waterloo, Canada.

Vachon, K., and Oelbermann, M. (2011). Crop Residue Input and Decomposition in a Temperate Maize-Soybean Intercrop System. *Soil Science* 176:157-163.

van Groenigen, K.J., Hastings, A., Forristal, D., Roth, B., Jones, M., and Smith, P. (2011). Soil C storage as affected by tillage and straw management: An assessment using field measurements and model predictions. *Agriculture, Ecosystems and Environment* 140:218-225.

Vance, E.D., Brookes, P.C., and Jenkinson, D.S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology & Biochemistry* 19:703-707.

Vigil, M.F., and Kissel, D.E. (1991). Equations for Estimating the Amount of Nitrogen Mineralized from Crop Residues. *Soil Science Society of America* 55:757-761.

Vitória, L., Otero, N., Soler, A., and Canals, À. (2004). Fertilizer Characterization: Isotopic Data (N, S, O, C, and Sr). *Environmental Science & Technology* 38:3254-3262.

Voroney, R.P., Brookes, P.C., and Beyaert, R.P. (2008). Soil Microbial Biomass C, N, P, and S. In Carter, M.R., and Gregorich, E.G. (Eds.), *Soil Sampling and Methods of Analysis.* Boca Raton, FL:CRC Press.

Wall, G.J., Pringle, E.A., and Sheard, R.W. (1991). Intercropping red clover with silage corn for soil erosion control. *Canadian Journal of Soil Science* 71:137-145.

Wang, Q., Li, Y., and Ashok, A. (2010). Cropping systems to improve carbon sequestration for mitigation of climate change. *Journal of Environmental Protect* 1:207-215.

Watve, M.G., and Gangal, R.M. (1996). Problems in Measuring Bacterial Diversity and a Possible Solution. *Applied and Environmental Microbiology* 62:4299-4301.

Wright, A.L., and Hons, F.M. (2005). Tillage impacts on soil aggregation and carbon and nitrogen sequestration under wheat cropping sequences. *Soil & Tillage Research* 84:67-75.

Wynn, J.G, and Bird, M.I. (2007). $C_4$-derived soil organic carbon decomposes faster than its $C_3$ counterpart in mixed $C_3/C_4$ soils. *Global Change Biology* 13:2206-2217.

Yakir, D., and Sternberg L. daS.L. (2000). The use of stable isotopes to study ecosystem gas exchange. *Oecologia* 123:297-311.

Yilmaz, S, Atak, M., and Erayman, M. (2008). Identification of Advantages of Maize-Legume Intercropping over Solitary Cropping through Competition Indices in the East Mediterranean Region. *Turkish Journal of Agriculture and Forestry* 32:111-119.

Yuan, H., Ge, T., Chen, C., O'Donnell, A.G., and Wu, J. (2012). Significant Role for Microbial Autotrophy in the Sequestration of Soil Carbon. *Applied and Environmental Microbiology* 78:2328-2336.

Zhao, W.Z., Xiao, H.L., Liu, Z.M., and Li, J. (2005). Soil degradation and restoration as affected by land use change in the semiarid Bashang area, northern China. *Catena* 59:173-186.

Zhou, X., Yu, G., and Wu, F. (2011). Effects of intercropping cucumber with onion or garlic on soil enzyme activities, microbial communities and cucumber yield. *European Journal of Soil Biology* 47:279-287.

Ziska, L.H., and Bunce, J.A. (2007). Predicting the impact of changing $CO_2$ on crop yields: some thoughts on food. *New Phytologist* 175:607-618.