Evaluation of maize and soybean intercropping on soil quality and nitrogen transformations in the Argentine Pampa

by

Alison Regehr

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, 2014

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

Agricultural intensification to increase food, feed, and fibre production has also resulted in environmental degradation, including poorer soil quality and high emissions of greenhouse gases (GHGs) like nitrous oxide (N_2O). Intercropping, an agroecosystem management practice where more than one crop is planted on the same plot of land at the same time, promotes the complementary use of soil nutrients, and may improve soil quality and increase the retention of inorganic nitrogen (N) in the soil, thereby reducing N_2O emissions. An experiment was conducted in Balcarce, Argentina to determine the impact of intercropping maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.), (either 1:2 or 2:3 rows of maize to soybean) on soil quality and soil N transformations after six cropping seasons. It was found that intercropping significantly improved soil quality over a six year period, as indicated by the soil organic carbon (SOC), soil total nitrogen (TN), soil light fraction organic matter (LF), and soil microbial biomass carbon (SMB-C). However, the soil quality also significantly improved in the sole crops over this time, and in 2012, only SMB-C was significantly ($p<0.05$) greater in the 2:3 intercrop than in the sole crops. Intercropping resulted in higher rates of gross nitrogen (N) mineralization than the sole crops, and the 2:3 intercrop resulted in higher rates of gross N immobilization than in the other treatments. However, the high rate of gross N mineralization resulted in a low relative NH_4^+ immobilization in both intercrops, signifying a lower potential for reducing soil NH_4^+ concentrations than in the sole crop treatments. Net N immobilization occurred in all treatment plots, which was desired at the end of the fallow period to reduce N losses from the soil. The 2:3 intercrop appeared to perform better than the 1:2 intercrop. However, further research needs to be conducted to determine the seasonal variations in N mineralization and immobilization, and to further examine the intercrop spatial arrangements to increase crop residue yield.

Acknowledgements

I could not have finished this thesis without the help of many people. Firstly, thank you to my advisor, Dr. Maren Oelbermann, for helping guide me through the process of researching and writing this thesis, providing helpful feedback along the way. Thank you also to my committee member, Dr. Len Tsuji, and my external examiner, Dr. Marney Isaac, for their time and comments on my thesis.

Thank you to Meaghan Wilton, who oriented me through my first days in Argentina, introduced me to her Argentine ‘family,’ helped with my field work, taught me how to use the elemental analyzer, and was moral support in the laboratory. Thanks also to Bethany Winstone, for helping me complete some of the more tedious laboratory work.

Me gustaría dar las gracias a la gente del Instituto Nacional de Tecnología Agropecuaría (INTA) en Balcarce, Argentina, especialmente a Dra. Laura Echarte, Dra. Cecilia Videla y Marta Latorraca. Ellas me dieron una bienvenida muy especiale a Balcarce y INTA, y compartieron su laboratorio conmigo. Sin ellas, no habría sido capaz de terminar mi trabajo en el campo y el laboratorio.

And finally, thank you to my family and friends who supported me throughout this long process, encouraging me throughout. Thanks especially to Jennifer Regehr for helping to edit this thesis.

Table of Contents

List of Figures

List of Tables

General introduction

From the onset of the Green Revolution, agricultural intensification has occurred rapidly at a global scale to meet the needs of a growing population. Modern, high-yielding crop varieties were developed to increase food production with only a limited increase in land area devoted to agriculture (Evenson and Gollin, 2003). Although greater food production has resulted, agricultural intensification has also contributed to environmental degradation, including soil erosion, water overconsumption, biodiversity and ecosystem service loss, greenhouse gas (GHG) emissions, land-use change, and reliance on fossil fuels (Weis, 2010).

In agriculture, nitrous oxide (N_2O) is one of the primary GHGs of concern. For example, in the United States, nearly 60% of agricultural GHG emissions came from N_2O in 2005, and approximately 80% of all N_2O emissions were from the agricultural sector (Snyder et al., 2009), indicating that N_2O is primarily associated with agriculture. Although the global emissions of N_2O are less than those of carbon dioxide (CO_2), N_2O has a global warming potential that is 296 times greater than that of CO_2 (Snyder et al., 2009). It is therefore important for the agricultural sector to take actions to mitigate N_2O emissions where possible, to reduce the effects on, and from, climate change.

Additionally, recent agricultural intensification has occurred at the expense of soil quality, negatively impacting soil biological and chemical characteristics such as soil organic carbon (SOC), nitrogen (N), soil microbial biomass (SMB), and organic matter light fraction (LF). SMB and LF are among the more sensitive indicators of soil conditions, and can act as signals for changes in land management practices and ecosystem integrity (Oelbermann and Echarte, 2011). As farmers attempt to improve land management practices, the temporal impacts of these practices on soil characteristics should be monitored to determine whether the predicted and desired results are occurring.

Intercropping occurs when more than one crop is planted on the same plot of land at the same time. Intercropping a legume and a non-legume, such as soybean (*Glycine max* (L.) Merr.) and maize (*Zea mays* L.), can potentially reduce N_2O emissions and increase soil quality as compared to sole crops of soybean and maize. The legume/cereal crop combination reduces fertilizer requirements, as the crops complement each other's N resources, and increase carbon (C) and N retention in the soil. However, there is still a lack of understanding of the impact of intercropping on soil quality and the processes which transform N and emit N_2O in maize/soybean intercrops. Therefore, a long-term research site was established to address these issues with a long-term goal to determine the processes of C stabilization and N transformations to minimize GHG emissions in complex agroecosystems, such as intercrops. The current study will help to meet this long-term goal through determining the impact of maize/soybean intercropping on soil quality and gross N mineralization and immobilization rates.

Thesis Outline

This thesis is arranged in five chapters, beginning with an introduction, literature review, and description of the study site. The two experiments are covered in chapters 3 and 4, and chapter 5 presents conclusions from the experiments as well as future research ideas.

Chapter 1: Introduces the literature relating to this thesis, including the effects of intercropping on soil quality and N mineralization and immobilization rates.

Chapter 2: Presents a description of the study site, including the study design, soil and climate characteristics, and the historical and regional context of the site.

Chapter 3: Examines the soil quality, as indicated by various biological and chemical characteristics, of the intercrops and sole crops after 6 years of intercropping.

Chapter 4: Examines the impact of soybean-maize intercropping on gross N mineralization and immobilization rates.

Chapter 5: Draws conclusions from the experiments described in chapters 3 and 4 and provides suggestions for further research.

Chapter 1: Literature review

1.1 Land-use change

Changes in land-use can have significant impacts on greenhouse gas (GHG) emissions, soil quality, and vegetation biomass. The conversion of forest to agriculture, and vice versa, has been, and will continue to be, one of the most significant land-use changes (Christensen et al., 2007). Unfortunately, most land that is well suited to agriculture has already been converted (Christensen et al., 2007), and marginal lands will have to be converted to meet the increased future demand for agricultural products (Adviento-Borbe et al., 2007).

Changing land-use modifies the properties associated with vegetation, soils, and water of the area, and hence can impact the local climate by changing radiation, cloudiness, and surface temperature (Denman et al., 2007). Through climate teleconnection processes, local climate changes can impact broader climate processes extending past the area of land-use change (Christensen et al., 2007). Not only do land-use transitions impact the climate through physical changes to the environment, but transitions to permanent agriculture contribute about 7% of the global warming potential (GWP) of anthropogenic GHG emissions (Ponsioen and Blonk, 2012). Deforestation has been the prime contributor to the CO_2 flux associated with land-use change as forests are removed to make way for agriculture, and can also increase N_2O and NO emissions by 30-250% (Denman et al., 2007). In Argentina, the average land-use change GWP values for maize were 2.3 tonnes CO_2 equivalent per ha, and for soybean were 8.9 tonnes CO_2 equivalent per ha (Ponsioen and Blonk, 2012). Of the most important crops in Argentina, comprising soybean, wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and sunflower (*Helianthus annuus* L.), the GWP values for soybean were the greatest (Ponsioen and Blonk, 2012), indicating that a land-use change to growing soybeans is one of Argentina's more significant agricultural contributions to anthropogenic climate change.

Land-use change from forest or grassland to agriculture can change land cover (Guo and Gifford, 2002), and hence decrease soil organic carbon (SOC) content (Guo and Gifford, 2002; Spohn and Giani, 2011); decrease soil aggregation (Spohn and Giani, 2011); increase soil bulk density and therefore change soil hydraulic properties such as hydraulic conductivity and soil water retention; and change the water budget of river catchments (Bormann et al., 2007). In the course of transitioning land-use to agriculture, carbon (C) that was stored in the original vegetation is released by burning or decomposition (Guo and Gifford, 2002). During agricultural production, most of the crop is harvested and little to no organic matter (OM) is returned to the soil, thereby reducing the SOC stocks (Guo and Gifford, 2002). Based on a meta-analysis of 537 observations from 16 countries around the world, Guo and Gifford (2002) reported that land-use change from native forest to crop production reduced soil C by 42%, and the conversion of pasture and grassland to crop production reduced soil C by 59%. They found that soil C loss was greatest 30-50 years after the land-use conversion from pasture and grassland to crops, indicating that the impacts of land-use change are very long-lasting. Additionally, Guo and Gifford (2002) reported that reverse land-use change from crops to grassland or forests rarely reaches original C stocks.

To meet the food and fibre needs of a growing global population, further land-use change to agriculture will likely be required (Vermeulen et al., 2012). Carreño et al. (2012) developed a series of criteria to assess land-use options through a trade-offs analysis of ecosystem and economic services provision. According to the results of the assessment, it was determined that the Argentine Pampa demonstrated a low capacity to provide ecosystem services in comparison to its ability to produce food and economic income. This result was strengthened when compared to a region such as Brazilian forests which have a very high capacity to provide ecosystem services. However, even in areas where there is a low capacity for ecosystem services, it is important for agricultural practices to minimize the negative aspects of land-use change (Carreño et al., 2012).

1.2 Climate change

Global climate patterns are based on solar energy and how the earth and atmosphere respond to it through reflection, absorption, and re-emission (Solomon et al., 2007). Therefore, changes in the properties of the atmosphere and earth surface can result in changes in the global climate. Since the Industrial Revolution, atmospheric concentrations of GHGs have increased significantly (Forster et al., 2007). Many GHGs occur naturally in the atmosphere, but anthropogenic sources of these gases have caused the concentrations to rise rapidly over the previous 250 years, while other GHGs are entirely from human actions (Solomon et al., 2007). GHGs can increase the atmospheric absorption of both incoming (solar) and outgoing (earth) radiation (Solomon et al., 2007) and hence result in climatic changes.

1.2.1 Greenhouse gas emissions

Carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) comprise the three long-lived GHGs which can influence the climate over the long term (Solomon et al., 2007). Anthropogenic emissions of all three gases have increased significantly since the 1700s and the Industrial Revolution (Solomon et al., 2007). Emissions of CO_2 are the highest among the GHGs and are increasing at a greater rate than the emissions of other GHGs (Solomon et al., 2007). In the 8000 years before industrialization, the concentration of CO_2 in the atmosphere increased by 20 ppm, while in the previous 260 years, the concentration has increased by nearly 100 ppm (Solomon et al., 2007). These increased CO_2 emissions can primarily be attributed to fossil fuel use and deforestation from land-use change (Forster et al., 2007).

Atmospheric CH_4 concentrations have more than doubled since pre-industrial times (Solomon et al., 2007), although the growth rate of CH_4 emissions has decreased to nearly zero since 1999 (Forster et al., 2007). Methane has a 100 year GWP 25 times greater than CO_2 and an atmospheric lifetime of 12 years (Forster et al., 2007). Primary sources for CH_4 emissions include landfills, natural gas distribution, wetlands, and agriculture, particularly ruminant animals, rice cultivation, and the burning of biomass (Forster et al., 2007; Solomon et al., 2007).

Globally, 6% of GHG emissions are N_2O, which has an atmospheric residence time of 120 years and a GWP which is 298 times greater than CO_2 (Frimpong et al., 2012). Since preindustrial times, N_2O emissions have increased by 40-50% due to anthropogenic activity (Snyder et al., 2009) and over half of the global anthropogenic N_2O emissions come from agriculture (Frimpong et al., 2012). Anthropogenic N_2O emissions are primarily due to fertilizer use and fossil fuel burning (Forster et al., 2007).

1.2.2 Climate change impacts

Already, impacts from climate change are observed around the world (Solomon et al., 2007). Not only has the global average surface temperature increased, but so have the temperature extremes (Trenberth et al., 2007). Of the twelve warmest years recorded since 1850, eleven occurred in the twelve-year period between 1995 and 2006 (Trenberth et al., 2007). In mid-latitude regions, the number of frost-free days has increased, the number of warm extremes (the warmest 10% of days or nights) has increased, the number of cold extremes (the coldest 10% of days or nights) has decreased, and heat waves have increased in duration (Solomon et al., 2007). These higher temperatures have decreased snow cover, snowpack, the area of seasonally frozen ground, and the area covered by Arctic sea ice; river and lake ice are forming later and breaking up earlier; and glaciers and icecaps are experiencing unprecedented melting (Solomon et al., 2007).

Precipitation is also impacted by climate change, showing a greater spatial and temporal variability. Some parts of the world have experienced increased precipitation, whereas others have experienced drying (Solomon et al., 2007). Droughts in the tropics and subtropics are longer and more intense since the 1970s, while in other areas the number of heavy precipitation events has increased (Trenberth et al., 2007).

1.2.3 Climate change predictions

Many models and scenarios are used to predict future climate change trends. Future GHG emissions vary among these modelled scenarios, and different algorithms are used in each model to account for the complexity inherent in the climate. These general circulation models

(GCMs) all therefore have inherent uncertainty in their predictions (Nelson et al., 2009; Osborne et al., 2012). Nonetheless, it was predicted that if the global atmospheric GHG concentrations do not increase above those from the year 2000, by 2090-2099, the global mean temperature will increase by up to 0.9°C (Solomon et al., 2007). Under a worst case scenario of increasing GHG emissions, the global mean temperature could increase by up to 6.4°C with an associated sea level rise of up to 0.59 m (Meehl et al., 2007). Regardless of the extent of temperature rise, snow cover will decrease, thaw depth in permafrost areas will increase, year-round ice cover in parts of the Arctic Ocean will cease, and the higher global temperatures will suppress the ability of the land and ocean to take up CO_2 (Solomon et al., 2007).

Regional predictions of climate change have been modeled based on the GCMs. It is predicted that by 2080-2099, southern South America, including Argentina, will increase in temperature by 2.5°C to 5°C from the 1980-1999 average, depending on future GHG emissions (Christensen et al., 2007). Precipitation changes are expected to be minimal, with a median annual increase of 3%, although extreme precipitation events are predicted to increase (Christensen et al., 2007).

1.2.4 Climate change and agriculture

Agriculture contributes to climate change through the emissions of CO_2, CH_4, and N_2O, yet agroecosystems themselves are extremely vulnerable to climate change (Nelson et al., 2009). In 2005, agriculture accounted for 10-12% of anthropogenic GHG emissions and emitted approximately 17% more CH_4 and N_2O than in 1990 (Smith et al., 2007). The higher temperatures and variable precipitation associated with climate change will make it more difficult to maintain agricultural productivity. Yields will be reduced, and weeds and pests will become more difficult to suppress (Nelson et al., 2009). Lower yields will increase crop and meat prices, reducing calorie intake among more vulnerable populations, and increasing child malnutrition (Nelson et al., 2009).

As the global population grows and becomes wealthier, the demand for food will increase. It is therefore expected that agriculture will expand to meet this demand, and agricultural GHG emissions will increase as well (Smith et al., 2007). However, mitigation of agricultural GHG emissions is possible. Emissions can be reduced through more efficient nutrient management (Bouwman, 2001), and atmospheric C can be removed and stored in the soil organic matter (SOM) of agricultural ecosystems (Smith et al., 2007). For example, it is estimated that by 2030, the mitigation potential of agricultural C sequestration will be between 5500-6000 MtCO_2-eq/year (Smith et al., 2007).

Soil N_2O emissions constitute the most significant source of non-CO_2 emissions from agriculture, at 38% (Smith et al., 2007). It is predicted that by 2020, agricultural N_2O emissions will increase by 50% compared to 1990 (Mosier and Kroeze, 2000). Efforts have therefore been made to improve the understanding of N_2O production and to determine how it can be reduced. It has been frequently observed that additions of nitrogen (N) fertilizer result in immediate increases in soil N_2O production (Adviento-Borbe et al., 2007; Frimpong et al., 2012; McSwiney and Robertson, 2005). The addition to soil of OM, particularly with high N content, also increases N_2O emissions once the OM has decomposed and been mineralized to free inorganic N (Frimpong et al., 2012; Snyder et al., 2009). This accumulation of inorganic N in the form of nitrate (NO_3^-) and ammonium (NH_4^+) increases the nitrification and denitrification rates, which are the primary processes which form N_2O (Frimpong et al., 2012;

McSwiney and Robertson, 2005). Other factors have been found to increase the rates of N_2O production, such as soil moisture (Frimpong et al., 2012), soil temperature (Adviento-Borbe et al., 2007), soil compaction (Steinbach and Alvarez, 2006), and lack of competition for soil N resources (Adviento-Borbe et al., 2007; McSwiney and Robertson, 2005).

Many of these drivers of N_2O production can be influenced by soil management practices. Reducing the content of inorganic N in the soil, and hence N_2O emissions, can be accomplished by including legumes in the crop rotation, applying precise quantities of N fertilizer to meet the crop requirements, only applying N fertilizer to the crop (Smith et al., 2007), and coordinating the timing of fertilizer placement with the peak nutrient needs of the plants (Adviento-Borbe et al., 2007). Returning higher quantities of crop residue to the soil can help increase C sequestration in the SOM. This can occur by using high-yielding crop varieties, using crops with high below-ground biomass, and minimizing the use of unplanted fallow periods (Smith et al., 2007). Since tillage accelerates soil C loss, no-till methods may help improve C sequestration (Abdalla et al., 2013).

1.2.5 Soil nitrogen mineralization and immobilization

The production of N_2O is part of a larger cycle of N transformations that occurs in the soil (Figure 1.1). N mineralization is the process by which organic N, such as proteins, amino acids and nucleic acids from dead organisms, or the excretions of urea and uric acid from living organisms, is degraded to ammonia (NH_3) or NH_4^+ by saprophytic microorganisms (Philippot and Germon, 2005). N mineralization is integral in determining the amount of inorganic soil N which is available for plant use or loss (Monaghan and Barraclough, 1997).

Once mineralized, the NH_4^+ can undergo other processes, such as fixation by clays; immobilization by SOM, plants, and microorganisms (Philippot and Germon, 2005; Trehan, 1996); or nitrification and denitrification into NO_3^-, NO, N_2O, or N_2 (Philippot and Germon, 2005). Immobilization is the process by which inorganic N, as NH_4^+ and NO_3^-, is incorporated into organic forms (Myrold and Bottomley, 2008). Although both mineralization and immobilization are primarily biologically mediated, physical and chemical reactions can also be involved (Myrold and Bottomley, 2008). Together, N mineralization and immobilization determine soil N availability (Recous et al., 1999).

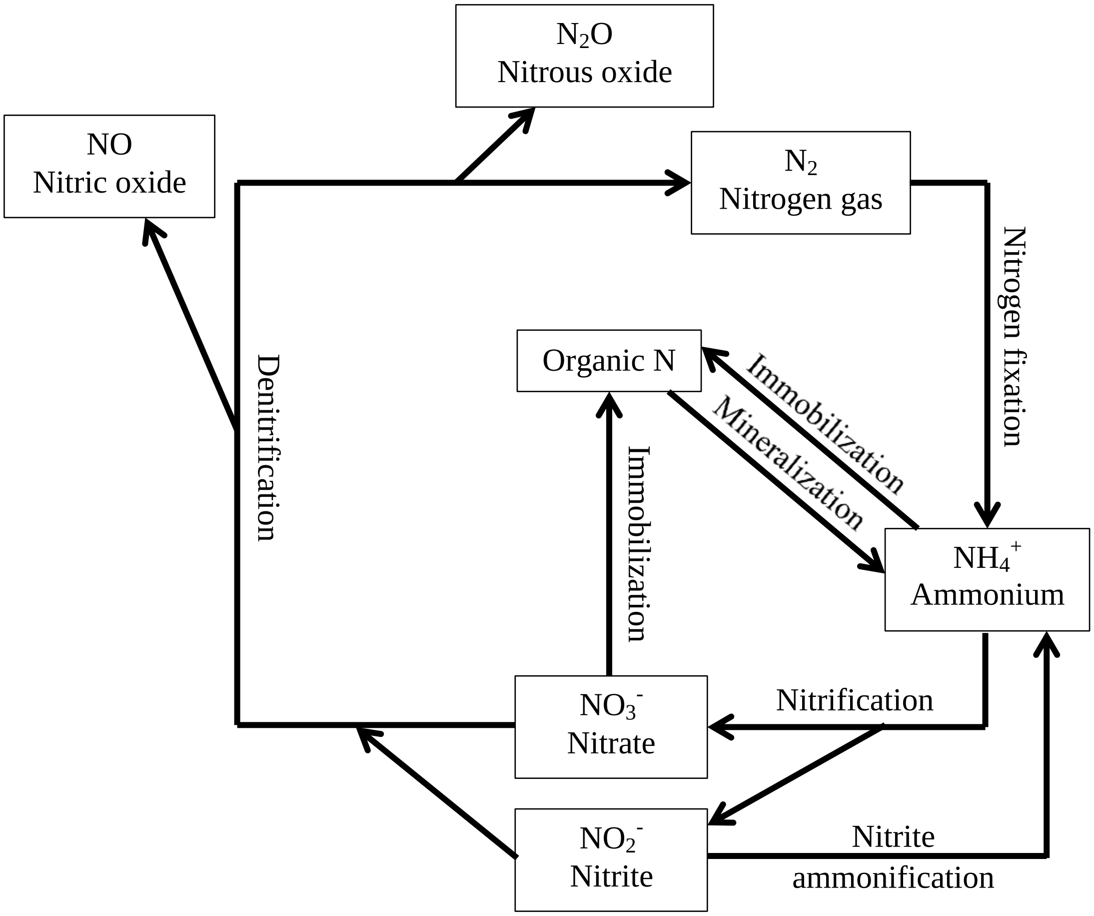

Figure 1.1 – Soil nitrogen transformations (adapted from Philippot and Germon, 2005)

Both mineralization and immobilization occur simultaneously and continuously in the soil. Mineralization-immobilization turnover (MIT) is a term used to describe the continual N cycling through the processes of gross mineralization and gross immobilization. Net mineralization (or net immobilization) is used to describe the difference between gross mineralization and gross immobilization (Andersen, 1999; Myrold and Bottomley, 2008).

The C/N ratio of the soil or organic substrate significantly impacts the rates of mineralization and immobilization, and can be used to predict whether net mineralization or immobilization will occur (Recous et al., 1999). Therefore, there is a strong connection between the MIT and the carbon cycle (Recous et al., 1999). Generally, net mineralization occurs when the substrate C/N ratio is about 20-25:1 or lower; net immobilization occurs when this ratio is greater and microbial activity is stimulated by the high quantities of labile C (Frimpong et al., 2012; Myrold and Bottomley, 2008). Net immobilization reduces N_2O emissions (Frimpong et al., 2012) since the N is not available for the processes of nitrification and denitrification until it has been mineralized.

However, plants require inorganic N from the soil, and excessive N immobilization results in insufficient quantities of N to be available to meet the plant needs. In fact, of the nutrient elements acquired by plants through the soil, N is most frequently limiting because it is continually lost (Philippot and Germon, 2005). It is therefore important to understand the processes of immobilization and mineralization to ensure that sufficient inorganic N is in the soil to meet plant and microbial needs, but that it is not in excess and producing N_2O or leaching out of the soil. Several studies have been conducted to investigate different management practices which would immobilize N when not needed but result in sufficient N mineralization to meet the N requirements. One method is to combine the residues of a cereal and a legume to balance the C/N ratios of each. Incorporation into the soil of leguminous residues such as pigeon pea (*Cajanus cajan* (L.) Millsp.) (Sakala et al., 2000), alfalfa (*Medicago sativa* L.) (Paré et al., 2000), and cow pea (*Vigna unguiculata* L.) (Frimpong et al., 2012) were all found to result in net mineralization. Incorporation of cereal residues, such as maize, resulted in immediate net immobilization (Frimpong et al., 2012; Paré et al., 2000; Sakala et al., 2000). It was therefore hypothesized that combining the cereal and legume residues would moderate the MIT.

Combining pigeon pea residue and maize residue and incorporating them into the soil resulted in net immobilization for 300 days, even if three times the weight of pigeon pea as maize residues were applied, although limited re-mineralization began occurring after four weeks (Sakala et al., 2000). However, since Paré et al. (2000) found that residue mineralization occurred faster when there were plants growing, it is possible that the laboratory experiment of Sakala et al. (2000) overestimated the time required for net mineralization to occur. Kaewpradit et al. (2008) reported that combining rice (*Oryza sativa* L.) straw and groundnut (*Arachis hypogaea* L.) residues increased N immobilization during the fallow period, that N remineralization occurred at a rate sufficient to meet the rice N requirements, and that N_2O emissions were reduced as a result. However, CH_4 emissions from the rice crop increased and the reduction in N_2O emissions was not enough to counteract the increase in CH_4 emissions, resulting in a greater GWP of the crop. This would not be the case for all residue mixtures, as the anaerobic conditions of the rice production increased the CH_4 emissions. For example, Bavin et al. (2009) found that the oxidation or production of CH_4 was negligible from either maize or soybean crops.

Although the C/N ratio of the applied residues is a significant factor in determining the MIT, other factors are also important, including the lignin and polyphenol contents of the residues (Kaewpradit et al., 2008), soil temperature, soil moisture, soil texture, native inorganic N availability, (Andersen, 1999), the microbial content and composition (Ledgard et al., 1998), and the presence of growing plants (Gill, 2009; Paré et al., 2000). When describing residue quality, frequently only the C/N ratio is used, but it is important to look beyond this ratio to also examine the lignin and polyphenol content of the residue (Millar and Baggs, 2004). Residues may have a low C/N ratio, implying that the mineralization rate will be high, but if the lignin and polyphenol content is high, there may be little mineralization (Millar and Baggs, 2004). Lignin is a very recalcitrant substance and does not break down easily, and polyphenols can bind with proteins and immobilize N (Gentile et al., 2008). Gentile et al. (2008) reported a maize residue content of 3.1% lignin and 1.1% polyphenol, and Nakhone and Tabatabai (2008) reported a soybean residue content of 6.07% lignin and 0.96% polyphenol. Both residues have low lignin and polyphenol contents according to the criteria (<15% lignin; <4% polyphenol) outlined by Gentile et al. (2008), and hence these compounds will have little influence on the mineralization and immobilization of these residues.

Higher soil temperatures generally result in greater mineralization of SOM N until a maximum temperature is reached, at which point the rate of mineralization decreases (Griffin, 2008). Although rates of mineralization decrease at lower temperatures, significant decomposition can still occur below temperatures of 5°C (Andersen and Jensen, 2001). Andersen and Jensen (2001) found that immobilization was more sensitive to low temperatures than mineralization. Similarly, higher soil water content results in greater mineralization until a maximum water content is reached (Griffin, 2008). Mineralization is also greater in sandy soils than in clayey or silty soils, as there is generally less organic C or N present (Griffin, 2008).

Although microbial immobilization of both NH_4^+ and NO_3^- does occur, NH_4^+ immobilization occurs to a greater extent than NO_3^- immobilization (Andersen, 1999; Recous et al., 1999; Rice and Tiedje, 1989). Rice and Tiedje (1989) found that the presence of NH_4^+, even at such low concentrations as 0.1 µg N g^{-1} $soil^{-1}$ would inhibit microbial NO_3^- immobilization immediately. At higher concentrations, they found that NH_4^+ inhibited NO_3^- immobilization by 80%. Similarly, Recous et al. (1999) found that NO_3^- immobilization was only 30% of the total immobilization of NH_4^+ and NO_3^-. Therefore, Rice and Tiedje (1989) concluded that microbial immobilization of NO_3^- is likely to be unimportant, unless the soil NH_4^+ supply has been depleted such that there are NH_4^+-free microsites available in the soil.

There is poor agreement between studies regarding the impact of growing plants on N mineralization and immobilization. Paré et al. (2000) reported that those studies which found that the presence of growing plants slowed mineralization credited it to plant and microbe competition for scarce N, and microbicides released by plant roots. The studies which found that plant growth increased N mineralization attributed it to the ability of plant roots to supply energy to the microbes for N mineralization purposes. The root systems of plants can also affect the MIT by stimulating or reducing residue decomposition through rhizodeposits, changing the root structure, or changing soil water availability (Gill, 2009; Myrold and Bottomley, 2008).

1.3 Soil quality

Soil quality has long been vaguely conceptualized by the biomass produced by the soil, but a quantitative definition has been difficult to achieve (Lal, 2003). Many definitions of soil quality have been proposed, and one which is widely accepted (Gil-Sotres et al., 2005) is that of Karlen et al. (1997), where soil quality is defined as: “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation.” This definition recognizes that the characteristics of soil quality depend on the function provided by the soil. Therefore, many researchers will adapt the term to encompass the soil function and characteristics being investigated through their specific research (Bastida et al., 2008). In agriculture, the soil function is to support crop growth and maximize yield. Agricultural soil quality is therefore often measured indirectly by measuring the crop yield: higher yields are associated with higher soil quality (Lal, 2003). To measure soil quality more directly, different fractions of OM are quantified, and a higher OM content is associated with higher soil quality (Bastida et al., 2008). The more labile fractions of the OM, such as light fraction organic matter (LF) and soil microbial biomass (SMB) are more sensitive to changes within an agro-ecosystem, but measurements of the SOC and N are also representative of the SOM.

SOM plays a very important role in the soil. It provides nutrients for plants and soil organisms, improves soil structural stability, buffers against pollution, and sequesters CO_2 (Gosling et al., 2013). The SOM content is affected by the quantity and quality of OM inputs to the soil, and the rate of break-down and mineralization of these inputs (Ryan et al., 2009).

The decomposition of OM is dependent on temperature, soil moisture content, soil texture, and tillage (Franzluebbers, 2002; Gosling et al., 2013).

1.3.1 Soil organic carbon and soil total nitrogen

Soil organic C contains the largest C stock in the world; it contains twice as much C as the atmosphere, and nearly thrice that of vegetation (Wang et al., 2011). The C content of native soils is in a dynamic equilibrium between inputs and losses of OM (Barrios et al., 1996). However, when cultivated, the OM losses are typically greater than the inputs, and the SOC consequently decreases (Costantini et al., 2006). The decrease in SOC varies according to soil management practices, but a global meta-analysis found that a loss of 20-50% of SOC within the top 20-30 cm of soil is typical in the first 30-50 years of cropping (Berhongaray et al., 2013). Even with best management practices, Studdert and Echeverría (2000) reported that agricultural soils can never achieve their original level of SOC, unless the original SOC level was very low. Nonetheless, managing the soil to attain the highest possible SOC levels is integral to maintaining soil quality, as SOC can improve soil structure, increase microbial activity, and impact nutrient cycling (Bell et al., 2012).

Soil organic C and soil total nitrogen (TN) are controlled by many factors influencing the addition and decomposition of OM (Jobbágy and Jackson, 2000). Climate can affect the production of biomass, and hence the amount of OM added to the soil, as well as the rate of mineralization (Berhongaray et al., 2013). Specific crop types and/or genotypes can be used to increase SOC, if chosen for their high biomass production and C/N ratio (Studdert and Echeverría, 2000). Root distribution affects the deposition of SOC throughout the soil; limited root distribution limits the SOC (Jobbágy and Jackson, 2000). Tillage can decrease the SOC and TN, as the added aeration increases the rate of decomposition (Studdert and Echeverría, 2000). Nitrogen fertilization can increase crop biomass production, and if crop residue is returned to the soil, it can subsequently increase the SOC and TN (Diekow et al., 2005).

1.3.2 Light fraction organic matter

The LF is one of the more labile fractions of the SOM. The LF is described as having a density between 1.3-1.8 g cm^{-3} (Gosling et al., 2013) and generally includes incompletely decomposed, recently added OM (Six et al., 2002) such as plant residues, seeds, fungal hyphae, and spores (Six et al., 2002; Wander, 2004). The proportion of C in LF in mineral soil generally ranges from 2-30%, and N from 1.5-10% (Wander, 2004). Similar to SOC, the quantity of LF depends on the processes which add OM and which result in its subsequent breakdown. The addition of OM, such as manure, compost, or crop residues, increases the LF content of the soil (Gosling et al., 2013). As the breakdown of OM is microbially mediated, the soil LF content is dependent on the climate (Wingeyer, 2007), with warmer climates decreasing the LF (Gosling et al., 2013). Tillage increases the soil aeration and the rate of degradation, thus decreasing the amount of LF (Wander, 2004).

The soil LF is one of one of the more sensitive indicators to changes in soil management (Heitkamp et al., 2011; Wander, 2004). Because the LF is not protected from microbial degradation by processes such as mineral sorption, it responds quickly to management changes (Gosling et al., 2013). For example, Matos et al. (2012) studied LF in plantations of black locust (*Robinia pseudoacacia* L.*)*, and found that the LF-C in the top 3 cm of soil more than doubled from year 2 to year 3, whereas the SOC only increased by approximately 10%. After 14 years, the LF-C was about 14 times greater and the SOC was about 7 times greater than in year 2. Although the LF-C responded more strongly to the plantation growth over the entire experimental period, the SOC responded slowly at first, and increased its response with time.

1.3.3 Soil microbial biomass

The SMB, like the soil LF, is one of the labile pools of the SOM, forming 1-3% of the total SOM (Kaur et al., 2000). The SMB is primarily composed of bacteria and fungi, but also includes microfauna and stored C and other nutrients (Wardle et al., 1992). The bacteria and fungi of the SMB are important mediators of the various nutrient cycles taking place within the soil (Shi et al., 2013) and are the main sources of CH_4 and N_2O (Treseder, 2008). This turnover of C and N is significant within the global carbon and nitrogen cycles, because the SMB contains approximately 1.4% and 2.8% of the global terrestrial soil C and N stocks, respectively (Wardle et al., 1992).

Within the soil, the SMB acts as both a source and a sink of nutrients (Granatstein et al., 1987; Kaur et al., 2000; Leite et al., 2010). By mediating biogeochemical transformations, the SMB determines the availability of key labile nutrients such as C, N, phosphorus (P), and sulphur (S) (Leite et al., 2010). The availability of these nutrients influences crop growth (Granatstein et al., 1987), and the quality and quantity of the crop residues subsequently returned to the soil impacts the SMB (Kaur et al., 2000).

The addition of OM to the soil increases the SMB. Because C is the limiting nutrient for microbial growth, the SMB is highly dependent on SOM (Kallenbach and Grandy, 2011). There are many management practices which can increase the SMB by influencing the SOM. Organic amendments such as manure or mulch biomass add both C and N, and increase the SMB significantly (Wardle et al., 1992) and rapidly (Jannoura et al., 2013). After harvest, leaving the stubble on the soil results in a greater SMB than if the stubble was removed (Lou et al., 2011). However, the greater microbial respiration from the higher SMB results in increased CO_2 emissions (Jannoura et al., 2013). Tillage immediately causes a large increase in microbial activity, but this is a temporary effect (Balota et al., 2003). If N is added in the form of mineral fertilizer and no C is applied, the SMB is reduced. Through a global meta-analysis of 82 field studies, Treseder (2008) found that the addition of mineral N fertilizer reduced SMB by an average of 15%.

Crop growth can increase the SMB through the process of rhizodeposition (Liang et al., 2011; Murphy et al., 2007). Plant roots continually deposit C and N to the soil, which can then be used by the SMB (Wardle et al., 1992). Approximately 17% of the photosynthate produced by a plant is released by the roots, and much of this is available to the microbial community (Mandal et al., 2007). The different rhizodeposits from each plant species thus result in a varied SMB (Li et al., 2010).

1.4 Sustainable agriculture

The goal of sustainability is to meet the needs of the present without compromising the ability of future generations to meet their own needs (Spiertz, 2010). At present, sufficient food is produced to meet the needs of the global population of 7 billion people, although the distribution of this food is highly inequitable (Garnett, 2013). However, by 2050, the world population is predicted to be 9 billion people (Spiertz, 2010) and per capita consumption will increase as incomes rise in the developing world (Vermeulen et al., 2012). Agricultural production will therefore have to increase to meet this higher demand. Currently, approximately 40-50% of the Earth's land area is being used for agricultural purposes (Smith, 2012), but land that is appropriate for agriculture is limited and can only be increased by about 180 million ha (Spiertz, 2010). Therefore, in order to meet future agricultural needs, further agricultural intensification will be needed (Vermeulen et al., 2012). To achieve this in a sustainable way requires that it is economically profitable, ethically sound, and improves or maintains environmental quality (Spiertz, 2010).

Conventional tillage practices violate the environmental quality premise of sustainable agriculture, as they reduce SOC and increase soil CO_2 emissions (Abdalla et al., 2013). In conventional tillage, any cultivation practices which leave less than 30% of crop residues on the soil surface such as the mouldboard plough, results in the inversion and redistribution of the top soil (0-20 cm) leading to oxidation and enhanced microbial activity. However, conservation tillage, where more than 30% of the crop residues remain on the soil surface, reduces soil disturbance and oxidation (Abdalla et al., 2013). Although conservation tillage reduces soil CO_2 emissions and does not impact CH_4 emissions, it increases N_2O emissions

(Abdalla et al., 2013; Powlson et al., 2011). By increasing the soil bulk density and water content, conservation tillage, including no-tillage, increases the rate of nitrification and hence the rate of N_2O emissions (Abdalla et al., 2013). Powlson et al. (2011) recommend further research to determine whether N_2O emissions from conservation tillage can be reduced, as the benefits to the SOM are otherwise so substantial.

Maintaining or increasing SOM not only improves soil quality, but it also has the largest potential for climate change mitigation in agriculture (Muller et al., 2012). Soil organic matter can be increased by increasing OM input rates and reducing the rate of OM decomposition by practices such as moving SOM storage deeper in the soil, and protecting the SOM by intra-aggregate or organomineral complexes (Guo and Gifford, 2002). In addition, covering the soil with crop residues provides protection from water and wind erosion, reduces water loss, increases soil biodiversity, and improves soil aggregate stability (Verhulst et al., 2010; Vermeulen et al., 2012). Because soils can store such large quantities of SOM, the potential for C sequestration is equally large, and practices which maintain SOM could therefore contribute significantly to climate change mitigation (Smith et al., 2007).

Soil N is generally the limiting nutrient in food crop systems in temperate soils. Mineral N fertilizers were developed to meet this need, and their current use is seven times greater than it was in the 1960s (Spiertz, 2010). Unfortunately, the use of N fertilizers is highly inefficient, and between 30-80% of N applied to soils leaches into groundwater, runs off into surface water, or is released into the atmosphere (Spiertz, 2010). For example, Skinner et al. (2014) reported that excess soil N results in N_2O emissions, however, reducing N application rates lowers emissions of N_2O. Therefore, it is important to develop sustainable practices which use N more efficiently. If mineral N fertilizer is used, the quantity and timing of its application should be appropriately managed (Powlson et al., 2011). The timing of the fertilizer application needs to be synchronized with the crop N requirements and the quantity such that it is quickly immobilized in the soil (Powlson et al., 2011). Organic N fertilizers, such as compost, manure, and crop residues are also used in sustainable agriculture. Nitrogen can also be added to the soil through the incorporation of different crops in rotation, including legumes, green manures, and cover crops (Spiertz, 2010).

1.4.1 Intercropping

Intercropping is a sustainable agricultural practice which involves the simultaneous growth of more than one crop on the same plot of land (Pappa et al., 2011). Intercropping can involve alternating rows of each crop across the field, the sowing of one crop under another as a groundcover, or planting crops in between rows of trees (tree-based intercropping). Competition is inherent in intercropping, and can occur in three ways: mutual inhibition, mutual cooperation, and compensation (Abaidoo and Van Kessel, 1989). Ideally, compensation can be achieved, where both crops benefit. Previous research has identified many benefits of intercropping, including greater soil productivity (Carruthers et al., 2000), higher yield (Abaidoo and Van Kessel, 1989; Hauggaard-Nielsen et al., 2001; Mbah et al., 2007), pest and disease reduction (Martin et al., 1990; Muoneke et al., 2007), lower input costs and higher monetary returns (Abaidoo and Van Kessel, 1989; Mbah et al., 2007), efficient use of resources (Mbah et al., 2007), improvements in soil fertility (Abaidoo and Van Kessel, 1989), and decreased soil erosion (Kolawole, 2012).

Investigating the impact of intercropping on crop yield was the most common, but results have not been uniform. In Nepal, Clement et al. (1992) found that both soybean and maize yields were lower in an intercrop than in sole crops, whereas Gao et al. (2010) and Keswani et al. (1977) found in China and Tanzania, respectively, that maize yields were greater and soybean yields were lower in an intercrop than in sole crops. However, comparing the land equivalent ratio (LER) rather than the yield presents a more conclusive outcome. Ahmed and Rao (1982) define LER as "the sum of the relative land areas required by sole crops to produce the same yields as obtained from intercropping." Therefore, an LER greater than one indicates that an intercrop requires less land to produce the same yield as the sole crops of the components. Ahmed and Rao (1982), Carruthers et al. (2000), Clement et al. (1992), and Gao et al. (2010) all found LERs greater than one for intercropping, indicating more conclusively the benefits of intercrop on yield.

Intercrops have been used as a traditional agricultural practice, especially in the humid tropics (Ahmed and Rao, 1982). Farmers in this region have appreciated the many benefits associated with intercrops, particularly the reduced risk of crop failure that comes from growing diverse crops (Ahmed and Rao, 1982). In the temperate zone, a common intercrop has been the integration of corn and soybean, which have been commercially grown together in North America since the early 1900s (Martin et al., 1990). Although agricultural development has replaced intercropping with sole cropping, this exchange has occurred less frequently than predicted due to the many benefits of intercropping (Ahmed and Rao, 1982). Regardless, intercropping is much less common than it used to be, as the highly mechanized and fertilized modern agricultural paradigm has overtaken such traditional practices, which are viewed as irrelevant (Hauggaard-Nielsen et al., 2001).

Intercropping can be especially beneficial when a legume and non-legume, often a cereal, are planted together. Because legumes can assimilate N, there is reduced competition for soil N, and the increased residual N is available for a subsequent crop (Searle et al., 1981). Cereals tend to be more competitive than legumes for soil inorganic N, indicating that when cereals and legumes are intercropped, the cereal wins the soil N and forces the legume to rely more heavily on N_2 fixation (Hauggaard-Nielsen et al., 2001).

1.5 Historical and regional context of agriculture in Argentina

Until the late 19th century, the 52 million ha Pampa region of Argentina (Austin et al., 2006) was primarily native grassland, until colonization programs resulted in a transition toward cereal and oil crops, and dryland cattle production (Viglizzo and Frank, 2006; Viglizzo et al., 2003). Unsuitable cultivation practices, low rainfall, and high wind speed and frequency created a dust bowl effect during the 1930s and 1940s leading to severe erosion, culminating in high migration away from farming in the Pampas (Viglizzo and Frank, 2006). Reduced anthropogenic pressure on the environment and a return to normal precipitation and wind patterns enabled the lands to recover and hence prompted a return of grazing and cropland use in the 1960s (Viglizzo and Frank, 2006). Currently, most of the Pampas are used for mixed grain crop-livestock production (Viglizzo et al., 2003) and only around 25% of the land is uncultivated (Viglizzo and Frank, 2006). However, recent rapid agricultural expansion and intensification resulting in a greater national production of cereals and oil seed crops (Caviglia and Andrade, 2010) will continue to accelerate over the next 50 years (Austin et al., 2006).

Argentina, particularly the Pampas region, is now considered a major global agricultural region (Austin et al., 2006). Predominant crops include soybean (*Glycine max* (L.) Merr.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and sunflower (*Helianthus annuus* L.) (Austin et al., 2006; Viglizzo et al., 2003). However, the resurgence in agricultural activity is again deteriorating soil quality, increasing soil erosion and nutrient deficiency (Studdert and Echeverría, 2000) indicating that future agricultural practices must change in order to maintain high crop yields and reduce negative environmental impacts.

1.6 Research objectives

Currently intercropping is investigated in Argentina as a more sustainable agricultural practice, and includes intercrops of soybean with maize, sunflower, or wheat (e.g. Caviglia et al., 2010; Dyer et al., 2012; Echarte et al., 2011; Oelbermann and Echarte, 2011; Vachon and Oelbermann, 2011). Few investigations of intercropping in temperate environments have been conducted (Hauggaard-Nielsen et al., 2001), and intercropping research in Argentina is still in its infancy (Oelbermann and Echarte, 2011). Three seasons of intercropping maize and soybean resulted in increased carbon input to the soil (Dyer et al., 2012; Vachon and Oelbermann, 2011), decreased N mineralization (Vachon and Oelbermann, 2011), and decreased soil emissions of CO_2 and N_2O (Dyer et al., 2012) as compared to sole crops of maize and soybean. However, a minimum period of five years is needed in the Argentine Pampa to determine measurable differences in SOC (Alvarez et al., 1998), which were not evident in these earlier short-term studies. In addition, although intercropping decreased soil N_2O emissions, there is still a lack of understanding of the soil N transformations which caused this decrease. This study will help to meet these knowledge gaps through answering the following research question:

> How does maize/soybean intercropping impact soil quality as indicated by the SOC, TN, LF, and SMB, and gross N mineralization and immobilization rates?

The objectives therefore were:

1. To quantify the temporal changes in soil quality, as indicated by the SOC, TN, LF, and SMB, after six years of maize/soybean intercropping.
2. To quantify the soil gross N mineralization and immobilization rates from two maize/soybean intercropping systems, and two sole crop rotations of maize and soybean.

It was hypothesized that:

(1) The intercrops will demonstrate stronger positive changes in soil quality compared to the baseline data and the sole crops.

H_0: There will be no difference in soil quality between the intercrops, sole crops, and baseline data.

(2) The rate of gross N mineralization will be greatest in the soybean sole crops, and least in the maize sole crops, and vice versa for gross N immobilization. The rates of gross N mineralization and immobilization in the intercropping systems will fall in between those of sole soybean and maize.

H_0: There will be no difference in N mineralization and immobilization rates between the intercrops and sole crops.

Chapter 2: Study site location and experimental design

The research site was located at the Instituto Nacional de Tecnología Agropecuaria (INTA) in the rolling Pampas near Balcarce, Argentina (37°45’55’’S, 58°18’11’’W) at an elevation of 130 m above sea level (Figure 2.1). The Balcarce climate can be described by the Köpen classification as temperate humid without a dry season (Domínguez et al., 2009) or by the Thornthwaite classification as mesothermal subhumid-humid (Domínguez et al., 2009; Fabrizzi et al., 2003). Average temperatures during the growing season are low, and there is only a frost-free period of approximately 150 days (Andrade, 1995). About 80% of the annual precipitation occurs during the spring-summer growing season (Fabrizzi et al., 2003). From 1980-2010, annual precipitation was 960 mm and the mean air temperature was 13.9°C (Dyer, 2010) and the annual median potential evapotranspiration was 894 mm (Domínguez et al., 2009) (Figure 2.2).

The soil is characterized by a mixture of fine, mixed, thermic Luvic Phaozems of the *Mar del Plata* series and a fine, mixed or illitic, thermic Chernozemic Loam of the *Balcarce* series (Domínguez et al., 2009; Studdert and Echeverría, 2000). In the Petrocalcic Paleudoll soil, the petrocalcic horizon is below 0.7 m (Domínguez et al., 2009) which restricts root development (Coll et al., 2012). Both series have similar characteristics in the surface horizon, where the soil texture is loamy with approximately 41% sand, 36% silt, and 23% clay (Domínguez et al., 2009). The soil is moderately acid, has low available phosphorus, and a high content of organic carbon (Fabrizzi et al., 2003). The slope is 2%, indicating little to no erosion (Domínguez et al., 2009; Studdert and Echeverría, 2000).

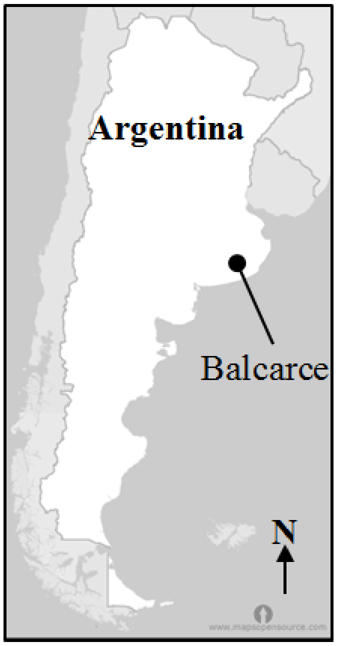

Figure 2.1 – Map of Argentina, showing location of study site at Balcarce. (http://www.mapsopensource.com/argentina-outline-map-black-and-white.html)

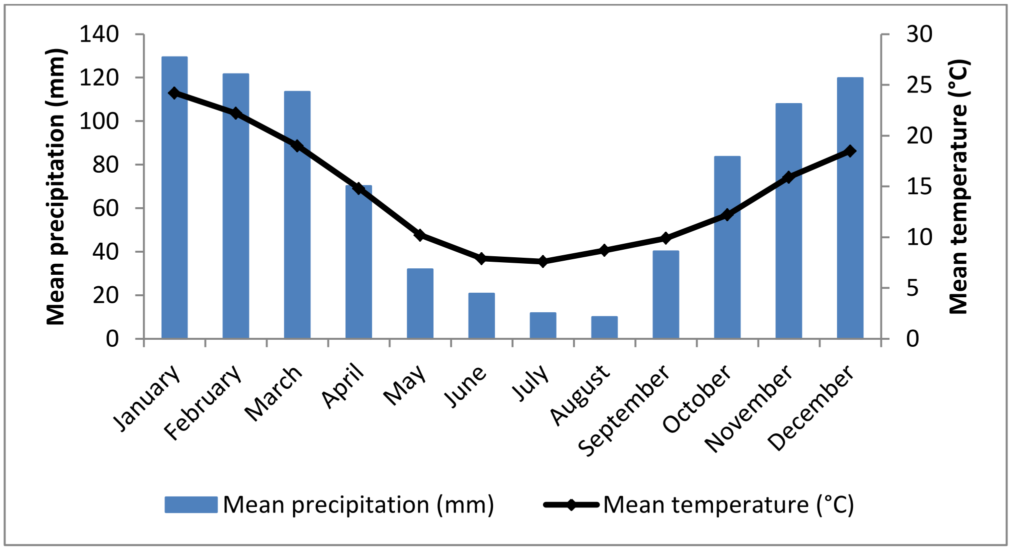

Figure 2.2 – Mean monthly temperature and precipitation (1980-2010) at the Instituto Nacional de Tecnología Agropecuaria (INTA) (adapted from Dyer, 2010).

The experimental plots were arranged in a randomized complete block design (RCBD) with three replicates of four treatments (Figure 2.3). The four treatments included: two annual rotations of sole cropped maize and soybean, one begun with maize in 2007, and one begun with soybean in 2007, 1:2 intercrop (1 row of maize and 2 rows of soybeans), and 2:3 intercrop (2 rows of maize and 3 rows of soybeans). These intercrop configurations are typical of those used in the region. The experimental plots were established in October 2007 on land that had previously been under sunflower (*Helianthus annuus* L.) production, and have been managed with conventional tillage. Nitrogen fertilizer was applied only to the maize plants at 150 kg N ha^{-1}, phosphorus (P) fertilizer was applied to all crops at 35 kg P ha^{-1}, soybeans were inoculated with *Bradyrhizobium japonicum*, and weeds were managed by N-phosphonomethyl glycine (Glyphosate). Maize was seeded in October and harvested in April, and soybeans were seeded in November and harvested in May. The maize density was 4.3 plants m^{-2} in the 1:2 intercrop, 5.3 plants m^{-2} in the 2:3 intercrop, and 8.0 plants m^{-2} in the maize sole crop. In all treatments, the soybean density was 29 plants m^{-2}. Inter-row distance was 0.52 m in all treatments. In the sole crops, the maize intra-row distance was 0.24 m and the soybean intra-row distance was 0.065 m. In the intercrops, the maize intra-row distance was 0.145 m and the soybean intra-row distance was 0.04 m.

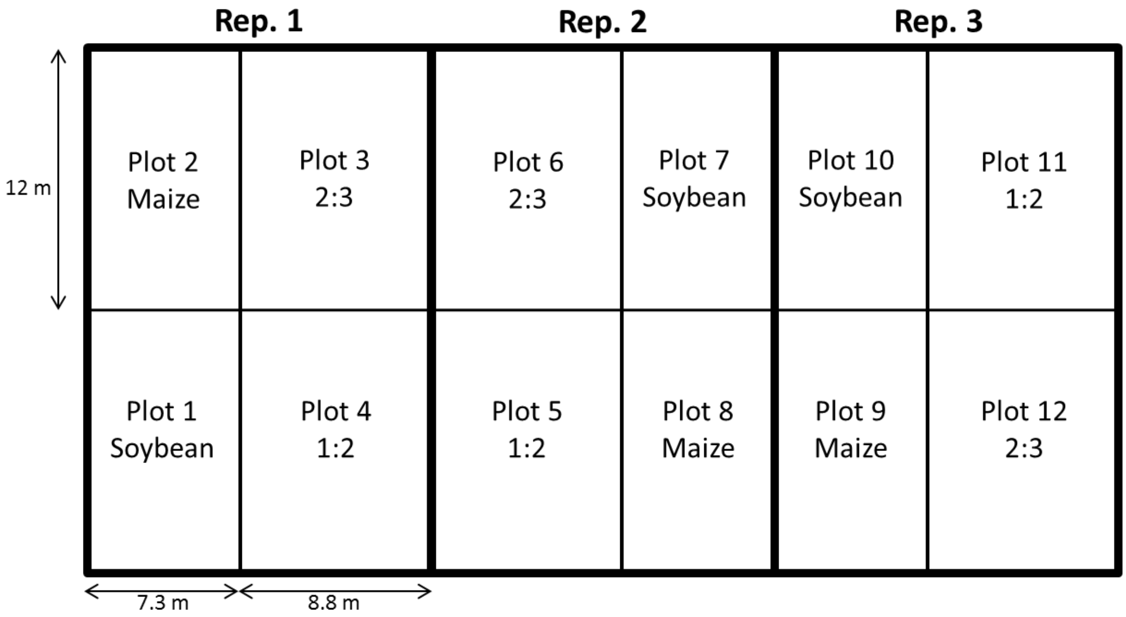

Figure 2.3 – RCBD of the experimental plots, showing three replications of four treatments in the 2012-2013 cropping season. Ratios indicate rows of maize to rows of soybeans.

Chapter 3: Effects of intercropping on soil quality characteristics

3.1 Introduction

Soil is fundamental to plant growth, and therefore it is important to maintain high soil quality for the production of food, fibre, and fuel, the three primary traditional uses of soil (Lal, 2003). In agriculture, there are three primary components to soil quality, including physical, chemical, and biological characteristics (Lal, 2003). Each component is impacted by a variety of attributes, or indicators (Table 3.1), many of which may be influenced by management practices. It is not feasible to analyze the soil for each of these indicators, so a subset must be selected (Gil-Sotres et al., 2005). This indicator subset should be composed of attributes which are highly sensitive to changes within the soil system, generally biological and biochemical attributes which respond to even minimal changes (Gil-Sotres et al., 2005). Various fractions of organic matter are used most frequently as indicators of soil quality, and soil microbial biomass carbon (SMB-C) is the most common of these (Bastida et al., 2008; Gil-Sotres et al., 2005; Lal, 2003). Additional indicators of physical, chemical, and biological soil characteristics are also required (Gil-Sotres et al., 2005), as SMB-C may vary among soils of similar quality (Bastida et al., 2008).

Conventional agriculture generally reduces soil quality (Lal, 2003). In these systems, organic matter (OM) inputs are low because much of the crop biomass is removed and tillage practices increase OM decomposition due to higher soil temperatures and increased aeration (Berhongaray et al., 2013; Costantini et al., 2006). This can result in losses of 30-50% of soil organic carbon (SOC) from the upper 20-30 cm of soil, whether in temperate or tropical ecosystems (Berhongaray et al., 2013). Agriculture also reduces the microbial biomass due to smaller carbon inputs and SOC content (Kallenbach and Grandy, 2011). Agricultural soil quality can be improved, primarily through two mechanisms, as outlined by Lal (2003). Firstly, the degraded soil can be converted to a non-agricultural land use, such as forestry. Secondly, crop management practices, particularly those impacting the soil organic matter (SOM), can be improved to reduce erosion, improve the soil structure, increase SOC retention, and enhance nutrient cycling.

Table 3.1 – Attributes of physical, chemical, and biological characteristics relating to soil quality (adapted from Lal, 2003).

	Attributes
Physical Quality	Texture, structure, porosity, and pore size distribution, available water holding capacity, infiltration capacity, internal drainage, effective rooting depth, and soil temperature
Chemical Quality	pH, cation exchange capacity (CEC)/anion exchange capacity (AEC), nutrient retention and availability, toxicity of some elements, and high concentration of soluble salts
Biological Quality	Soil organic carbon (SOC) and soil microbial biomass carbon (SMB) contents, soil respiration, microbial activity, and species diversity of soil fauna and flora

Soil organic matter is one of the most important components of soil due to its many varying roles. Soil organic matter provides nutrients for plants and soil organisms, promotes nutrient sorption, increases soil structural stability, buffers the soil from environmental pollution, and sequesters atmospheric CO_2 (Gosling et al., 2013). Organic matter inputs to the soil determine the makeup of the SOM (Ryan et al., 2009) which can include soil organisms, plant residues, animal fragments, and soluble compounds formed from microbially-mediated decay processes (Gosling et al., 2013). The proportion of each of these SOM components varies according to the soil and its management processes (Gosling et al., 2013). Therefore, as each component of SOM performs a different role in the soil, management practices can change the relative importance of each of these components and ideally increase the soil quality.

Each component of the SOM acts on a different time scale. Since the active, or labile, SOM has a half-life of days to a few years (Wander, 2004), it is of the greatest importance in managing agroecosystems (Gosling et al., 2013). The active SOM can include the soil microbial biomass (SMB), chloroform-labile SOM, and other labile substrates such as plant residues and nonaggregate protected particulate organic matter (POM) (Wander, 2004). Slow SOM and recalcitrant SOM have half-lives ranging from a few years to centuries, and thus are less able to indicate changes in soil quality (Wander, 2004).

Light fraction soil organic matter (LF) and particulate organic matter (POM) are components of the active SOM. Soil LF and POM are conceptually similar, sharing similar characteristics as they both originate from recent, partly decomposed plant residues (Six et al., 2002). The soil LF is distinguished by its density, and POM by its size, and both encompass larger, lighter particles (Gosling et al., 2013). As they are derived from recent plant residues, they are among the fastest SOM fractions to respond to changes in soil management practices (Gosling et al., 2013). Due to these similarities, it is redundant to measure both LF and POM when studying agricultural soil quality; LF is typically measured when studying the impact of management practices on biologically available C or N (Wander, 2004).

Soil microbial biomass (SMB) is another component of the active SOM which is highly sensitive to changes in soil management (Granatstein et al., 1987; Kallenbach and Grandy, 2011). Both a source and a sink for labile nutrients (Leite et al., 2010), SMB has a short turnover time (Kallenbach and Grandy, 2011) which therefore promotes a fast response to changes in soil quality. Soil microbial biomass is particularly important in its central role in the decomposition of OM, as it regulates nutrient cycling by immobilizing and then releasing labile nutrients, making them available to other soil biota (Wardle, 1998).

Despite the negative impacts of agriculture, levels of SOM can be maintained or increased through a variety of management practices, such as: enhancing the quantities of crop residue returned to the soil, increasing the C/N ratio of these crop residues, adding organic or mineral fertilizers, and reducing tillage intensity (Studdert and Echeverría, 2000). These practices can alter the balance between mineralization and immobilization rates, influencing levels of SOM. Intercropping is an agroecosystems management practice which may help to increase the level of SOM, and thus soil quality, by increasing the crop residue returned to the soil, and increasing the C/N ratio of these residues. Due to a more efficient use of soil resources, intercropping can increase biomass production when compared to a sole crop (Baldé et al., 2011). Additionally, the combination of residues from a cereal (maize) and a legume (soybean) in an intercropping system will increase the C/N ratio as compared to a sole crop of soybean, although the C/N ratio in the intercrop will be lower than that of a sole crop of maize.

Although intercropping may reduce the yield of each intercropped species as compared to the corresponding sole crops, the land equivalent ratio (LER) is generally used for a more thorough comparison (Muoneke et al., 2007). The land equivalent ratio (LER) is a useful method for conceptualizing the land area that would be required by sole crops to obtain the same yield (i.e. grain yield, or plant biomass) as that obtained by an intercrop (Vandermeer, 1989). An LER greater than one indicates that the intercrop can result in higher yields than a comparable land area that is sole cropped by the same species. All previous studies on intercropping maize and soybean (Table 3.2) found an LER greater than 1, regardless of the study location, intercrop design, soil texture, or soil quality, indicating that biomass production in these intercrops has been consistently greater than in the corresponding sole crops. These previous studies have focussed primarily on the impacts of intercropping on yield (e.g. Carruthers et al., 2000; Martin et al., 1998) and root dynamics (Bethlenfalvay et al., 1991; Gao et al., 2010; Keswani et al., 1977). Of the surveyed studies, only Searle et al. (1981) investigated soil properties in a maize/soybean intercrop, and they only measured exchangeable soil nitrogen. Therefore, there is a clear gap in the literature regarding the impact of maize and soybean intercropping on soil quality. By using SOC, total nitrogen (TN), LF and SMB-C and N as indicators of soil quality, an experiment was carried out to determine the impact of maize and soybean intercropping on soil quality, as compared to a maize and soybean sole crop rotation. The objectives of this research were:

1. To quantify the LER and crop biomass production between the 1:2 and 2:3 maize/soybean intercrops and the rotated maize and soybean sole crops.
2. To quantify differences in SOC, TN, LF, and SMB-C between the 1:2 and 2:3 maize/soybean intercrops and the rotated maize and soybean sole crops.
3. To quantify and compare changes in LER, SOC, TN, LF, and SMB-C that have occurred over a 6 year period (2007 – 2013) in the 1:2 and 2:3 maize/soybean intercrops and the rotated maize and soybean sole crops.

Table 3.2 – Intercrop layout and land equivalent ratio in previous intercropping systems of maize and soybean

Source	Intercrop ratio*	Sole crop interrow spacing (cm)	Intercrop interrow spacing (cm)**	LER	Study location
Ahmed and Rao (1982)	1:2	M – 100 S – 50	25	1.62	China, India, Philippines, Sri Lanka, Thailand, Hawaii (USA), Australia
Carruthers et al. (2000)	1:1 1:2	M – 75 S – 37.5	37.5	1.02 1.04	Quebec (Canada)
Clement et al. (1992)	1:2 2:3	M – 80 S – 40	25 50	1.08 1.01	Nepal
Gao et al. (2010)	1:3	M – 50 S – 30	30	1.31	China
Kolawole (2012)	2:2	M – 100 S – 50	25	1.78	Nigeria
Martin et al. (1998)	1:1	M – 76 S – 38	38	1.14	Nova Scotia, New Brunswick (Canada)
Mbah et al. (2007)	1:1	M – 75 S – 75	37.5	1.31	Nigeria
Muoneke et al. (2007)	1:1	M – 75 S – 75	37.5	1.32	Nigeria
Searle et al. (1981)	1:2	M – 100 S – 50	25	1.36	Australia
Undie et al. (2012)	1:1 1:2 2:2	M – 75 S – 75	37.5 25 37.5	1.45 1.53 1.58	Nigeria

* Ratio of rows of maize (M) to rows of soybean (S)

** Distance between nearest rows of maize and soybean

3.2 Materials and methods

3.2.1 Experimental design

Three random locations were chosen for sampling the soil profile from each replicate plot within each treatment. Soil was collected from plots 8-12 on December 5, 2012, and from plots 1-7 on December 12, 2012 (Figure 2.3). Soil was sampled from five depth increments: 0-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, and 60-80 cm. Soil from each depth increment from the three sampling locations was combined to form one sample per depth per plot. The samples were then air-dried and sieved (2mm).

3.2.2 Crop biomass production and land equivalent ratio

Shoot biomass was determined for each of the experiment plots at the time of harvest. Three 1 m^2 areas were sampled in each replicate per treatment, and the above-ground crop residue was dried for one week at 60°C, weighed and averaged after removal of cobs and pods. The LER was then calculated based on the dried relative shoot weights in the intercrops and sole crops:

$$LER = \frac{soy\ biomass\ (intercrop)}{soy\ biomass\ (sole\ crop)} + \frac{maize\ biomass\ (intercrop)}{maize\ biomass\ (sole\ crop)} \qquad \text{(Equation 3.1)}$$

3.2.3 Soil physical and chemical characteristics

Equipment restrictions prevented the collection of soil for bulk density (BD) in December 2012. Therefore, values of soil BD from soil samples collected between May 6-12, 2012 within the same treatments and replications were used to quantify bulk density. Soil BD was evaluated by extracting soil samples with a soil corer of 5 cm inner diameter. The collected soil samples were oven-dried for 24 hours at 105°C and weighed. Bulk density was calculated using the oven dry weight, the inner diameter of the corer, and the respective segment depth of each soil sample. To prepare the air-dried and sieved (2 mm) soil samples for analysis of soil SOC and TN, carbonates were first removed by acid washing according to Dyer et al. (2012) and Midwood and Boutton (1998). A 50 ml aliquot of 0.5 M HCl was added to 2 g of soil, and was shaken three times (Heidolph Unimax 1010 DT, Schwabach, Germany) at approximately 350 rpm for 10 minutes each over 24 hours. Once the soil settled, it was washed by pipetting the acid from the soil, and adding 50 ml ultrapure water. The soil was washed daily for four days, at which point it was dried at 40°C for 2 days. Once dry, the soil was ground in a ball mill (Retsch® ZM1, Haan, Germany) to 250 µm. Subsamples of 12-20 mg were weighed into tin capsules (Costech, 5x9 mm), and analyzed for SOC and TN concentrations (%) using an elemental analyzer (Costech 4010, Cernusco, Italy). From these results, the C/N ratio, SOC stock, and TN stock (g m^{-2}) were calculated. Soil organic carbon and TN stocks were calculated using the bulk density, SOC, or TN concentrations to a depth of 60 cm based on a 10 cm depth increment. Prior to removal of soil carbonates, soil pH was quantified using a 1:1 soil:water suspension (accumet AB15, Singapore).

3.2.4 Soil light fraction carbon and nitrogen

Soil organic matter light fraction (LF) was determined according to Gregorich and Beare (2008) for soil collected at 0-10 cm and 10-20 cm. For each soil sample, 25 g of air-dried and sieved (2 mm) soil was shaken with 50 ml of NaI solution with a specific gravity of 1.7. After standing for 48 hours, the LF was vacuumed off the surface of the NaI, and rinsed with 75 ml of 0.01 M $CaCl_2$ and 75 ml of distilled water to eliminate any NaI residue. The recovered LF was dried at 60°C for 48 hours and ground in a ball mill (Retsch® ZM1, Haan, Germany) to 250 µm. Subsamples of approximately 5 mg were weighed into tin capsules (Costech, 5x9 mm), and analyzed for SOC and TN concentration (%) using an elemental analyzer (Costech 4010, Cernusco, Italy). The total LF (mg LF/g soil), percent nitrogen in the LF (LF-N), percent carbon in the LF (LF-C), C/N ratio (LF-C/N), proportion of LF-C in the SOC (LF-C/SOC), proportion of LF-N in the TN (LF-N/TN), the LF-C stock, and the LF-N stock (g m^{-2}) were then calculated for the depth intervals of 0-10 cm and 10-20 cm.

3.2.5 Soil microbial biomass carbon

Soil microbial biomass-C was determined according to Voroney et al. (2008). Air-dried and sieved (2mm) soil samples were incubated for 7 days at 25°C and 45% water-holding capacity. Approximately 25 g of the moist soil was weighed into a 100 ml glass bottle and placed in a desiccator containing 50 ml $CHCL_3$ and boiling chips. The desiccator was evacuated for 1-2 minutes after the $CHCl_3$ boiled vigorously, and was then sealed and stored in the dark for 24 hours at room temperature. Residual $CHCl_3$ vapour was subsequently removed by three evacuations of 5 minutes each, and one evacuation of 20 minutes. Once removed from the desiccator, 50 ml of 0.5 M K_2SO_4 was added to the sample and shaken for 1 hour (Heidolph Unimax 1010 DT, Schwabach, Germany) at approximately 350 rpm. After shaking, the soil suspension was filtered through Whatman GF 934-AH filter paper. The filtrate was then freeze-dried (Mandel ModulyoD-115, Ashville, NC), and 25-40 mg weighed into tin capsules

(Costech, 5x9 mm) for analysis using an elemental analyzer (Costech 4010, Cernusco, Italy). SMB-C was calculated as follows:

$$WS = \frac{wet\ weight\ -\ dry\ weight}{dry\ weight} \times 100$$ Equation 3.2

$$MS\ = \frac{wet\ weight\ \times 100}{100 + WS}$$ Equation 3.3

$$VS = \frac{wet\ weight - dry\ weight + extractant\ volume}{1\ g/mL}$$ Equation 3.4

$$C_F, C_U = organic\ C\ \times \frac{VS}{MS}$$ Equation 3.5

$$SMB - C = \frac{C_F - C_U}{K_{EC}}$$ Equation 3.6

where WS is soil water content expressed on an oven-dry basis (%); soil weights are expressed in g; MS is the weight of the subsample taken for CFE labile C analysis (g); VS is the total volume of solution in the extracted soil (ml); C_F is the total weight of extractable C in the fumigated sample (µg g^{-1} soil); C_U is the total weight of extractable C in the unfumigated sample (µg g^{-1} soil); organic C is the content of C present in the soil extract (µg ml^{-1}); K_{EC} is the conversion factor, which for C was 0.35 (Voroney et al., 2008).

Soil microbial biomass nitrogen (SMB-N) was similarly calculated in 2007, using nitrogen values and a conversion factor K_{EN} of 0.5 (Voroney et al., 2008). In 2012, SMB-N was unable to be calculated as only the N_F values (total weight of extractable N in the fumigated sample in µg g^{-1} soil) were obtained. The 2007 SMB-N and 2012 N_F results are presented in Appendix B.

3.2.6 Statistical analysis

Statistical analyses were carried out using Statistica (StatSoft, Inc.; v.8.0, 2007). All data were examined for normal distribution using the Shapiro-Wilk (SW) test and for homogeneity of variance using the Levene test and the Hartley F-max, Cochran C, and Bartlett Chi-square tests. To achieve normality and/or homogeneity of variance, the following data transformations were conducted:

- Homogeneity of variance could not be achieved for BD, so BD was analyzed using nonparametric measures. A Kruskal-Wallis (K-W) ANOVA was therefore used.
- SOC and TN were found to be bimodally distributed. The first population was from the 0-40 cm depth increments, and the second population from the 40-80 cm depth increments. Within these populations, the data were normally distributed with homogeneity of variance.
- C/N ratio was found to be bimodally distributed. The first population was from the 0-60 cm depth increments, and the second population from the 60-80 cm depth increments. Within these populations, the data were normally distributed with homogeneity of variance.
- SOC stock and N stock were normalized by being squared.

A general linear model (two-way analysis of variance [ANOVA]) was run to compare differences between the different depths and treatments. When significant main effects or interactions were identified from the ANOVA, a Tukey's post-hoc multiple comparisons test was used to identify the differences with a p-value of 0.05.

3.2.7 Baseline data comparison

The intercropping system was established in the spring of 2007 (October and November). Oelbermann and Echarte (2011) analyzed soil samples collected in October 2007 after the first growing season to establish a baseline data set with which to compare future soil analyses. Soil samples were analyzed from the depth intervals of 0-10 cm, 10-20 cm, 20-40 cm, and 40-80 cm. The 2012 results from the depth intervals of 40-60 cm and 60-80 cm were averaged to represent the depth interval of 40-80 cm to enable direct comparisons to the 2007 data. To compare the 2007 and 2012 data, a two-way repeated measures ANOVA was run after examining the data for normal distribution using the Shapiro-Wilk (SW) test and for homogeneity of variance using the Levene test and the Hartley F-max, Cochran C, and Bartlett Chi-square tests. To achieve normality and/or homogeneity of variance, the following data transformations were conducted:

- Although 2007 BD was normally distributed with homogeneity of variance, 2012 BD was not. Therefore, comparisons of the grand mean were made using Friedman's ANOVA and Kendall's Coefficient of Concordance.
- As SOC, TN and C/N ratio were bimodally distributed in 2007 and 2012, they were separated into two populations of 0-40cm, and 40-80cm which were normally distributed with homogeneity of variance in both populations.
- 2007 C and N stock were bimodally distributed, while the 2012 C and N stock were negatively skewed. To best achieve normality and homogeneity of variance, the C and N stock were divided into two populations, from 0-40 cm, and 40-80 cm. Each population was normally distributed with homogeneity of variance.

- 2007 and 2012 SMB-C were normalized by taking the square root.

Following the repeated measures ANOVA, any significant main effects or interactions were further examined using a Tukey's post-hoc multiple comparisons test with a p-value of 0.05.

3.3 Results

3.3.1 Soil quality from samples collected in 2012

The shoot biomass was greatest in the sole crop maize plots and least in the sole crop soybean plots (Table 3.3). However, as the sole crops were rotated annually, the average shoot biomass produced over the six cropping seasons was similar for each of the sole crops, and slightly higher in the 1:2 and 2:3 intercrops. With the exception of the first cropping season (2007-2008), the biomass produced was greater in the 2:3 intercrop than the 1:2 intercrop. With the exception of 2011-2012, the biomass produced in each intercrop increased during the period from 2007 through 2013. Nonetheless, the LER for both intercrops was low, ranging from 0.82 in the 1:2 intercrop in 2012-2013 to a high of 1.22 in the 2:3 intercrop in 2009-2010 (Table 3.3).

Table 3.3 – Shoot biomass and land equivalent ratio of sole cropped and intercropped soybean and maize over six cropping seasons from 2007-2013.

Year	Shoot Biomass ($g\ m^{-2}$)				LER	
	Soybean*	**Maize****	**1:2**	**2:3**	**1:2**	**2:3**
2007-2008	2532	1104	2069	1993	1.04	1.02
2008-2009	682	2187	1477	1674	0.85	0.94
2009-2010	2405	569	1675	1783	1.17	1.22
2010-2011	1189	2442	1766	1829	0.89	0.92
2011-2012	1162	736	1229	1295	1.14	1.20
2012-2013	665	2693	1914	2074	0.82	0.93
Mean	**1439**	**1622**	**1688**	**1775**	**0.99**	**1.04**

* These treatment plots were planted with soybean in the 2012-2013 cropping season.
** These treatment plots were planted with maize in the 2012-2013 cropping season.

No significant differences were found between treatments for bulk density or pH (Table 3.4). Bulk density tended to increase with depth in each of the four treatments, but this increase was not significant. Interaction effects of treatment by depth were not significant for either bulk density or pH. The pH increased significantly with depth in all four treatments. When considering the entire depth interval (0-80 cm), the pH was highest in the 2:3 intercrop and lowest in the soybean sole crop, although not significantly different.

The SOC and soil TN stock were only quantified to a depth of 60 cm, as bulk density could not be measured at greater depths. Interaction effects of treatment by depth were not significant for SOC or TN stock. Although SOC and TN stock did not differ significantly between treatments, it was observed that both SOC and soil TN stocks were lowest in the maize sole crop and highest in the soybean sole crop (Table 3.6). The SOC and soil TN stocks were both greater in the 2:3 intercrop than in the 1:2 intercrop. SOC and N stock both increased slightly from 0-10 cm to 10-20 cm, and then decreased significantly with depth.

Table 3.4 – Soil bulk density and pH in maize and soybean sole crops and 1:2 and 2:3 intercrops, 2012. Standard errors are given in parentheses.

	Depth (cm)	**Treatment**			
		Soybean	**Maize**	**1:2**	**2:3**
Bulk Density (g cm^{-3})	0-10	1.33 (0.05)^{A,a}	1.27 (0.06) ^{A,a}	1.33 (0.05) ^{A,a}	1.27 (0.06) ^{A,a}
	10-20	1.53 (0.03) ^{A,a}	1.46 (0.04) ^{A,a}	1.47 (0.04) ^{A,a}	1.36 (0.05) ^{A,a}
	20-40	1.48 (0.03) ^{A,a}	1.44 (0.03) ^{A,a}	1.53 (0.03) ^{A,a}	1.53 (0.02) ^{A,a}
	40-60	1.53 (0.03) ^{A,a}	1.34 (0.05) ^{A,a}	1.50 (0.04) ^{A,a}	1.52 (0.03) ^{A,a}
	0-60 mean	**1.47 (0.02) ^{A}**	**1.38 (0.02) ^{A}**	**1.46 (0.02) ^{A}**	**1.43 (0.02) ^{A}**
pH	0-10	5.32 (0.12) ^{A,a}	5.64 (0.12) ^{A,a}	5.56 (0.12) ^{A,a}	5.52 (0.12) ^{A,a}
	10-20	5.55 (0.12) ^{A,ab}	5.74 (0.12) ^{A,ac}	5.70 (0.11) ^{A,ab}	5.85 (0.11) ^{A,ab}
	20-40	5.83 (0.12) ^{A,abc}	5.86 (0.12) ^{A,ac}	5.83 (0.12) ^{A,abc}	5.92 (0.11) ^{A,abc}
	40-60	6.21 (0.12) ^{A,bc}	6.13 (0.12) ^{A,ac}	6.23 (0.12) ^{A,bc}	6.32 (0.12) ^{A,bc}
	60-80	6.35 (0.12) ^{A,c}	6.35 (0.12) ^{A,bc}	6.35 (0.12) ^{A,c}	6.46 (0.11) ^{A,c}
	0-80 mean	**5.85 (0.06) ^{A}**	**5.94 (0.06) ^{A}**	**5.94 (0.05) ^{A}**	**6.01 (0.05) ^{A}**

Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$. Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$.

Soil organic C and TN were found to be bi-modally distributed with depth, and hence were analyzed in two depth increments: 0-40 cm and 40-80 cm. In both depth increments, interaction effects of treatment by depth were not significant. SOC and TN were significantly ($p=0.0001$) different between these two depth increments, although within each increment, no significant differences were found with depth or treatment (Table 3.5). At both depth increments, the SOC and TN were slightly greater in the 2:3 intercrop than in the 1:2 intercrop. In general, SOC and TN declined with depth within each treatment. The C/N ratio was also bi-modally distributed, and was hence separated into two depth increments from 0-60 cm and from 60-80 cm (Table 3.5). In both depth increments, interaction effects of treatment by depth were not significant. The C/N ratio was significantly ($p=0.0001$) lower in the 60-80 cm increment than in the 0-60 cm depth increment.

Table 3.5 – SOC and TN concentrations (0-40 cm; 40-80 cm), and C/N ratio (0-60 cm; 60-80 cm) in maize and soybean sole crops and 1:2 and 2:3 intercrops, 2012. Standard errors are given in parentheses.

	Depth (cm)	**Treatment**			
		Soybean	**Maize**	**1:2**	**2:3**
SOC (%)	0-10	3.29 (0.19) ^{A,a}	3.06 (0.19) ^{A,a}	3.09 (0.19) ^{A,a}	3.19 (0.19) ^{A,a}
	10-20	3.17 (0.19) ^{A,a}	2.86 (0.19) ^{A,a}	3.00 (0.17) ^{A,a}	3.11 (0.17) ^{A,a}
	20-40	2.70 (0.19) ^{A,a}	2.44 (0.19) ^{A,a}	2.54 (0.19) ^{A,a}	2.67 (0.19) ^{A,a}
	0-40 mean	**3.05 (0.11)** ^{A}	**2.79 (0.11)** ^{A}	**2.88 (0.11)** ^{A}	**2.99 (0.11)** ^{A}
	40-60	1.46 (0.19) ^{A,b}	1.45 (0.19) ^{A,b}	1.40 (0.19) ^{A,b}	1.59 (0.16) ^{A,b}
	60-80	1.07 (0.19) ^{A,b}	0.90 (0.19) ^{A,b}	0.88 (0.19) ^{A,b}	1.04 (0.16) ^{A,b}
	40-80 mean	**1.26 (0.13)** ^{A}	**1.18 (0.13)** ^{A}	**1.14 (0.13)** ^{A}	**1.31 (0.11)** ^{A}
TN (%)	0-10	0.24 (0.01) ^{A,a}	0.23 (0.01) ^{A,a}	0.23 (0.01) ^{A,a}	0.24 (0.01) ^{A,a}
	10-20	0.24 (0.01) ^{A,a}	0.21 (0.01) ^{A,a}	0.22 (0.01) ^{A,a}	0.23 (0.01) ^{A,a}
	20-40	0.21 (0.01) ^{A,a}	0.18 (0.01) ^{A,a}	0.19 (0.01) ^{A,a}	0.20 (0.01) ^{A,a}
	0-40 mean	**0.23 (0.01)** ^{A}	**0.21 (0.01)** ^{A}	**0.21 (0.01)** ^{A}	**0.22 (0.01)** ^{A}
	40-60	0.12 (0.01) ^{A,b}	0.11 (0.01) ^{A,b}	0.11 (0.01) ^{A,b}	0.13 (0.01) ^{A,b}
	60-80	0.09 (0.01) ^{A,b}	0.08 (0.01) ^{A,b}	0.08 (0.01) ^{A,b}	0.09 (0.01) ^{A,b}
	40-80 mean	**0.10 (0.01)** ^{A}	**0.09 (0.01)** ^{A}	**0.09 (0.01)** ^{A}	**0.11 (0.01)** ^{A}
C/N ratio	0-10	13.55 (0.18) ^{A,a}	13.16 (0.18) ^{A,a}	13.31 (0.18) ^{A,a}	13.31 (0.18) ^{A,a}
	10-20	13.31 (0.18) ^{A,a}	13.43 (0.18) ^{A,a}	13.49 (0.16) ^{A,a}	13.31 (0.16) ^{A,a}
	20-40	13.07 (0.18) ^{A,a}	13.60 (0.18) ^{A,a}	13.41 (0.18) ^{A,a}	13.25 (0.18) ^{A,a}
	40-60	12.79 (0.22) ^{A,a}	13.00 (0.31) ^{A,a}	13.45 (0.31) ^{A,a}	12.79 (0.18) ^{A,a}
	0-60 mean	**13.18 (0.09)** ^{A}	**13.30 (0.11)** ^{A}	**13.42 (0.11)** ^{A}	**13.16 (0.09)** ^{A}
	60-80 mean	**11.61 (0.57)** ^{A,b}	**11.22 (0.57)** ^{A,b}	**10.91 (0.57)** ^{A,b}	**11.00 (0.49)** ^{A,b}

Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$. Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$.

Table 3.6 – SOC and TN stock (0-60 cm) in maize and soybean sole crops and 1:2 and 2:3 intercrops, 2012. Standard errors are given in parentheses.

	Depth (cm)	**Treatment**			
		Soybean	**Maize**	**1:2**	**2:3**
SOC stock (g m^{-2})	0-10	4336 (247) ^{A,a}	3871 (277) ^{A,ac}	4048 (265) ^{A,ac}	4060 (264) ^{A,ac}
	10-20	4825 (222) ^{A,a}	4216 (254) ^{A,a}	4408 (243) ^{A,a}	4314 (215) ^{A,a}
	20-40	4003 (189) ^{A,a}	3684 (190) ^{A,a}	3896 (213) ^{A,ac}	4099 (185) ^{A,a}
	40-60	2253 (476) ^{A,b}	2008 (534) ^{A,bc}	2347 (560) ^{A,bc}	2541 (366) ^{A,bc}
	0-60 mean	**3974 (126) ^{A}**	**3549 (140) ^{A}**	**3758 (144) ^{A}**	**3819 (122) ^{A}**
TN stock (g m^{-2})	0-10	320 (19) ^{A,a}	294 (20) ^{A,a}	304 (20) ^{A,ac}	305 (20) ^{A,ac}
	10-20	362 (17) ^{A,a}	314 (19) ^{A,a}	331 (18) ^{A,a}	325 (16) ^{A,a}
	20-40	306 (14) ^{A,a}	273 (14) ^{A,a}	292 (16) ^{A,ac}	309 (14) ^{A,a}
	40-60	180 (34) ^{A,b}	147 (41) ^{A,b}	169 (44) ^{A,bc}	201 (26) ^{A,bc}
	0-60 mean	**300 (9) ^{A}**	**265 (11) ^{A}**	**281 (11) ^{A}**	**289 (9) ^{A}**

Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$. Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$.

Interaction effects of treatment by depth were not significant for any of the LF characteristics. No significant differences were noted between treatments for any of the LF characteristics (Table 3.7). The quantity of LF was greatest in the soybean sole crop, and least in the maize sole crop, and tended to decrease with depth. In the maize sole crop, the quantity of LF was significantly lower in the 10-20 cm increment than in the 0-10 cm increment. The quantity of LF did not appear to be correlated to the quality of LF, as both LF-N and LF-C were greatest in the maize sole crop and least in the 2:3 intercrop. The LF-C/N ratio was very similar in all the treatments, and tended to increase with depth. The exception was the 1:2 intercrop, where the C/N ratio decreased with depth. The LF-C/N ratio was greater than the soil C/N ratio (Table 3.5). The proportion of LF-C in SOC (LF-C/SOC) and LF-N in TN (LF-N/TN), as well as the LF-C and LF-N stock decreased with depth in all treatments, although not significantly.

All were greatest in the soybean sole crop and least in the maize sole crop. When comparing the two intercropping systems, the LF characteristics were all higher in the 1:2 intercrop than the 2:3 intercrop, with the exception of the LF-C/N ratio, although none of these differences were significant.

Interaction effects of treatment by depth were not significant for SMB-C. SMB-C was significantly lower in the maize sole crop than in the 2:3 intercrop in the 0-10 cm depth increment (Table 3.8). In both the 1:2 and 2:3 intercrops, the SMB-C was significantly greater than in the maize sole crop. Although the SMB-C tended to decrease with depth, this trend was only significant in the 2:3 intercrop.

Table 3.7 – LF characteristics (0-20 cm) in maize and soybean sole crops and 1:2 and 2:3 intercrops, 2012. Standard errors are given in parentheses.

	Depth (cm)	**Treatment**			
		Soybean	**Maize**	**1:2**	**2:3**
LF (mg g^{-1})	0-10	5.74 (0.60) ^{A,a}	4.74 (0.49) ^{A,a}	5.51 (0.49) ^{A,a}	4.97 (0.49) ^{A,a}
	10-20	3.43 (0.60) ^{A,a}	1.95 (0.49) ^{A,b}	2.90 (0.42) ^{A,a}	3.12 (0.49) ^{A,a}
	0-20 mean	**4.59 (0.42)** ^{A}	**3.34 (0.35)** ^{A}	**4.20 (0.32)** ^{A}	**4.04 (0.35)** ^{A}
LF-N (%)	0-10	1.40 (0.13) ^{A,a}	1.36 (0.11) ^{A,a}	1.30 (0.11) ^{A,a}	1.32 (0.11) ^{A,a}
	10-20	1.35 (0.13) ^{A,a}	1.47 (0.11) ^{A,a}	1.31 (0.09) ^{A,a}	1.18 (0.11) ^{A,a}
	0-20 mean	**1.38 (0.09)** ^{A}	**1.41 (0.08)** ^{A}	**1.30 (0.07)** ^{A}	**1.25 (0.08)** ^{A}
LF-C (%)	0-10	24.18 (1.91) ^{A,a}	23.19 (1.56) ^{A,a}	23.80 (1.56) ^{A,a}	23.23 (1.56) ^{A,a}
	10-20	25.16 (1.91) ^{A,a}	27.03 (1.56) ^{A,a}	22.97 (1.35) ^{A,a}	22.19 (1.56) ^{A,a}
	0-20 mean	**24.67 (1.35)** ^{A}	**25.11 (1.10)** ^{A}	**23.39 (1.03)** ^{A}	**22.71 (1.10)** ^{A}
LF-C/N ratio	0-10	17.25 (0.81) ^{A,a}	17.23 (0.66) ^{A,a}	18.37 (0.66) ^{A,a}	17.67 (0.66) ^{A,a}
	10-20	18.54 (0.81) ^{A,a}	18.48 (0.66) ^{A,a}	17.56 (0.57) ^{A,a}	19.00 (0.66) ^{A,a}
	0-20 mean	**17.89 (0.57)** ^{A}	**17.86 (0.47)** ^{A}	**17.97 (0.44)** ^{A}	**18.33 (0.47)** ^{A}
LF-C/ SOC (%)	0-10	4.07 (0.58) ^{A,a}	3.73 (0.47) ^{A,a}	4.21 (0.47) ^{A,a}	3.64 (0.47) ^{A,a}
	10-20	2.71 (0.58) ^{A,a}	1.84 (0.47) ^{A,a}	2.36 (0.41) ^{A,a}	2.27 (0.47) ^{A,a}
	0-20 mean	**3.39 (0.41)** ^{A}	**2.79 (0.33)** ^{A}	**3.28 (0.31)** ^{A}	**2.95 (0.33)** ^{A}
LF-N/TN (%)	0-10	3.20 (0.51) ^{A,a}	2.92 (0.41) ^{A,a}	3.06 (0.41) ^{A,a}	2.75 (0.41) ^{A,a}
	10-20	1.93 (0.51) ^{A,a}	1.33 (0.41) ^{A,a}	1.81 (0.36) ^{A,a}	1.65 (0.41) ^{A,a}
	0-20 mean	**2.56 (0.36)** ^{A}	**2.12 (0.29)** ^{A}	**2.44 (0.27)** ^{A}	**2.20 (0.29)** ^{A}
LF-C stock (g m^{-2})	0-10	176.55 (19.42) ^{A,a}	144.44 (15.86 ^{A,a}	170.47 (15.86) ^{A,a}	145.52 (15.86) ^{A,a}
	10-20	124.57 (19.42) ^{A,a}	76.61 (15.86) ^{A,a}	98.47 (13.73) ^{A,a}	89.19 (15.86) ^{A,a}
	0-20 mean	**150.56 (13.73)** ^{A}	**110.53 (11.21)** ^{A}	**134.47 (10.49)** ^{A}	**117.36 (11.21)** ^{A}
LF-N stock (g m^{-2})	0-10	10.24 (1.26) ^{A,a}	8.52 (1.03) ^{A,a}	9.32 (1.03) ^{A,a}	8.26 (1.03) ^{A,a}
	10-20	6.74 (1.26) ^{A,a}	4.11 (1.03) ^{A,a}	5.62 (0.89) ^{A,a}	4.79 (1.03) ^{A,a}
	0-20 mean	**8.49 (0.89)** ^{A}	**6.31 (0.73)** ^{A}	**7.47 (0.68)** ^{A}	**6.53 (0.73)** ^{A}

Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$. Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$.

Table 3.8 – SMB-C (0-80 cm) in maize and soybean sole crops and 1:2 and 2:3 intercrops, 2012. Standard errors are given in parentheses.

	Depth (cm)	**Treatment**			
		Soybean	**Maize**	**1:2**	**2:3**
SMB-C (ug g^{-1})	0-10	356.74 (35.80) ^{AB,a}	217.16 (35.80) ^{B,a}	380.27 (35.80) ^{AB,a}	436.45 (43.84) ^{a,a}
	10-20	355.58 (35.80) ^{A,a}	274.79 (31.01) ^{A,a}	303.73 (35.80) ^{A,a}	355.31 (31.01) ^{a,ac}
	20-40	213.63 (35.80) ^{A,a}	197.72 (43.85) ^{A,a}	197.86 (35.80) ^{A,a}	353.54 (35.80) ^{A,acd}
	40-60	185.54 (35.80) ^{A,a}	137.16 (35.80) ^{A,a}	255.20 (35.80) ^{A,a}	218.68 (31.01) ^{A,bc}
	60-80	193.38 (35.80) ^{A,a}	181.56 (35.80) ^{A,a}	221.33 (35.80) ^{A,a}	188 49 (31.01) ^{A,bd}
	0-80 mean	**260.97 (16.01) ^{AB}**	**201.68 (16.41) ^{A}**	**271.68 (16.01) ^{B}**	**310.49 (15.61) ^{B}**

Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$. Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$.

3.3.2 Comparison of soil quality between 2007 and 2012

Since the bulk density was analyzed using non-parametric tests, only the means (0-80 cm) were compared from 2007 and 2012, and it was found that the mean BD increased significantly ($p<0.05$) in all treatments, with the greatest increases in 1:2 and 2:3 (Table 3.9). Interaction effects of treatment by year were significant for pH [$F(3, 48) = 4.54$, $p = 0.007$]. The mean pH (0-80 cm) decreased significantly from 2007 to 2012 in the soybean sole crop and the 1:2 intercrop (Table 3.9). However, although insignificant, the greatest decreases in pH were found in the shallower depths, particularly in the soybean sole crop. In the top two depth increments in the maize sole crop, the pH actually increased from 2007 to 2012.

Interaction effects of treatment by depth or year were not significant for SOC, TN, and C/N ratio. The mean SOC, TN, and C/N ratio increased significantly in the 0-40 cm depth increment from 2007 to 2012 in all treatments (Table 3.10). With the exception of the 20-40 cm depth increment in the maize sole crop, SOC increased significantly in all treatments and all depth increments within 0-40 cm. SOC, TN, and C/N ratio increased in the 40-80 cm depth increment from 2007 to 2012, although not significantly. The greatest increase in SOC was in the 2:3 intercrop for the 0-40 cm depth increment, and in the soybean sole crop for the 40-80 cm depth increment. Within the 0-40 cm depth increment, TN only increased significantly in the 20-40 cm increment in the soybean sole crop and the 1:2 intercrop, and in the 10-20 cm increment in the 2:3 intercrop. The increases in TN in the 40-80 cm depth interval were not significant in all four treatments. The C/N ratio increased significantly in all three increments in the 0-40 cm depth increment. Although the C/N ratio increased in the 40-80 cm depth increment, these increases were not significant.

Table 3.9 - Comparison of bulk density and pH (0-80 cm) between 2007 and 2012 in maize and soybean sole crops and 1:2 and 2:3 intercrops. Standard errors are given in parentheses.

	Year	Depth (cm)	Treatment			
			Soybean	**Maize**	**1:2**	**2:3**
Bulk Density* (g cm^{-3})	**2007**	0-10	1.21 (0.08) ^{A,a}	1.11 (0.08) ^{A,a}	1.17 (0.08) ^{A,a}	1.13 (0.08) ^{A,a}
		10-20	1.24 (0.08) ^{A,a}	1.24 (0.08) ^{A,a}	1.18 (0.08) ^{A,a}	1.15 (0.08) ^{A,a}
		20-40	1.25 (0.08) ^{A,a}	1.27 (0.08) ^{A,a}	1.16 (0.08) ^{A,a}	1.24 (0.08) ^{A,a}
		40-80	1.32 (0.08) ^{A,a}	1.25 (0.08) ^{A,a}	1.25 (0.10) ^{A,a}	1.19 (0.08) ^{A,a}
		0-80 mean	**1.25 (0.04)** ^{A†}	**1.24 (0.04)** ^{A†}	**1.19 (0.04)** ^{A†}	**1.18 (0.04)** ^{A†}
	2012	0-10	1.33 (0.05) ^{A,a}	1.27 (0.06) ^{A,a}	1.33 (0.05) ^{A,a}	1.27 (0.06) ^{A,a}
		10-20	1.53 (0.03) ^{A,a}	1.46 (0.04) ^{A,b}	1.47 (0.04) ^{A,a}	1.36 (0.05) ^{A,a}
		20-40	1.48 (0.03) ^{A,a}	1.44 (0.03) ^{A,ab}	1.53 (0.03) ^{A,a}	1.53 (0.02) ^{A,a}
		40-80	1.53 (0.03) ^{A,a}	1.34 (0.05) ^{A,ab}	1.50 (0.04) ^{A,a}	1.52 (0.03) ^{A,a}
		0-80 mean	**1.47 (0.02)** ^{A†}	**1.38 (0.02)** ^{A†}	**1.46 (0.02)** ^{A†}	**1.43 (0.02)** ^{A†}
pH	**2007**	0-10	5.83 (0.20) ^{A,a}	5.54 (0.20) ^{A,a}	5.91 (0.20) ^{A,a}	5.76 (0.20) ^{A,a}
		10-20	5.90 (0.20) ^{A,a}	5.62 (0.20) ^{A,a}	5.89 (0.20) ^{A,a}	5.68 (0.20) ^{A,a}
		20-40	6.13 (0.20) ^{A,a}	5.88 (0.20) ^{A,a}	6.13 (0.20) ^{A,a}	5.97 (0.20) ^{A,a}
		40-80	6.63 (0.20) ^{A,a}	6.38 (0.20) ^{A,a}	6.57 (0.20) ^{A,a}	6.56 (0.20) ^{A,a}
		0-80 mean	**6.12 (0.10)** ^{A†}	**5.86 (0.10)** ^{A}	**6.12 (0.10)** ^{A†}	**5.99 (0.10)** ^{A}
	2012	0-10	5.32 (0.12) ^{A,a}	5.64 (0.12) ^{A,a}	5.56 (0.12) ^{A,a}	5.52 (0.12) ^{A,a}
		10-20	5.55 (0.12) ^{A,ac}	5.74 (0.12) ^{A,a}	5.70 (0.11) ^{A,a}	5.85 (0.11) ^{A,ac}
		20-40	5.83 (0.12) ^{A,ac}	5.86 (0.12) ^{A,a}	5.83 (0.12) ^{A,a}	5.92 (0.11) ^{A,ac}
		40-80	6.28 (0.09) ^{A,bc}	6.24 (0.09) ^{A,a}	6.29 (0.09) ^{A,a}	6.40 (0.08) ^{A,bc}
		0-80 mean	**5.74 (0.06)** ^{A†}	**5.87 (0.06)** ^{A}	**5.85 (0.06)** ^{A†}	**5.92 (0.05)** ^{A}

^{A,a} Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$. Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$.

* 2007 and 2012 values of bulk density were only compared for the 0-80 mean.

† indicates the values were significantly different between years.

Interaction effects of treatment by depth or year were not significant for SOC stock or N stock. SOC stock increased significantly from 2007 to 2012 in all treatments and depth increments within the 0-40 cm depth increment (Table 3.11). The greatest increases in SOC stock were observed in the 1:2 and 2:3 intercrops. There were similar increases in the 40-80 cm depth increment, but these increases were not found to be significant despite the increase being greater than 100% in the soybean sole crop and the 1:2 and 2:3 intercrops. The mean N stock (0-40 cm) also increased significantly from 2007 to 2012, as did the N stock in most of the depth increments in each of the treatments, excepting soybean 0-10 cm and maize 20-40 cm. In the 40-80 cm depth increment, only the increase in TN stock in the 2:3 intercrop was significant; this increase was greater than 100%.

Interaction effects of treatment by year were not significant in all LF characteristics. In all treatments, all LF characteristics increased between 2007 and 2012 except for the LF quantity in the maize treatment, which decreased (Table 3.12). The only significant increases were in LF-C in the maize sole crop and LF-C stock in the 1:2 intercrop. All LF characteristics showed greater increases in the 1:2 intercrop than in the 2:3 intercrop.

Interaction effects of treatment by year were significant for SMB-C [F(3,31) = 8.98, p = 0.0002]. SMB-C increased from 2007 to 2012 in nearly all treatments and depths (Table 3.13). The exception was in the 0-10 cm depth increment in the maize sole crop treatment, which decreased over this time period. These increases were significant in the 0-80 cm mean in the 1:2 and 2:3 intercrops, as well as in the 40-80 cm depth increment in the 1:2 intercrop, and in the 0-10 cm, 10-20 cm, and 40-80 cm increments in the 2:3 intercrop. The largest increases in SMB-C occurred in the 2:3 intercrop.

Table 3.10 – Comparison of SOC and TN concentrations and C/N ratio (0-40 cm; 40-80 cm) between 2007 and 2012 in maize and soybean sole crops and 1:2 and 2:3 intercrops. Standard errors are given in parentheses.

	Year	Depth (cm)	Treatment			
			Soybean	**Maize**	**1:2**	**2:3**
SOC (%)	**2007**	0-10	2.57 (0.12) ^{A,a†}	2.48 (0.12) ^{A,a†}	2.40 (0.12) ^{A,a†}	2.39 (0.12) ^{A,a†}
		10-20	2.49 (0.12) ^{A,a†}	2.22 (0.12) ^{A,a†}	2.20 (0.12) ^{A,a†}	2.27 (0.12) ^{A,a†}
		20-40	1.74 (0.12) ^{A,a†}	1.92 (0.12) ^{A,a}	1.79 (0.12) ^{A,a†}	1.80 (0.12) ^{A,a†}
		0-40 mean	**2.27 (0.07)** ^{A†}	**2.21 (0.07)** ^{A†}	**2.13 (0.07)** ^{A†}	**2.15 (0.07)** ^{A†}
		40-80 mean	**0.80 (0.11)** ^{A,b}	**0.89 (0.11)** ^{A,b}	**0.79 (0.11)** ^{A,b}	**0.87 (0.11)** ^{A,b}
	2012	0-10	3.29 (0.20) ^{A,a†}	3.06 (0.20) ^{A,a†}	3.09 (0.20) ^{A,a†}	3.19 (0.20) ^{A,a†}
		10-20	3.17 (0.20) ^{A,a†}	2.86 (0.20) ^{A,a†}	2.98 (0.20) ^{A,a†}	3.09 (0.20) ^{A,a†}
		20-40	2.70 (0.20) ^{A,a†}	2.44 (0.20) ^{A,a}	2.54 (0.20) ^{A,a†}	2.67 (0.20) ^{A,a†}
		0-40 mean	**3.05 (0.12)** ^{A†}	**2.79 (0.12)** ^{A†}	**2.87 (0.12)** ^{A†}	**2.98 (0.12)** ^{A†}
		40-80 mean	**1.26 (0.18)** ^{A,b}	**1.18 (0.18)** ^{A,b}	**1.14 (0.18)** ^{A,b}	**1.27 (0.18)** ^{A,b}
TN (%)	**2007**	0-10	0.22 (0.01) ^{A,a}	0.21 (0.01) ^{A,a}	0.20 (0.01) ^{A,a}	0.20 (0.01) ^{A,a}
		10-20	0.21 (0.01) ^{A,a}	0.19 (0.01) ^{A,a}	0.19 (0.01) ^{A,a}	0.19 (0.01) ^{A,a†}
		20-40	0.15 (0.01) ^{A,a†}	0.16 (0.01) ^{A,a}	0.15 (0.01) ^{A,a†}	0.15 (0.01) ^{A,a}
		0-40 mean	**0.19 (0.01)** ^{A†}	**0.19 (0.01)** ^{A†}	**0.18 (0.01)** ^{A†}	**0.18 (0.01)** ^{A†}
		40-80 mean	**0.08 (0.01)** ^{A,b}	**0.08 (0.01)** ^{A,b}	**0.07 (0.01)** ^{A,b}	**0.07 (0.01)** ^{A,b}
	2012	0-10	0.24 (0.01) ^{A,a}	0.23 (0.01) ^{A,a}	0.23 (0.01) ^{A,a}	0.24 (0.01) ^{A,a}
		10-20	0.24 (0.01) ^{A,a}	0.21 (0.01) ^{A,a}	0.22 (0.01) ^{A,a}	0.23 (0.01) ^{A,a†}
		20-40	0.21 (0.01) ^{A,a†}	0.18 (0.01) ^{A,a}	0.19 (0.01) ^{A,a†}	0.20 (0.01) ^{A,a}
		0-40 mean	**0.23 (0.01)** ^{A†}	**0.21 (0.01)** ^{A†}	**0.21 (0.01)** ^{A†}	**0.22 (0.01)** ^{A†}
		40-80 mean	**0.10 (0.01)** ^{A,b}	**0.09 (0.01)** ^{A,b}	**0.09 (0.01)** ^{A,b}	**0.11 (0.01)** ^{A,b}
C/N ratio	**2007**	0-10	11.77 (0.11) ^{A,a†}	11.86 (0.14) ^{A,a†}	11.93 (0.11) ^{A,a†}	11.75 (0.11) ^{A,a†}
		10-20	11.80 (0.11) ^{A,a†}	11.76 (0.11) ^{A,a†}	11.78 (0.11) ^{A,a†}	11.83 (0.11) ^{A,a†}
		20-40	12.03 (0.11) ^{A,a†}	12.01 (0.11) ^{A,a†}	12. 02 (0.11) ^{A,a†}	12.04 (0.11) ^{A,a†}
		0-40 mean	**11.87 (0.06)** ^{A†}	**11.88 (0.07)** ^{A†}	**11.91 (0.06)** ^{A†}	**11.87 (0.06)** ^{A†}
		40-80 mean	**10.82 (0.35)** ^{A,b}	**11.22 (0.35)** ^{A,b}	**10.71 (0.35)** ^{A,b}	**11.25 (0.35)** ^{A,b}
	2012	0-10	13.55 (0.17) ^{A,a†}	13.07 (0.21) ^{A,a†}	13.31 (0.17) ^{A,a†}	13.31 (0.17) ^{A,a†}
		10-20	13.31 (0.17) ^{A,a†}	13.43 (0.17) ^{A,a†}	13. 42 (0.17) ^{A,a†}	13.29 (0.17) ^{A,a†}
		20-40	13.07 (0.17) ^{A,a†}	13.60 (0.17) ^{A,a†}	13.41 (0.17) ^{A,a†}	13.25 (0.17) ^{A,a†}
		0-40 mean	**13.31 (0.10)** ^{A†}	**13.37 (0.11)** ^{A†}	**13.38 (0.10)** ^{A†}	**13.28 (0.10)** ^{A†}
		40-80 mean	**12.03 (0.60)** ^{A,b}	**12.25 (0.60)** ^{A,b}	**11.99 (0.60)** ^{A,b}	**11.57 (0.60)** ^{A,b}

Values followed by the same upper case letters, compare treatments within each depth, are not significantly different at $p<0.05$. Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$.
† indicates the values were significantly different between years.

Table 3.11 – Comparison of SOC and TN stock (0-40 cm; 40-80 cm) between 2007 and 2012 in maize and soybean sole crops and 1:2 and 2:3 intercrops. Standard errors are given in parentheses.

	Year	**Depth (cm)**	**Treatment**			
			Soybean	**Maize**	**1:2**	**2:3**
SOC stock (g m^{-2})	**2007**	0-10	3094 (263) $^{A,a\,\dagger}$	2723 (263) $^{A,a\,\dagger}$	2791 (263) $^{A,a\,\dagger}$	2684 (263) $^{A,a\,\dagger}$
		10-20	3099 (263) $^{A,a\,\dagger}$	2772 (263) $^{A,a\,\dagger}$	2626 (263) $^{A,a\,\dagger}$	2627 (263) $^{A,a\,\dagger}$
		20-40	2228 (263) $^{A,a\,\dagger}$	2448 (263) $^{A,a\,\dagger}$	2088 (263) $^{A,a\,\dagger}$	2237 (263) $^{A,a\,\dagger}$
		0-40 mean	**2807 (152)** $^{A\,\dagger}$	**2648 (152)** $^{A\,\dagger}$	**2502 (152)** $^{A\,\dagger}$	**2516 (152)** $^{A\,\dagger}$
		40-80 mean	**1060 (275)** ^{A,b}	**1208 (275)** ^{A,b}	**1156 (336)** ^{A,b}	**1020 (275)** ^{A,b}
	2012	0-10	4335 (281) $^{A,a\,\dagger}$	3871 (281) $^{A,a\,\dagger}$	4043 (281) $^{A,a\,\dagger}$	4044 (281) $^{A,a\,\dagger}$
		10-20	4811 (281) $^{A,a\,\dagger}$	4194 (281) $^{A,a\,\dagger}$	4387 (281) $^{A,a\,\dagger}$	4156 (281) $^{A,a\,\dagger}$
		20-40	3983 (281) $^{A,a\,\dagger}$	3515 (281) $^{A,a\,\dagger}$	3874 (281) $^{A,a\,\dagger}$	4074 (281) $^{A,a\,\dagger}$
		0-40 mean	**4376 (162)** $^{A\,\dagger}$	**3860 (162)** $^{A\,\dagger}$	**4102 (162)** $^{A\,\dagger}$	**4091 (162)** $^{A\,\dagger}$
		40-80 mean	**2227 (336)** ^{A,b}	**1945 (336)** ^{A,b}	**2347 (412)** ^{A,b}	**2341 (336)** ^{A,b}
TN stock (g m^{-2})	**2007**	0-10	265 (24) ^{A,a}	230 (24) $^{A,a\,\dagger}$	237 (24) $^{A,a\,\dagger}$	228 (24) $^{A,a\,\dagger}$
		10-20	257 (24) $^{A,a\,\dagger}$	233 (24) $^{A,a\,\dagger}$	223 (24) $^{A,a\,\dagger}$	220 (24) $^{A,a\,\dagger}$
		20-40	188 (24) $^{A,a\,\dagger}$	204 (24) ^{A,a}	175 (24) $^{A,a\,\dagger}$	197 (24) $^{A,a\,\dagger}$
		0-40 mean	**237 (13)** $^{A\,\dagger}$	**223 (13)** $^{A\,\dagger}$	**212 (13)** $^{A\,\dagger}$	**212 (13)** $^{A\,\dagger}$
		40-80 mean	**102 (24)** ^{A,b}	**108 (24)** ^{A,b}	**100 (30)** ^{A,b}	**86 (24)** $^{A,b\,\dagger}$
	2012	0-10	320 (24) ^{A,a}	294 (24) $^{A,a\,\dagger}$	304 (24) $^{A,a\,\dagger}$	304 (24) $^{A,a\,\dagger}$
		10-20	361 (24) $^{A,a\,\dagger}$	312 (24) $^{A,a\,\dagger}$	330 (24) $^{A,a\,\dagger}$	313 (24) $^{A,a\,\dagger}$
		20-40	305 (24) $^{A,a\,\dagger}$	259 (24) ^{A,a}	289 (24) $^{A,a\,\dagger}$	307 (24) $^{A,a\,\dagger}$
		0-40 mean	**329 (12)** ^{A}	**289 (12)** $^{A\,\dagger}$	**307 (12)** $^{A\,\dagger}$	**308 (12)** $^{A\,\dagger}$
		40-80 mean	**178 (24)** ^{A,b}	**144 (24)** ^{A,b}	**168 (30)** ^{A,b}	**189 (24)** $^{A,b\,\dagger}$

Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$. Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$.

† indicates the values were significantly different between years.

Table 3.12 – Comparison of LF characteristics (averaged over 0-20 cm) in maize and soybean sole crops and 1:2 and 2:3 intercrops in Balcarce, Argentina between 2007 and 2012. Standard errors are given in parentheses.

	Year	Treatment			
		Soybean	**Maize**	**1:2**	**2:3**
LF (mg g^{-1})	**2007**	4.40 (0.39) ^{A}	3.95 (0.32) ^{A}	3.63 (0.32) ^{A}	3.62 (0.32) ^{A}
	2012	4.59 (0.49) ^{A}	3.35 (0.40) ^{A}	4.24 (0.40) ^{A}	4.04 (0.40) ^{A}
LF-C (%)	**2007**	17.13 (1.21) ^{A}	16.72 (0.99) ^{A†}	16.66 (0.99) ^{A}	17.35 (0.99) ^{A}
	2012	24.67 (1.49) ^{A}	25.11 (1.22) ^{A†}	23.51 (1.22) ^{A}	22.71 (1.22) ^{A}
LF N (%)	**2007**	1.14 (0.01) ^{A}	1.06 (0.01) ^{A}	0.99 (0.01) ^{A}	0.97 (0.01) ^{A}
	2012	1.38 (0.09) ^{A}	1.41 (0.08) ^{A}	1.31 (0.08) ^{A}	1.25 (0.08) ^{A}
LF-C/N	**2007**	15.07 (1.06) ^{A}	15.74 (0.87) ^{A}	16.87 (0.87) ^{A}	17.91 (0.87) ^{A}
	2012	17.89 (0.55) ^{A}	17.86 (0.45) ^{A}	17.96 (0.45) ^{A}	18.33 (0.45) ^{A}
LF-C/SOC (%)	**2007**	3.03 (0.29) ^{A}	2.83 (0.24) ^{A}	2.62 (0.24) ^{A}	2.69 (0.24) ^{A}
	2012	3.39 (0.46) ^{A}	2.79 (0.38) ^{A}	3.31 (0.38) ^{A}	2.95 (0.38) ^{A}
LF-N/TN (%)	**2007**	2.40 (0.27) ^{A}	2.09 (0.22) ^{A}	1.82 (0.22) ^{A}	1.77 (0.22) ^{A}
	2012	2.56 (0.40) ^{A}	2.12 (0.33) ^{A}	2.46 (0.33) ^{A}	2.20 (0.33) ^{A}
LF-C stock (g m^{-2})	**2007**	90.94 (10.54) ^{A}	77.90 (8.60) ^{A}	71.63 (8.60) ^{A†}	71.79 (8.60) ^{A}
	2012	154.13 (13.67) ^{A}	115.77 (11.16) ^{A}	138.27 (11.16) ^{A†}	118.14 (11.16) ^{A}
LF-N stock (g m^{-2})	**2007**	6.10 (0.65) ^{A}	4.95 (0.53) ^{A}	4.23 (0.53) ^{A}	4.02 (0.53) ^{A}
	2012	8.62 (0.89) ^{A}	6.54 (0.73) ^{A}	7.72 (0.73) ^{A}	6.53 (0.73) ^{A}

Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$. Values followed by † are significantly different between years.

Table 3.13 – Comparison of SMB-C (0-80 cm) between 2007 and 2012 in maize and soybean sole crops and 1:2 and 2:3 intercrops. Standard errors are given in parentheses.

	Year	**Depth (cm)**	**Treatment**			
			Soybean	**Maize**	**1:2**	**2:3**
SMB-C (ug g^{-1})	**2007**	0-10	294.26 (30.46) ^{A,a}	241.16 (27.58) ^{A,a}	340.71 (32.78) ^{A,a}	259.40 (28.60) $^{A,a\,\dagger}$
		10-20	266.75 (29.00) ^{A,ab}	247.65 (27.94) ^{A,a}	276.14 (29.51) ^{A,ac}	180.47 (23.86) $^{A,ab\,\dagger}$
		20-40	174.38 (23.45) ^{A,ab}	136.13 (22.93) ^{A,ab}	130.15 (20.26) ^{A,bc}	177.87 (23.68) ^{A,ab}
		40-80	124.57 (19.82) ^{A,b}	103.43 (18.06) ^{A,b}	92.19 (17.05) $^{A,b\,\dagger}$	77.08 (15.59) $^{A,b\,\dagger}$
		0-80 mean	209.18 (18.16) ^{AB}	176.25 (17.17) ^{AB}	196.60 (17.61) $^{A\,\dagger}$	166.77 (16.22) $^{B\,\dagger}$
	2012	0-10	354.60 (38.68) ^{AB,a}	210.55 (29.80) ^{B,a}	378.63 (39.96) ^{AB,a}	469.04 (44.48) $^{A,a\,\dagger}$
		10-20	355.08 (38.70) ^{A,a}	276.31 (34.14) ^{A,a}	298.49 (35.48) ^{A,ab}	282.53 (40.17) $^{A,ba\,\dagger}$
		20-40	212.87 (29.97) ^{A,a}	197.67 (31.96) ^{A,a}	197.28 (28.85) ^{A,b}	347.18 (38.27) ^{A,ab}
		40-80	187.89 (28.15) ^{A,a}	156.17 (25.67) ^{A,a}	238.24 (31.70) $^{A,ab\,\dagger}$	213.55 (30.01) $^{A,b\,\dagger}$
		0-80 mean	272.02 (23.95) ^{A}	208.00 (21.57) ^{A}	274.03 (24.04) $^{A\,\dagger}$	346.53 (27.04) $^{B\,\dagger}$

Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$. Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$.

† indicates the values were significantly different between years.

3.4 Discussion

3.4.1 Crop biomass production and land equivalent ratio

Biomass residue returned to the soil is the primary factor impacting the levels of SOC, and thus soil quality (Studdert and Echeverría, 2000). The shoot biomass produced by the 1:2 and 2:3 intercrops was not significantly different from that produced by the maize and soybean sole crops (Table 3.3). The shoot biomass was used to calculate the LER for each of the intercrops, and it was found that during the six seasons of intercropping maize and soybeans in Balcarce, Argentina, the LER for both the 1:2 and 2:3 intercrops was below 1 for three growing seasons (Table 3.3). Therefore, intercropping did not appear to have a consistent impact on the quantity of biomass residue returned to the soil, the most important factor influencing soil quality. Previous studies demonstrated a clear benefit to crop yield and biomass production from intercropping maize and soybean (Table 3.2), findings that were not supported by the present study. The studies outlined in Table 3.2 varied by location, climate, soil texture, and soil quality, and yet they all found an LER greater than 1. The primary differences between these previous investigations and the current investigation were in the intercrop design: the inter-row spacing (the distance between the nearest maize and soybean rows) and the time of planting. In the intercropping systems described in Table 3.2, the inter-row distance was generally 25 cm or 37.5 cm; in the current study, it was 52 cm. Additionally, in the these previous investigations, the inter-row spacing in the intercrops was always less than the intercrop spacing in the sole crop, whereas in the current study, the inter-row spacing was the same in the intercrops and sole crops. In the previous investigations, the maize and soybean were planted at the same time, whereas in the current study, the maize was sown one month prior to the soybean in both the intercrops and the sole crops. The staggered planting time and large inter-row spacing likely reduced the beneficial interactions between the maize and soybean.

Clement et al. (1992) conducted one experiment in a 2:3 intercrop, where the row spacing between maize and soybean was 50 cm. However, the two maize rows in this intercrop were 30 cm apart from each other and the soybean rows were 35 cm apart, still allowing for a greater plant density. This differed from the present study, where the inter-row distance of 52 cm was maintained in between all rows, and the plant density therefore remained low. Clement et al. (1992) still obtained an LER greater than 1, although it was less than the LER found from a 1:2 intercrop with 25 cm inter-row spacing. Carruthers et al. (2000) examined the impact of delaying soybean seeding by three weeks in a temperate climate and found that simultaneous seeding of maize and soybean resulted in a higher LER than delayed seeding.

Clement et al. (1992) found that closer associations between the intercropped species resulted in higher yields because the maize was able to make better use of available N. Closer proximity of the maize and soybean allows for beneficial root interactions (Hinsinger et al., 2011). Due to competition for soil nutrients and water, the roots may spread farther and eventually encompass a greater volume of soil, enabling greater uptake of N, P, and K in maize intercrops (Kolawole, 2012). In addition, the nitrogenous excretions from soybean roots could be taken up by nearby maize roots, allowing for better maize plant nutrition (Kolawole, 2012). However, if the soybeans are planted a month after the maize, the maximum soybean root exudations won’t occur until after the maize has flowered, which is past the time of peak demand from the maize (Coll et al., 2012; Liang et al., 2011). Therefore, in the current study, the soybeans were likely planted too late to provide these beneficial root exudations to maize. Based on the typical growing season in the Argentine Pampa (Coll et al., 2012), the maize would likely have already passed its peak nutrient demand by the time the soybean root exudations reached their maximum.

3.4.2 Soil quality

3.4.2.1 Soil pH

The pH values are in general agreement with previous studies at nearby sites to that of the current study area, where a range in surficial pH from 5.3 to 6.0 was reported (Studert and Echeverría, 2000; Domínguez et al., 2009; Oelbermann and Echarte, 2011). Urea, applied as a nitrogen fertilizer, increases soil acidity over the long term. Urea was applied to the maize sole crops and the intercrops at a rate of 150 kg N ha^{-2}, likely decreasing the pH in all treatments, but significantly in the soybean and 1:2 treatments. The increase in maize pH in the 0-20 cm depth intervals was likely a short term increase, as soybean was the most recent crop, and thus not fertilized. It is likely the pH will decrease in the following field season, when urea will again be applied to maize. In general, the pH increased with depth in all four treatments, as the urea was applied to the surface and little of it would reach the lower depths. Additionally, carbonate accumulations with depth and the presence of a petrocalcic horizon below depths of 0.7 m (Domínguez et al., 2009) would buffer the influence of the urea, and maintain a higher pH.

3.4.2.2 Soil organic carbon and soil total nitrogen

In all four treatments, SOC and TN were greatest at the surface (0-10 cm), and declined with depth. In a global meta-analysis, Jobbágy and Jackson (2000) reported that this is a typical SOC distribution, showing that of the carbon within the top meter of soil, 42% was found within the top 20 cm, 22% from 20-40 cm, 16% from 40-60 cm, 11% from 60-80 cm, and 9% from 80-100 cm. The SOC distribution can be attributed to root distribution within the soil, and carbon inputs from the surface (Berhongaray et al., 2013).

Although the quantity of residues returned to the soils is the most important contributor to SOC, it can also be impacted by the residue quality (Dick and Gregorich, 2004). Different organic compounds in the residue, such as cellulose, hemicellulose, lignin, and protein, all impact the recalcitrance of the plant residues (Dick and Gregorich, 2004). For example, lignin is decomposed more slowly than cellulose and hemicellulose, so residues with high lignin content would result in higher SOC levels (Dick and Gregorich, 2004). Soybean residues are higher in lignin and lower in cellulose and hemicellulose than corn residues (Johnson et al., 2007). However, as the lignin concentration in both soybean and maize residues is relatively low, it does not play a large role in determining the rate to decomposition of these residues (Gentile et al., 2008). The low C/N ratio of the soybean, similar to other legumes, will result in faster decomposition than maize residues. For example, when investigating the impact of intercropping maize with either lablab (*Lablab purpureus* L.) or pigeon pea over a period of 17 years in Brazil, Diekow et al. (2005) found that both the maize/lablab and maize/pigeon pea intercrops resulted in significantly higher SOC and TN concentrations in the top 7.5 cm of soil than in the corresponding maize crop. It was therefore expected that similar results would be found in the current study, and that the presence of soybean in the intercrops would increase the soil SOC and TN above the sole crops. However, significant increases in SOC and TN were observed in both the intercropping and sole cropping systems, with no significant difference between any of the treatments. This may be due to the maize and soybean rotation in the sole crops providing a similar quality of crop residues as the intercrops.

Typically, the establishment of crops reduces SOC stocks, particularly when conventional tillage is used (Batlle-Bayer et al., 2010). However, cropping systems that include a legume in the crop rotation or intercrop have been found to meet or surpass the native C and N stocks (Diekow et al., 2005), although this is not possible if conventional tillage is maintained (Studdert and Echeverría, 2000). Since the establishment of the current experiment, both C and N stocks have increased significantly in all treatments. It is anticipated that with continued return of both maize and soybean residues to the soil, the SOC and TN, and hence the C and N stocks, will continue to increase in all treatments, although they may not reach the grassland levels if the conventional mouldboard plowing continues.

3.4.2.3 Light fraction carbon and nitrogen

Although the quantity of LF did not change significantly with time, the LF-C and LF-N in the 1:2 and maize treatments did increase significantly from 2007 to 2012. Similarly, in southern Africa, Beedy et al. (2010) found that intercropping maize with *Gliricidia* (*Gliricidia sepium*), a leguminous tree, increased LF-C and LF-N by 62% and 86%, respectively, over the sole maize plot after 14 years of intercropping. Although the increases in LF-C and LF-N were not so high in the present study, the time scale in Beedy et al. (2010) was over twice that of this study. However, significant changes in LF have typically been seen within four years, whether in temperate or tropical climates (Barrios et al., 1996; Matos et al., 2012). When comparing a maize sole crop, maize and pigeon pea rotations, and maize and pigeon pea intercropping in Kenya, Barrios et al. (1996) found that LF-C and LF-N were greatest in the rotation, followed by the intercrop, and least in the maize sole crop. This finding is comparable to the present study, where the greatest LF-C and LF-N values were found in the soybean and maize rotations, followed by the 1:2 and 2:3 intercrops.

The LF-C/N ratio is highly dependent on the quality of crop residues returned to the soil. If the crop is primarily a grain crop and no manure is added or legumes included in a rotation, the LF-C/N ratio will be higher (Wander, 2004). For example, Beedy et al. (2010) found that the LF-C/N ratio decreased 14% from sole maize if the legume *Gliricidia* was intercropped with the maize. However, no significant differences in the LF-C/N ratios were noted in the present study. The LF-C/N ratios from each of the four treatments were very similar, ranging from 17.86 in the maize treatment to 18.33 in the 2:3 intercrop. Previously, Vachon and Oelbermann (2011) found that the above-ground crop residue C/N ratio of the maize sole crop, 1:2, and 2:3 intercrops was not significantly different, ranging from 56 in the 1:2 intercrop to 66 in the maize sole crop, while it was 31 in the soybean sole crop. It is therefore likely that the more recalcitrant maize residue dominated the LF, as the presence of different quantities of soybean residue did not significantly reduce the LF-C/N ratio.

3.4.2.4 Soil microbial biomass carbon

Soil microbial biomass-C values found in this study were similar to previously reported values from the Argentine Pampa, and followed a similar trend of decreasing with depth (Alvarez et al., 1998). No significant differences were found in SMB-C between the soybean and maize sole crops, and the 1:2 intercrop, but the SMB-C was significantly higher in the 2:3 intercrop than in the other treatments in 2012. Similarly, Balota et al. (2003) found no difference in SMB-C of the surface soils between maize and soybean planted in rotation. Although maize produces more biomass, which is the most important factor influencing SMB (Kallenbach and Grandy, 2011), the lower C/N ratio of soybean is preferential for microbial growth (Balota et al., 2003). In an experiment of intercropping maize and faba bean (*Vicia faba* L.), Song et al. (2007) found significant differences in SMB-C in the third season of a maize/faba bean intercrop. SMB-C was greatest in the faba bean sole crop, followed by the intercrop, and least in the maize sole crop. In the present study, SMB-C was found to be the greatest in the 2:3 intercrop, and that the maize and soybean sole crops were both low. This difference is likely due to the rotation of maize and soybean in sole crops in the current study (Balota et al., 2003). The significantly higher SMB-C in the 2:3 intercrop was likely due to the different soil microbiological context that would have emerged in the intercrop rhizosphere (Song et al., 2007). Anderson and Domsch (1989) reported that organic matter that is more heterogeneous results in more complex microbial communities which can more effectively decompose SOC. It can thus be deduced that the spatial arrangement of the 2:3 intercrop increased the complexity of the microbial community, and hence resulted in a higher SMB-C.

3.5 Conclusions

Based on the analysis of SMB-C, intercropping in a 2:3 ratio of maize to soybean improved soil quality to a greater extent than the sole crops or the 1:2 intercrop in the period from 2007 to 2012. The other indicators of soil quality, including SOC, TN and LF, did not indicate that intercropping improved soil quality more than the sole crops. The soil quality, as indicated by

SOC, TN, LF, and SMB-C, did improve significantly in all four treatments from 2007 to 2012, and C sequestration clearly occurred as evidenced by the greater SOC stocks. However, the large inter-row distance in the intercrops combined with the delayed planting of the soybean one month after the maize likely reduced the many previously reported benefits of intercropping. The quantity of biomass returned to the soil did not differ between the intercrops and the sole crop maize, and it is the increased quantity and quality of biomass that typically improves soil quality in an intercropping system.

Chapter 4: Effects of intercropping on soil nitrogen mineralization and immobilization

4.1 Introduction

Soil inorganic nitrogen (N), generally in the form of ammonium (NH_4^+) or nitrate (NO_3^-), is a requirement for crop growth, but is also a significant contributor to agricultural pollution (Frimpong et al., 2012). Although NH_4^+ is relatively immobile, NO_3^- is highly mobile and can leach or run off from crops, resulting in excessively high levels of NO_3^- in rivers, lakes, and groundwater (Philippot and Germon, 2005). Ammonium can be nitrified to NO_3^-, which can then undergo denitrification and form nitrous oxide (N_2O), a potent greenhouse gas (GHG) (Figure 1.1). In order to minimize the environmental contamination associated with these N losses from the soil system, accumulation and leaching of NO_3^- and NH_4^+ must be avoided.

Given the current reality of climate change, understanding N_2O emissions from agriculture is particularly important. Due to anthropogenic activity, global N_2O emissions have increased by 40-50% since preindustrial times (Snyder et al., 2009). Nitrous oxide has a global warming potential (GWP) which is 296 times greater than carbon dioxide (CO_2) (Snyder et al., 2009). Over half of global anthropogenic N_2O emissions come from agriculture (Frimpong et al., 2012), so it is therefore important for the agricultural sector to take actions to mitigate N_2O emissions where possible. Although there are many factors which influence N_2O emissions, one of the primary factors is a lack of competition for soil N resources (Adviento-Borbe et al., 2007; McSwiney and Robertson, 2005). When plants are more successful than soil microorganisms in the competition for N resources, there is a low N_2O flux until the plant demand is reduced, at which time the microbes can become more active and N_2O emissions increase (McSwiney and Robertson, 2005). N_2O emissions are also impacted by temperature, moisture, pH, and the concentration of C and N (Adviento-Borbe et al., 2007). External sources of N which are applied to the soil, especially mineral N fertilizers, increase N_2O emissions (Frimpong et al., 2012). McSwiney and Robertson (2005) found that at low levels of N fertilizer application, soil N_2O emissions were correspondingly low. However, once the N additions reached a threshold value (101 kg N ha^{-1}), there was a sharp increase in N_2O emissions, suggesting that the competition for N maintained low N_2O emissions until inorganic N was in excess in the soil and the micro-organisms were able to use it.

The concentration of free inorganic N in the soil is regulated by the mineralization-immobilization turnover (MIT). Gross N mineralization is the process of forming NH_4^+ during the decomposition of soil organic matter (SOM) (Murphy et al., 2003), generally released by microorganisms (Andersen, 1999). The reverse process, gross N immobilization, is the assimilation of NH_4^+ and NO_3^- by soil microorganisms (Andersen, 1999). Both processes occur simultaneously and continuously in the soil, and the resulting equilibrium is the MIT. The rate of the MIT is impacted by the forms and quantity of N and C which are available in the soil; less complex, easily oxidizable C increases the rate of the MIT, whereas more complex sources of C slow it down (Gill, 2009). The MIT is also impacted by soil temperature, moisture, texture, and pH; the size of the soil microbial biomass (SMB); and the availability of inorganic N (Andersen, 1999). If the MIT can be synchronized with the crop demand for N, free inorganic N can be reduced along with the negative environmental consequences associated with it (Hauggaard-Nielsen et al., 2003). Ideally, net immobilization would then occur during the fallow period, and the immobilized N would then be mineralized in springtime to meet the nutrient needs of the new crop (McSwiney et al., 2010).

The C/N ratio of the soil and any added organic matter is frequently used to predict whether net mineralization or immobilization will occur in the soil. If the C/N ratio is below approximately 20-25:1, net mineralization will likely occur; higher ratios promote net immobilization (Myrold and Bottomley, 2008). Although it has been reported that maize, a high C/N ratio material, delays N mineralization (Frimpong et al., 2012), it is also argued that merely considering the C/N ratio is too simplistic (Haugaard-Nielsen et al., 2003). Lignin and polyphenol contribute to residue recalcitrance to decomposition regardless of the C/N ratio (Andersen, 1999; Kaewpradit et al., 2008), and must also be considered when predicting net N mineralization or immobilization. However, since both maize and soybean have low lignin and polyphenol contents (<15% lignin; <4% polyphenol), these compounds will have little influence on the mineralization and immobilization of these residues (Gentile et al., 2008; Nakhone and Tabatabai, 2008).

Combining crop residues and returning them to the soil results in organic matter of a different quantity and quality than if the residues had not been combined. This combination of crop residues can occur in an intercropping system, and particularly if a cereal and legume are intercropped together. Higher rates of mineralization and N_2O emissions are reported from leguminous sole crops (Pappa et al., 2011), whereas with sole cropped cereals like rice (*Oryza sativa* L.) and maize (*Zea mays* L.), net immobilization is more likely to occur (Kaewpradit et al., 2008; Sakala et al., 2000). If cereal and legume residues are combined, immediate net N immobilization delays the onset of net mineralization (Frimpong et al., 2012). For example, Pappa et al. (2011) found that intercropping spring barley (*Hordeum vulgare* cv. Westminster) and spring pea (*Pisum sativum* cv. Zero 4) in Scotland reduced N_2O emissions and NO_3^-

leaching. Similarly, combining rice straw and groundnut (*Arachis hypogaea* L.) residues reduced N_2O emissions in Thailand (Kaewpradit et al., 2008).

Gross N mineralization and immobilization rates are generally measured using the ^{15}N pool dilution technique, the calculations for which were developed by Kirkham and Bartholomew (1954). In this method, ^{15}N, a tracer isotope, is added to the soil, and the change in the soil isotope pool is measured over a period of time as mineralization adds ^{14}N to the NH_4^+ pool and immobilization removes both ^{14}N and ^{15}N (Schimel, 1996). In ^{15}N studies, the terms gross mineralization and immobilization are used to describe the production and consumption of NH_4^+ (Murphy et al., 2003; Myrold and Bottomley, 2008). Immobilization, as measured using the Kirkham and Bartholomew (1954) equations, also includes nitrification and volatilization (Andersen, 1999). However, when the soil pH is below 7, it can be assumed that volatilization is negligible (Sorensen, 2001), and Shammas (1986) reported that nitrification stops at a pH between 5-5.5. In the current study, the pH in the top 10 cm of all four treatments ranged from 5.32-5.64 (Table 3.4), indicating that the rates of volatilization and nitrification can also be assumed to be negligible. Therefore, the term immobilization will continue to be used, as it is by far the most significant contributor to NH_4^+ consumption.

Several other assumptions are made in the isotope pool dilution technique. It is assumed that ^{14}N and ^{15}N isotopes are equally used in all soil N transformation processes (Murphy et al., 2003; Schimel, 1996). However, if the incubation time is short (i.e., within 5 days), isotopic discrimination can be assumed to be negligible (Murphy et al., 2003). Similarly, it is assumed that the applied and the indigenous N pools are treated equally in all soil N transformation processes (Murphy et al., 2003). The first time point for sampling the soil should therefore be

24 h after applying the isotope tracer to allow the applied N pool to begin to equilibrate with the indigenous pool (Hood et al., 2003). It is also assumed that when the ^{15}N is applied to the soil, it is uniformly distributed throughout the soil column (Murphy et al., 2003; Schimel, 1996). If using a multiple injection system to distribute the isotope tracer through the soil column, Luxhøi et al. (2004) found that the measured rate of mineralization was not significantly different from the true mineralization rate. Since NH_4^+ is preferentially immobilized over NO_3^- (Rice and Tiedje, 1989), it can be assumed that nitrate immobilization is negligible (Sorensen, 2001). Finally, it is assumed that the rates of mineralization and immobilization are constant over the course of the experiment, which is a true assumption if the first sample is taken 24 h after the tracer application and the second sample is taken 72 hours later (Hood et al., 2003).

Little research has been conducted on how intercropping a cereal and a legume, such as maize and soybean, impacts the rates of gross N mineralization and immobilization. An experiment was therefore carried out to determine the impact of maize and soybean intercropping on gross N mineralization and immobilization, as compared to maize and soybean sole crops. The objectives of this research were:

1. To quantify differences in gross N mineralization and immobilization between the 1:2 and 2:3 maize/soybean intercrops and the rotated maize and soybean sole crops.
2. To quantify the changes in gross N mineralization and immobilization that have occurred over six cropping seasons in the 1:2 and 2:3 maize/soybean intercrops and the rotated maize and soybean sole crops.

4.2 Materials and methods

4.2.1 Experimental protocol

Gross N mineralization and immobilization rates were quantified using the isotope pool dilution technique, a method which minimizes soil disturbance (Davidson et al., 1991). The experiment was first conducted in November 2007 and repeated in November 2012 to compare gross mineralization and immobilization rates before the intercropping agroecosystems were established (2007), and after six years of intercropping (2012). The experiments were carried out in November, just prior to the delayed sowing maize, to minimize the differential impacts of the plants on the mineralization and immobilization rates. In 2007, four PVC cylinders were inserted in each treatment plot of the maize and soybean sole crops and the 2:3 intercrop. In 2012, six PVC cylinders were randomly inserted in each treatment plot of the maize and soybean sole crops and the 1:2 and 2:3 intercrops. Only two replicates per treatment were used in 2007 due to financial limitations. The cylinders were white PVC with an inner diameter of 6 cm and a length of 13 cm. The cylinders extended 12 cm into the soil, with 1 cm remaining above the soil. Ammonium sulphate ($(NH_4)_2SO_4$) fertilizer labelled with a % excess ^{15}N of 10% was applied to the soil within the PVC cylinders. A multi-injection system based on Monaghan (1995) was used to apply the ^{15}N as uniformly as possible throughout the soil within the PVC cylinder. Seven needles on the multi-injection system were inserted in the soil to a depth of 8 cm. Soil was prevented from entering the needles by the positioning of wires inside the needles (Figure 4.1). The wires were removed and the syringes were attached to the needles (Figure 4.2). A solution of $(^{15}NH_4^+)_2SO_4$ (~350 µg N ml^{-1}, 10% ^{15}N) was injected at a rate of approximately 10 µg N per g of dry soil. The volume of solution to be injected was dependent on the soil moisture content, and was adjusted to not increase the soil moisture content by more than 20-25% (Monaghan, 1995). The injection system was pulled up as the syringes were depressed, allowing for columns of solution to remain in the soil.

Figure 4.1 – Needles of the multi-injection system with wires partially inserted.

Figure 4.2 – Multi-injection system containing the ($^{15}NH_4^+$)$_2SO_4$ solution immediately prior to injection.

After 24 hours, the soil from half of the cylinders (two in 2007, three in 2012) was removed from each treatment plot and processed. After 96 hours, the soil from the remaining cylinders was removed and processed. The first samples were taken after 24 hours to ensure that the initial immobilization flux that follows N application had finished (Hood et al., 2003). The second time point, at 96 hours, was chosen to ensure sufficient time for N transformations to occur, but before re-mineralization began (Hood et al., 2003). The soil from each cylinder was placed in a plastic bag, crumbled, and thoroughly mixed. In the laboratory, a subsample of about 20 g of soil was taken from the bag and extracted with 100 ml of 2 M KCl. The solution of soil and KCl was shaken on an oscillating shaker at about 70 rev min^{-1} for 1 hour, and then gravity filtered (Whatman ashless grade 42). The soil extracts from 2007 were then frozen until 2012, at which time they were analyzed with the samples collected in 2012.

A diffusion process was used to determine the $^{15}NH_4^+$ concentration in each soil extract solution (Brooks et al., 1989; Chen and Dittert, 2008; Goerges and Dittert, 1998; Kirkham and Bartholomew, 1954). A 20 ml aliquot of the soil extract solution was pipetted into a clean 100 ml plastic urine sample jar. A folded piece of Teflon® tape (2 cm x 20 cm) enclosing a 7 mm diameter filter paper disc (Whatman GF/D) previously acidified with 10 µL of 5 M H_2SO_4 was laid across the top of the sample jar (Figure 4.3). Approximately 0.2 g MgO heavy powder was added to the jar, and the lid immediately screwed in place (Figure 4.4). The jar was slowly swirled to ensure full mixing of the MgO powder and the soil extract solution, and then shaken in the dark at 30°C on an oscillating shaker for 72 hours at about 50 rev min^{-1} (Mulvaney et al., 1997). After shaking, the discs were dried overnight in a desiccator over silica gel and 50 ml concentrated H_2SO_4. Once dry, the discs were stored in an Elisa plate until immediately prior to analysis, at which point they were weighed into tin capsules (Costech, 5x9 mm). The ^{15}N content of the discs was measured by direct combustion on 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.

Figure 4.3 – Teflon® tape enclosing the acidified filter disc and laid across the top of the diffusion container.

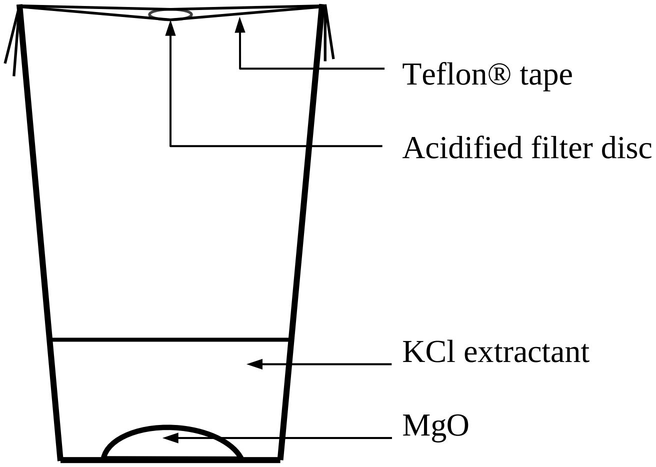

Figure 4.4 – Diffusion container set-up immediately prior to capping.

To verify and validate the process, a known quantity of $^{15}NH_4^+$ was dissolved in distilled water and processed as above to gauge the ^{15}N and NH_4^+ recovery by the discs. Acidified filter paper discs were processed as above, using KCl solution in the diffusion process, to gauge N contamination during the analysis period. In between uses, all equipment was immersed overnight in 10% HCl and rinsed with DI water.

The gross N mineralization and immobilization rates were calculated using the following formulae based on Kirkham and Bartholomew (1954):

$$m = \frac{(AT_1 - AT_2)}{\Delta t} \times \frac{\ln(AL_1/AL_2)}{\ln(AT_1/AT_2)} \qquad \text{(Equation 4.1)}$$

$$i = \frac{(AT_1 - AT_2)}{\Delta t} \times \frac{\ln(AT_1AL_1)/\ln(AT_2AL_2)}{\ln(AT_1/AT_2)} \qquad \text{(Equation 4.2)}$$

where m is the gross N mineralization rate (µg N g^{-1} soil d^{-1}); i is the gross N immobilization rate (µg N g^{-1} soil d^{-1}); AT is the total soil ammonium content (µg N g^{-1} soil); Δt is the related time interval (days); AL is the ^{15}N abundance within the ammonium pool (at % exc.); and the subscripts indicate the two time points (1 = 1 day, 2 = 4 days). The net rate of immobilization was calculated by subtracting the gross rate of mineralization from the gross rate of immobilization. The relative NH_4^+ immobilization was calculated by dividing the gross rate of N immobilization by the gross rate of N mineralization.

4.2.2 Statistical analysis

Statistical analyses were carried out using Statistica (StatSoft, Inc.; v.8.0, 2007). All data were examined for normal distribution using the Shapiro-Wilk (SW) test and for homogeneity of variance using the Levene test. Outliers were identified and removed using an outlier coefficient of 1.5. A repeated measures analysis of variance (ANOVA) was run to compare differences between the different treatments and to determine how the gross mineralization and immobilization rates changed between 2007 and 2012. Significant differences were further examined using Tukey's test with a p-value of 0.05.

4.3 Results

In 2007, no significant differences were found between treatments for gross N mineralization or immobilization rates (Table 4.1). In 2012, the gross N mineralization rate was significantly ($p<0.05$) greater in the 2:3 intercrop compared to the other treatments. The gross N immobilization rate was significantly greater in the 2:3 intercrop than in the soybean and 1:2 treatments in 2012. Although the 2012 gross N immobilization rate was greater in the 2:3 intercrop than the maize sole crop, this difference was not found to be significant. The gross N immobilization rate increased significantly ($p<0.05$) from 2007 to 2012 in the soybean sole crop and 2:3 intercrop. The gross N mineralization rate increased ($p=0.052$) from 2007 to 2012 in the 2:3 intercrop.

Table 4.1 – Soil nitrogen transformations in maize and soybean sole crops and 1:2 and 2:3 intercrops, 2012. Standard errors are given in parentheses.

	Year	**Treatment**			
		Maize	**Soybean**	**1:2 intercrop**	**2:3 intercrop**
Gross mineralization (µg N g^{-1} soil d^{-1})	2007	0.65 (0.09) ^{A}	0.63 (0.09) ^{A}	--	0.38 (0.13) ^{A}
	2012	0.89 (0.11) ^{A}	0.79 (0.08) ^{A}	0.97 (0.08) ^{A}	1.42 (0.08) ^{B}
Gross immobilization (µg N g^{-1} soil d^{-1})	2007	1.96 (0.29) ^{A}	1.48 (0.29) ^{A†}	--	1.48 (0.41) ^{A†}
	2012	2.92 (0.24) ^{BC}	2.46 (0.18) ^{AC†}	2.63 (0.18) ^{AC}	3.38 (0.19) ^{B†}
Net immobilization (µg N g^{-1} soil d^{-1})	2007	0.86 (0.19) ^{A}	1.99 (0.19) ^{B}	--	1.23 (0.24) ^{AB}
	2012	1.45 (0.10) ^{A}	2.03 (0.13) ^{BC}	1.66 (0.10) ^{AC}	1.96 (0.10) ^{BC}
Relative NH_4^+ immobilization	2007	2.23 (0.28) ^{A}	3.66 (0.33) ^{B}	--	2.26 (0.56) ^{AB}
	2012	2.87 (0.10) ^{AC}	3.07 (0.14) ^{AC}	2.72 (0.10) ^{BC}	2.45 (0.09) ^{B}

Values followed by the same upper case letters, comparing treatments, are not significantly different at $p<0.05$.
† indicates the values were significantly different ($p<0.05$) between years.
^{15}N and NH_4^+ values used to calculate the above rates are presented in Appendix B.

In both years and in all four treatments, net N immobilization occurred (Table 4.1). In 2007 and 2012, the rate of net immobilization was significantly lower in the maize sole crop than in the soybean and 2:3 treatment plots. In both years, net immobilization was greatest in the maize sole crop, although not significantly greater than in the intercrops. Although not significant, the rate of net N immobilization increased from 2007 to 2012 in all treatment plots. Similarly, the relative NH_4^+ immobilization was greatest in the soybean sole crop. In 2007, it was significantly greater in the soybean sole crop than in the maize sole crop. In 2012, the relative NH_4^+ immobilization was significantly lower in the 2:3 intercrop than in the maize and soybean sole crops. The percent recovery of $^{15}NH_4^+$ by the acidified discs was 86.6%. The measured atom percent of the ^{15}N recovered was 9.60%, only slightly lower than the actual 10%.

4.4 Discussion

Net immobilization was measured in all treatments in 2007 and 2012 (Table 4.1), which generally occurs when the C/N ratio of the residue is above 20-25 (Myrold and Bottomley, 2008). As the lignin and polyphenol contents of maize and soybean residues are low (Gentile et al., 2008; Nakhone and Tabatabai, 2008), these compounds likely had little influence on the mineralization and immobilization of these residues (Gentile et al., 2008). Therefore, the C/N ratio of the residue could be used to predict net mineralization and immobilization rates without excessive oversimplification (Haugaard-Nielsen et al., 2003). At the current study site in 2011, the measured C/N ratio for soybean residue was 32.0 and for maize residue was 63.9 (Bichel, 2012), both of which are above the threshold for net immobilization. Based on these C/N ratios, it would be expected that the gross mineralization rate would be lowest in the maize treatments and highest in the soybean treatments.

In 2007, the experimental plots were just established, and the intercropping treatments likely had not yet had a chance to impact the rates of mineralization and immobilization. However, in 2012 the gross mineralization rate was significantly higher in the 2:3 intercrop compared to the other treatments, and was lowest in the soybean sole crop. Similarly, it would be expected that the gross immobilization rate would be highest in the sole crop maize treatments and lowest in the sole crop soybean treatments. In 2012 the highest gross immobilization rate was from the 2:3 intercrop, although it was not significantly different from that of the maize sole crop, and lowest in the soybean sole crop. It is likely that the rotation of maize and soybean in the sole crop treatment plots resulted in greater rates of mineralization than in sole cropped maize, and lower rates of mineralization than in sole cropped soybean, as was found by Barrios et al. (1996), who reported that the gross rate of mineralization was slightly greater in a maize and cow pea (*Vigna unguiculata* L.) rotation than in a maize sole crop.

As the soil samples were collected in November at planting time, in 2012 maize residues had most recently been applied to the soybean sole crop plots, and soybean residues had most recently been applied to the maize plots. Ehaliotis et al. (1998) report that in temperate regions, approximately 30% of legume residues are not decomposed during one cropping season; this increases to 50-85% for poorer quality residues like maize. In a previous study at the current study site, Vachon (2008) found that 312 days after applying crop residues, about 33% of residues remained in the soybean sole crop, 40% remained in both intercrops, and 55% remained in the maize sole crop. Therefore, both maize and soybean residues were present at different ratios in the maize and soybean sole crops, and likely interacted in complex ways to modify the gross mineralization and immobilization rates (Barrios et al., 1996).

The quantity of maize and soybean residues returned to the soil may have impacted gross N mineralization and immobilization rates. Due to the different C/N ratios of maize and soybean, the quantity of each residue applied to the soil contributed to the varying gross N mineralization and immobilization rates. The much greater C/N ratio of maize residues prompted a higher rate of immobilization than that of the soybean residues. For example, Sakala et al. (2000) found that in a laboratory experiment simulating an intercrop system where maize stover was applied at three times the weight of pigeon pea (*Cajanus cajan* L.) leaves, net N immobilization occurred over the entire 500 days of the study. If twice the weight of pigeon pea leaves than maize stover was applied to the soil, net immobilization only occurred for 300 days, indicating that a very large quantity of legume residue must be applied to the soil in order for net mineralization to occur (Sakala et al., 2000). In the present study, when crop residue was returned to the soil at the end of the 2011-2012 growing season, biomass input from maize residues was 6.3 times greater than that from soybean residues in the 2:3 intercrop, and 6.6 times greater in the 1:2 intercrop (Table 3.3). Although pigeon pea residues contain more lignin than soybean residues (Gentile et al., 2008; Sakala et al., 2000), and hence have a lower mineralization rate, it is still likely that the greater biomass input from maize residues may contribute to long-term immobilization in the intercropping systems.

Not only does crop residue contribute to the rates of gross N mineralization and immobilization, but the light fraction (LF) does as well. Compton and Boone (2002) proposed that LF and crop residues have similar mineralization and immobilization dynamics, indicating that the quantity and quality of LF are influential to these processes. In the current study, the C/N ratio of the LF ranged from 17.86 in the soybean sole crop to 18.33 in the 2:3 intercrop (Table 3.7). These ratios indicated the potential for net mineralization to occur, as they are lower than the threshold of 20-25 (Myrold and Bottomley, 2008). However the quantity of crop residue applied to the soil was much greater than the quantity of LF, the C/N ratio of the residue was dominant over that of the LF, and net immobilization occurred.

The size of the SMB impacts the rates of gross N mineralization and immobilization (Andersen, 1999). The greatest SMB-C was found in the 2:3 intercrop (Table 3.8), as were the highest rates of gross N mineralization and immobilization, and the second highest rate of net N immobilization (Table 4.1). Similarly, Tracy and Frank (1998) found a significant correlation ($r^2 = 0.85$) between the rate of net N mineralization and SMB-C in a grassland study in Wyoming, and Zaman et al. (1999) found a significant correlation ($r^2 = 0.23$) between the rate of gross N mineralization and the SMB-C in a fertilization study in New Zealand.

Very few studies have been published investigating the impact of intercropping on N mineralization and immobilization rates. Frimpong et al. (2012) and Sakala et al. (2000) simulated intercrops by combining different quantities of maize and legume residues to evaluate the impact of intercropping maize and a legume on net mineralization rates. Both studies found that net mineralization occurred when sole legume residues were added to soil, and net immobilization occurred when sole maize residues were added to the soil. However, Frimpong et al. (2012) found that net mineralization occurred when equal quantities of legume and maize residues were added, whereas Sakala et al. (2000) found that net immobilization occurred when equal quantities were added. This is different from the current study, where net immobilization was found in the maize and soybean sole crops, as well as in both intercrops. However, the sole crops in this study were rotations of maize and soybean instead of monocropped as in the study by Sakala et al. (2000) and Frimpong et al. (2012), suggesting that the maize residues likely caused the net immobilization.

It is expected that the rates of gross N mineralization and immobilization will not be constant throughout the year. For example, Recous et al. (1999) found that the rate of gross N immobilization was highest in the autumn when straw residues were applied, and this rate decreased until spring, at which point it began to increase again. In the same study, gross mineralization followed a similar pattern, although it decreased less over the winter than the gross immobilization rate, meaning that net mineralization occurred from late autumn until late summer (Recous et al., 1999). Therefore, although it is useful to know the pattern of mineralization and immobilization at the time of crop planting, as in the current study, it is also necessary to determine the seasonal fluctuation in mineralization and immobilization rates.

The relative NH_4^+ immobilization is also referred to as the immobilization/mineralization ratio. In both 2007 and 2012, it was found to be greatest in the soybean sole crop and least in the 2:3 intercrop, indicating that there was a larger difference between the gross immobilization and mineralization rates in the soybean sole crop compared to the other treatments. The relative NH_4^+ immobilization was lowest in the intercrops, due to a more equal quantity of maize and soybean residues, as compared to the sole crops which were primarily either maize or soybean. Nonetheless, since the relative NH_4^+ immobilization values were greater than 1 in all four treatments, each had a high potential for decreasing the NH_4^+ pool (Vervaet et al., 2004), although this was least in the 2:3 intercrop.

From 2007 to 2012, gross mineralization, gross immobilization, net immobilization, and relative NH_4^+ immobilization increased in all treatments, except for the relative NH_4^+

immobilization in the soybean sole crop, which decreased during this time. Given that mineralization and immobilization were not quantified in the 1:2 intercrop in 2007, a comparison to the 2012 data was not possible. These changes were only significant for the gross immobilization rate in the soybean sole crop and 2:3 intercrop. However, the increase does indicate a more active microbial population in 2012 (Accoe et al., 2004), and hence a reduced potential for inorganic N loss.

4.5 Conclusions

Each of the sole crops and intercrops generally resulted in higher gross mineralization rates, gross immobilization rates, net immobilization rates, and relative NH_4^+ immobilization during the experimental time period in 2007 and 2012. Only in the soybean sole crop did the relative NH_4^+ immobilization decrease, signifying the increased importance of mineralization as compared to immobilization. Net N immobilization occurred in all four treatment plots, at significantly higher rates in the soybean sole crop and the 2:3 intercrop, which is desired during the crop fallow period.

Chapter 5: Conclusions and recommendations

5.1 Summary and conclusions

In order to meet increasing population demand for food, feed, and fibre, agricultural intensification has been occurring at a global scale. However, agricultural intensification has also resulted in environmental degradation, including poorer soil quality and high emissions of greenhouse gases (GHGs) like nitrous oxide (N_2O). Sustainable agroecosystem management practices such as intercropping, where more than one crop is planted on the same plot of land at the same time, promotes the complementary use of soil nutrients particularly when a legume and cereal are intercropped. It was therefore hypothesized that intercropping maize and soybean would improve soil quality and increase inorganic nitrogen (N) retention in the soil, thereby reducing N_2O emissions. The objectives of the experiment were therefore to quantify the soil gross N mineralization and immobilization rates, and the temporal changes in soil biological and chemical characteristics after six years of maize/soybean intercropping.

It was found that intercropping significantly improved soil quality from 2008 to 2012, as indicated by the soil organic C (SOC), total nitrogen (TN), light fraction organic matter (LF), and soil microbial biomass carbon (SMB-C). However, only SMB-C showed that intercropping was significantly better than the control sole crop treatments of rotated maize and soybean, which also increased the soil quality over the same time period. The other indicators, including SOC, TN, and LF, demonstrated improved soil quality in the intercrops which was comparable to the improvements seen in the control plots. This lack of significant differences between treatments was likely due to the fact that the quantity of biomass produced by the intercrops was similar to the quantity produced by the sole crops. Previous intercropping experiments found that intercrops produced more biomass than the corresponding sole crops, a finding which did not occur in the present study. The large inter-row spacing and delayed planting of soybean likely meant that the full yield benefits of intercropping could not be observed in the present study.

Intercropping in the 1:2 and 2:3 intercrops resulted in higher rates of gross mineralization than the sole crops, and the 2:3 intercrop resulted in higher rates of gross immobilization than in the other treatments. However, the high rate of gross mineralization resulted in a low relative NH_4^+ immobilization in both intercrops, signifying a lower potential for reducing soil NH_4^+ concentrations than in the sole crop treatments. Net N immobilization occurred in all treatment plots, which was desired at the end of the fallow period to reduce N losses from the soil. Based on the desire for high net N immobilization during the fallow period, the 2:3 intercrop appeared to perform better than the 1:2 intercrop.

5.2 Recommendations for future research

The spatial and temporal design of planting the maize and soybean was different in this experiment from those of most other intercropping experiments. The inter-row spacing was greater than average, and the soybean was planted a month after the maize. Previous studies have nearly all identified a positive impact of intercropping on crop yield and residue biomass; however this was not observed over the six years of intercropping in Balcarce, Argentina. It is speculated that the inter-row spacing and the staggered planting time impacted the crop yield and biomass production, and hence soil quality and the ability for C sequestration. It is therefore recommended to investigate the impact of row spacing and staggered planting time on biomass production, soil quality, and C sequestration. The optimal inter-row spacing and time of planting could then be determined in order to maximize the biomass production, and hence the land equivalent ratio (LER). Greater quantities of crop residue returned to the soil would likely result in greater benefits from intercropping on soil quality.

Although this study determined the gross rates of N mineralization and immobilization at the time of planting in 2007 and 2012, these data only characterize soil N transformations at one time in the seasonal cycle. In 2012, the soil N transformations were only determined at one time point to enable an initial comparison between 2007 and 2012. Net N immobilization was found in all four cropping systems in November 2007 and 2012; however it is unknown whether net N immobilization occurred throughout the preceding fallow period, or whether the rate of gross N mineralization increased during the subsequent growing season to result in net mineralization. It is therefore recommended that the gross mineralization and immobilization rates be studied throughout the year to allow for a more thorough understanding of the impact of intercropping on soil N transformations.

References

Abaidoo, R. C., & Van Kessel, C. (1989). ^{15}N-uptake, N_2-fixation and rhizobial interstrain competition in soybean and bean, intercropped with maize. *Soil Biology & Biochemistry, 21*(1), 155–159.

Abdalla, M., Osborne, B., Lanigan, G., Forristal, D., Williams, M., Smith, P., & Jones, M. B. (2013). Conservation tillage systems: a review of its consequences for greenhouse gas emissions. *Soil Use and Management, 29*, 199–209.

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

Adviento-Borbe, M. A. A., Haddix, M. L., Binder, D. L., Walters, D. T., & Dobermann, A. (2007). Soil greenhouse gas fluxes and global warming potential in four high-yielding maize systems. *Global Change Biology*, *13*, 1972–1988.

Ahmed, S., & Rao, M. R. (1982). Performance of maize-soybean intercrop combination in the tropics: Results of a multi-location study. *Field Crops Research, 5*, 147–161.

Andersen, M. K. (1999). *Short term N mineralization-immobilization turnover in soil after incorporation of green manures as a function of plant litter quality and soil temperature - A comparison of analytical models with the numerical model FLUAZ* [Master's thesis]. University of Aarhus.

Andersen, M., & Jensen, L. (2001). Low soil temperature effects on short-term gross N mineralization–immobilization turnover after incorporation of a green manure. *Soil Biology and Biochemistry, 33*, 511–521.

Anderson, T.-H., & Dousch, 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.

Adviento-Borbe, M. A. A., Haddix, M. L., Binder, D. L., Walters, D. T., & Dobermann, A. (2007). Soil greenhouse gas fluxes and global warming potential in four high-yielding maize systems. *Global Change Biology*, *13*, 1972–1988.

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

Austin, A. T., Piñeiro, G., & Gonzalez-Polo, M. (2006). More is less: agricultural impacts on the N cycle in Argentina. *Biogeochemistry*, *79*, 45–60.

Baldé, A. B., Scopel, E., Affholder, F., Corbeels, M., Da Silva, F. A. M., Xavier, J. H. V., & Wery, J. (2011). Agronomic performance of no-tillage relay intercropping with maize under smallholder conditions in Central Brazil. *Field Crops Research*, *124*, 240–251.

Balota, E. L., Colozzi-Filho, A., Andrade, D. S., & Dick, R. P. (2003). Microbial biomass in soils under different tillage and crop rotation systems. *Biology and Fertility of Soils*, *38*, 15–20.

Barrios, E., Buresh, R. J., & Sprent, J. I. (1996). Nitrogen mineralization in density fractions of soil organic matter from maize and legume cropping systems. *Soil Biology and Biochemistry*, *28*, 1459–1465.

Bastida, F., Zsolnay, A., Hernández, T., & García, C. (2008). Past, present and future of soil quality indices: A biological perspective. *Geoderma*, *147*, 159–171.

Bavin, T. K., Griffis, T. J., Baker, J. M., & Venterea, R. T. (2009). Impact of reduced tillage and cover cropping on the greenhouse gas budget of a maize/soybean rotation ecosystem. *Agriculture, Ecosystems & Environment*, *134*, 234–242.

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

Bell, L. W., Sparling, B., Tenuta, M., & Entz, M. H. (2012). Soil profile carbon and nutrient stocks under long-term conventional and organic crop and alfalfa-crop rotations and re-established grassland. *Agriculture, Ecosystems & Environment*, *158*, 156–163.

Berhongaray, G., Alvarez, R., De Paepe, J., Caride, C., & Cantet, R. (2013). Land use effects on soil carbon in the Argentine Pampas. *Geoderma*, *192*, 97–110.

Bichel, A. (2013). *Applied soybean and maize residue contributions to soil organic matter in a temperate soybean / maize intercropping system*. [Master's thesis]. University of Waterloo.

Bormann, H., Breuer, L., Gräff, T., & Huisman, J. A. (2007). Analysing the effects of soil properties changes associated with land use changes on the simulated water balance: A comparison of three hydrological catchment models for scenario analysis. *Ecological Modelling*, *209*, 29–40.

Bouwman, A. F. (2001). *Global estimates of gaseous emissions of NH_3, NO and N_2O from agricultural land* (p. 106). Rome.

Brooks, P. D., Stark, J. M., McInteer, B. B., & Preston, T. (1989). Diffusion method to prepare soil extracts for automated nitrogen-15 analysis. *Soil Science Society of America Journal, 53*, 1707–1711.

Campbell, C. A., Biederbeck, V. O., Wen, G., Zentner, R. P., Schoenau, J., & Hahn, D. (1999). Seasonal trends in selected soil biochemical attributes: Effects of crop rotation in the semiarid prairie. *Canadian Journal of Soil Science*, *79*, 73–84.

Carreño, L., Frank, F. C., & Viglizzo, E. F. (2012). Tradeoffs between economic and ecosystem services in Argentina during 50 years of land-use change. *Agriculture, Ecosystems & Environment*, *154*, 68–77.

Carruthers, K., Prithiviraj, B., Fe, Q., Cloutier, D., Martin, R. C., & Smith, D. L. (2000). Intercropping corn with soybean, lupin and forages: yield component responses. *European Journal of Agronomy, 12*(2), 103–115.

Caviglia, O. P., & Andrade, F. H. (2010). Sustainable intensification of agriculture in the Argentinean Pampas: Capture and use efficiency of environmental resources. *The Americas Journal of Plant Science and Biotechnology, 3*(Special Issue 1), 1–8.

Chen, R. R., & Dittert, K. (2008). Diffusion technique for ^{15}N and inorganic N analysis of low-N aqueous solutions and Kjeldahl digests. *Rapid Communications in Mass Spectrometry, 22*, 1727–1734.

Christensen, J. H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., et al. (2007). Regional climate projections. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, et al. (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 847–940). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Clement, A., Chalifour, F. P., Bharati, M. P., & Gendron, G. (1992). Effects of nitrogen supply and spatial arrangement on the grain yield of a maize/soybean intercrop in a humid subtropical climate. *Canadian Journal of Plant Science*, *72*, 57–67.

Coll, L., Cerrudo, A., Rizzalli, R., Monzon, J. P., & Andrade, F. H. (2012). Capture and use of water and radiation in summer intercrops in the south-east Pampas of Argentina. *Field Crops Research*, *134*, 105–113.

Compton, J. E., & Boone, R. D. (2002). Soil nitrogen transformations and the role of light fraction organic matter in forest soils. *Soil Biology and Biochemistry, 34*, 933–943.

Costantini, A., De-Polli, H., Galarza, C., Rossiello, R. P., & Romaniuk, R. (2006). Total and mineralizable soil carbon as affected by tillage in the Argentinean Pampas. *Soil and Tillage Research*, *88*, 274–278.

Davidson, E. A., Hart, S. C., Shanks, C. A., & Firestone, M. K. (1991). Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. *Journal of Soil Science, 42*, 349–355.

Denman, K. L., Brasseur, G., Chidthaisong, A., Ciais, P., Cox, P. M., Dickinson, R. E., Hauglustaine, D., et al. (2007). Couplings between changes in the climate system and biogeochemistry. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, et al. (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 499–587). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Dick, W. A., & Gregorich, E. G. (2004). Developing and maintaining soil organic matter levels. In P. Schjønning, S. Elmholt, & B. T. Christensen (Eds.), *Managing Soil Quality: Challenges in Modern Agriculture* (pp. 103–120). Cambridge: CABI Publishing.

Diekow, J., Mielniczuk, J., Knicker, H., Bayer, C., Dick, D. P., & Kögel-Knabner, I. (2005). Soil C and N stocks as affected by cropping systems and nitrogen fertilization in a southern Brazil Acrisol managed under no-tillage for 17 years. *Soil and Tillage Research, 81*(1), 87–95.

Dominguez, G., Diovisalvi, N., Studdert, G., & Monterubbianesi, M. (2009). Soil organic C and N fractions under continuous cropping with contrasting tillage systems on mollisols of the southeastern Pampas. *Soil and Tillage Research*, *102*, 93–100.

Dyer, L. (2010). *Evaluation of soil chemical and physical characteristics in a complex agroecosystem in the Argentine Pampa*. [Master's thesis]. University of Waterloo.

Dyer, L., Oelbermann, M., & 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.

Ehaliotis, C., Cadisch, G., & Giller, K. E. (1998). Substrate amendments can alter microbial dynamics and N availability from maize residues to subsequent crops. *Soil Biology and Biochemistry*, *30*, 1281–1292.

Evenson, R. E., & Gollin, D. (2003). Assessing the impact of the green revolution, 1960 to 2000. *Science*, *300*, 758–762.

Fabrizzi, K. P., Morón, A., & García, F. O. (2003). Soil carbon and nitrogen organic fractions in degraded vs. non-degraded mollisols in Argentina. *Soil Science Society of America Journal*, *67*, 1831–1841.

Forster, P. V., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D. W., Haywood, J., et al. (2007). Changes in atmospheric constituents and in radiative forcing. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, et al. (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 129–234). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Frimpong, K. A., Yawson, D. O., Agyarko, K., & Baggs, E. M. (2012). N_2O emission and mineral N release in a tropical acrisol incorporated with mixed cowpea and maize residues. *Agronomy*, *2*, 167–186.

García-Orenes, F., Guerrero, C., Mataix-Solera, J., Navarro-Pedreño, J., Gómez, I., & Mataix-Beneyto, J. (2005). Factors controlling the aggregate stability and bulk density in two different degraded soils amended with biosolids. *Soil and Tillage Research*, *82*, 65–76.

Garnett, T. (2013). Food sustainability: problems, perspectives and solutions. *Proceedings of the Nutrition Society*, *72*, 29–39.

Gao, Y., Duan, A., Qiu, X., Liu, Z., Sun, J., Zhang, J., & Wang, H. (2010). Distribution of roots and root length density in a maize/soybean strip intercropping system. *Agricultural Water Management*, *98*(1), 199–212.

Gentile, R., Vanlauwe, B., Chivenge, P., & Six, J. (2008). Interactive effects from combining fertilizer and organic residue inputs on nitrogen transformations. *Soil Biology and Biochemistry*, *40*(9), 2375–2384.

Gill, S. (2009). *Understanding mechanisms responsible for root-induced nitrogen mineralization in some leguminous and non-leguminous crops* (Doctoral dissertation). Bahauddin Zakariya University.

Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M. C., & Seoane, S. (2005). Different approaches to evaluating soil quality using biochemical properties. *Soil Biology and Biochemistry*, *37*, 877–887.

Goerges, T., & Dittert, K. (1998). Improved diffusion technique for ^{15}N: ^{14}N analysis of ammonium and nitrate from aqueous samples by stable isotope spectrometry. *Communications in Soil Science and Plant Analysis*, *29*, 361–368.

Gosling, P., Parsons, N., & Bending, G. D. (2013). What are the primary factors controlling the light fraction and particulate soil organic matter content of agricultural soils? *Biology and Fertility of Soils*, *49*, In press.

Granatstein, D. M., Bezdicek, D. F., Cochran, V. L., Elliott, L. E., & Hammel, J. (1987). Long-term tillage and rotation effects on soil microbial biomass, carbon and nitrogen. *Biology and Fertility of Soils*, *5*(3), 265–270.

Gregorich, E. G., & Beare, M. H. (2008). Physically uncomplexed organic matter. In M. R. Carter & E. G. Gregorich (Eds.), *Soil Sampling and Methods of Analysis* (2nd ed., pp. 607–616). Boca Raton: Taylor & Francis Group.

Griffin, T. S. (2008). Nitrogen availability. In J. S. Schepers, W. R. Raun, R. F. Follett, R. H. Fox, & G. W. Randall (Eds.), *Nitrogen in Agricultural Systems* (pp. 613–646). Madison: American Society of Agronomy.

Guo, L. B., & Gifford, R. M. (2002). Soil carbon stocks and land use change: A meta analysis. *Global Change Biology*, *8*, 345–360.

Halpern, M. T., Whalen, J. K., & Madramootoo, C. A. (2010). Long-term tillage and residue management influences soil carbon and nitrogen dynamics. *Soil Science Society of America Journal*, *74*(4), 1211–1217.

Hauggaard-Nielsen, H., Ambus, P., & 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., Ambus, P., & Jensen, E. S. (2003). The comparison of nitrogen use and leaching in sole cropped versus intercropped pea and barley. *Nutrient Cycling in Agroecosystems*, *65*, 289–300.

Heitkamp, F., Raupp, J., & Ludwig, B. (2011). Effects of fertilizer type and rate on labile soil fractions of a sandy Cambisol - long-term and short-term dynamics. *Journal of Plant Nutrition and Soil Science*, *174*, 121–127.

Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J., … Zhang, F. (2011). P for two, sharing a scarce resource: soil phosphorus acquisition in the rhizosphere of intercropped species. *Plant Physiology*, *156*, 1078–1086.

Hood, R., Bautista, E., & Heiling, M. (2003). Gross mineralization and plant N uptake from animal manures under non-N limiting conditions, measured using ^{15}N isotope dilution techniques. *Phytochemistry Reviews*, *2*, 113–119.

Jannoura, R., Bruns, C., & Joergensen, R. G. (2013). Organic fertilizer effects on pea yield, nutrient uptake, microbial root colonization and soil microbial biomass indices in organic farming systems. *European Journal of Agronomy*, *49*, 32–41.

Jensen, E. S., & Hauggaard-Nielsen, H. (2003). How can increased use of biological N_2 fixation in agriculture benefit the environment? *Plant and Soil*, *252*, 177–186.

Jobbágy, E. G., & Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. *Ecological Applications*, *10*, 423–436.

Johnson, J. M.-F., Barbour, N. W., & Weyers, S. L. (2007). Chemical composition of crop biomass impacts its decomposition. *Soil Science Society of America Journal*, *71*, 155–162.

Kaewpradit, W., Toomsan, B., Vityakon, P., Limpinuntana, V., Saenjan, P., Jogloy, S., Patanothai, A., et al. (2008). Regulating mineral N release and greenhouse gas emissions by mixing groundnut residues and rice straw under field conditions. *European Journal of Soil Science*, *59*(4), 640–652.

Kallenbach, C., & Grandy, A. S. (2011). Controls over soil microbial biomass responses to carbon amendments in agricultural systems: A meta-analysis. *Agriculture, Ecosystems & Environment*, *144*, 241–252.

Karlen, D. L., Mausbach, M. J., Doran, J. W., Cline, R. G., Harris, R. F., & Schuman, G. E. (1997). Soil quality: A concept, definition, and framework for evaluation. *Soil Science Society of America Journal*, *61*, 4–10.

Kaur, B., Gupta, S. R., & Singh, G. (2000). Soil carbon, microbial activity and nitrogen availability in agroforestry systems on moderately alkaline soils in northern India. *Applied Soil Ecology*, *15*, 283–294.

Kirkham, D., & Bartholomew, W. V. (1954). Equations for following nutrient transformations in soil, utilizing tracer data. *Soil Science Society of America Journal*, *18*(1), 33–34.

Kolawole, G. O. (2012). Effect of phosphorus fertilizer application on the performance of maize / soybean intercrop in the southern Guinea savanna of Nigeria. *Archives of Agronomy and Soil Science*, *58*(2), 189–198.

Lal, R. (2003). Cropping systems and soil quality. *Journal of Crop Production*, *8*, 33–52.

Ledgard, S. F., Jarvis, S. C., & Hatch, D. J. (1998). Short-term nitrogen fluxes in grassland soils under different long-term nitrogen management regimes. *Soil Biology and Biochemistry*, *30*, 1233–1241.

Leite, L. F. C., Oliveira, F. C., Araujo, A. S. F., Galvao, S. R. S., Lemos, J. O., & Silva, E. F. L. (2010). Soil organic carbon and biological indicators in an Acrisol under tillage systems and organic management in north-eastern Brazil. *Australian Journal of Soil Research*, *48*, 258–265.

Liang, B., Yang, X., He, X., & Zhou, J. (2011). Effects of 17-year fertilization on soil microbial biomass C and N and soluble organic C and N in loessial soil during maize growth. *Biology and Fertility of Soils*, *47*, 121–128.

Luxhøi, J., Nielsen, N. E., & Jensen, L. S. (2004). Effect of soil heterogeneity on gross nitrogen mineralization measured by ^{15}N-pool dilution techniques. *Plant and Soil*, *262*, 263–275.

Mandal, A., Patra, A. K., Singh, D., Swarup, A., & Ebhin Masto, R. (2007). Effect of long-term application of manure and fertilizer on biological and biochemical activities in soil during crop development stages. *Bioresource Technology*, *98*, 3585–3592.

Martin, R. C., Astatkie, T., & Cooper, J. M. (1998). The effect of soybean variety on corn-soybean intercrop biomass and protein yields. *Canadian Journal of Plant Science*, *78,* 289–294.

Martin, R. C., Voldeng, D., & Smith, D. L. (1990). Intercropping corn and soybean for silage in a cool-temperate region: Yield, protein and economic effects. *Field Crops Research*, *23*, 295–310.

Matos, E. S., Freese, D., Böhm, C., Quinkenstein, A., & Hüttl, R. F. (2012). Organic matter dynamics in reclaimed lignite mine soils under Robinia pseudacacia L plantations of different ages in Germany. *Communications in Soil Science and Plant Analysis*, *43*, 745–755.

Mbah, E. U., Muoneke, C. O., & Okpara, D. A. (2007). Effect of compound fertilizer on the yield and productivity of soybean and maize in soybean/maize intercrop in southeastern Nigeria. *Tropical and Subtropical Agroecosystems*, *7*, 87–95.

McSwiney, C. P., & Robertson, G. P. (2005). Nonlinear response of N_2O flux to incremental fertilizer addition in a continuous maize (Zea mays L.) cropping system. *Global Change Biology*, *11*, 1712–1719.

McSwiney, C. P., Snapp, S. S., & Gentry, L. E. (2010). Use of N immobilization to tighten the N cycle in conventional agroecosystems. *Ecological Applications*, *20*(3), 648–662.

Meehl, G. A., Stocker, T. F., Collins, W. D., Friedlingstein, P., Gaye, A. T., Gregory, J. M., … Zhao, Z.-C. (2007). Global climate projections. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, … H. L. Miller (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 747–845). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Midwood, A. J., & Boutton, T. W. (1998). Soil carbonate decomposition by acid has little effect on delta 13C of organic matter. *Soil Biology & Biochemistry*, *30*(10/11), 1301–1307.

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

Monaghan, R. (1995). Errors in estimates of gross rates of nitrogen mineralization due to non-uniform distributions of ^{15}N label. *Soil Biology & Biochemistry*, *27*, 855–859.

Monaghan, R., & Barraclough, D. (1997). Contributions to N mineralization from soil macroorganic matter fractions incorporated into two field soils. *Soil Biology & Biochemistry, 29*, 1215–1223.

Muller, A., Olesen, J., Davis, J., Smith, L., Dytrtová, K., Gattinger, A., … Niggli, U. (2012). Reducing global warming and adapting to climate change: The potential of organic agriculture. Frick, Switzerland.

Mulvaney, R. L., Khan, S. A., Stevens, W. B., & Mulvaney, C. S. (1997). Improved diffusion methods for determination of inorganic nitrogen in soil extracts and water. *Biology and Fertility of Soils*, *24*, 413–420.

Muoneke, C. O., Ogwuche, M. A. O., & Kalu, B. A. (2007). Effect of maize planting density on the performance of maize / soybean intercropping system in a guinea savannah agroecosystem. *African Journal of Agricultural Research, 2*, 667–677.

Murphy, D. V, Recous, S., Stockdale, E. A., Fillery, I. R. P., Jensen, L. S., Hatch, D. J., & Goulding, K. W. T. (2003). Gross nitrogen fluxes in soil: Theory, measurement and application of ^{15}N pool dilution techniques. *Advances in Agronomy*, *79*, 69–118.

Myrold, D. D., & Bottomley, P. J. (2008). Nitrogen mineralization and immobilization. In J. S. Schepers, W. R. Raun, R. F. Follett, R. H. Fox, & G. W. Randall (Eds.), *Nitrogen in Agricultural Systems* (pp. 157–172). Madison: American Society of Agronomy.

Nakhone, L. N., & Tabatabai, M. A. (2008). Nitrogen mineralization of leguminous crops in soils. *Journal of Plant Nutrition and Soil Science, 171*, 231–241.

Nelson, G. C., Rosegrant, M. W., Koo, J., Robertson, R., Sulser, T., Zhu, T., Ringler, C., et al. (2009). *Climate change: Impacts on agriculture and costs of adaptation*. *Food Policy* (p. 19). Washington, D.C.

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

Osborne, T., Rose, G., & Wheeler, T. (2012). Variation in the global-scale impacts of climate change on crop productivity due to climate model uncertainty and adaptation. *Agricultural and Forest Meteorology, In Press*.

Pappa, V. A., Rees, R. M., Walker, R. L., Baddeley, J. A., & Watson, C. A. (2011). Nitrous oxide emissions and nitrate leaching in an arable rotation resulting from the presence of an intercrop. *Agriculture, Ecosystems & Environment*, *141*(1-2), 153–161.

Paré, T., Gregorich, E. G., & Nelson, S. D. (2000). Mineralization of nitrogen from crop residues and N recovery by maize inoculated with vesicular-arbuscular mycorrhizal fungi. *Plant and Soil, 218*, 11–20.

Philippot, L., & Germon, J. C. (2005). Contribution of bacteria to intial input and cycling of nitrogen in soils. In F. Buscot & A. Varma (Eds.), *Soil Biology, Volume 3 Microorganisms in Soils: Roles in Genesis and Functions* (Vol. 3, pp. 159–176). Berlin, Heidelberg: Springer-Verlag.

Ponsioen, T. C., & Blonk, T. J. (2012). Calculating land use change in carbon footprints of agricultural products as an impact of current land use. *Journal of Cleaner Production*, *28*, 120–126.

Powlson, D. S., Gregory, P. J., Whalley, W. R., Quinton, J. N., Hopkins, D. W., Whitmore, A. P., … Goulding, K. W. T. (2011). Soil management in relation to sustainable agriculture and ecosystem services. *Food Policy*, *36*, S72–S87.

Puget, P., & Lal, R. (2005). Soil organic carbon and nitrogen in a Mollisol in central Ohio as affected by tillage and land use. *Soil and Tillage Research*, *80*, 201–213.

Recous, S., Aita, C., & Mary, B. (1999). In situ changes in gross N transformations in bare soil after addition of straw. *Soil Biology & Biochemistry*, *31*, 119–133.

Rice, C. W., & Tiedje, J. M. (1989). Regulation of nitrate assimilation by ammonium in soils and in isolated soil microorganisms. *Soil Biology and Biochemistry*, *21*(4), 597–602.

Ryan, J., Masri, S., & Singh, M. (2009). Seasonal changes in soil organic matter and biomass and labile forms of carbon as influence by crop rotations. *Communications in Soil Science and Plant Analysis*, *40*, 188–188.

Sakala, W. D., Cadisch, G., & Giller, K. E. (2000). Interactions between residues of maize and pigeonpea and mineral N fertilizers during decomposition and N mineralization. *Soil Biology and Biochemistry*, *32*, 679–688.

Schimel, J. (1996). Assumptions and errors in the $^{15}NH_4^+$ pool dilution technique for measuring mineralization and immobilization. *Soil Biology & Biochemistry*, *28*(6), 827–828.

Searle, P. G. E., Comudom, Y., Shedden, D. C., & Nance, R. A. (1981). Effect of maize + legume intercropping systems and fertilizer nitrogen on crop yields and residual nitrogen. *Field Crops Research*, *4*, 133–145.

Shammas, N. K. (1986). Interactions of temperature, pH, and biomass on the nitrification process. *Journal (Water Pollution Control Federation)*, *58*, 52–59.

Shi, Y., Lalande, R., Hamel, C., Ziadi, N., Gagnon, B., & Hu, Z. (2013). Seasonal variation of microbial biomass, activity, and community structure in soil under different tillage and phosphorus management practices. *Biology and Fertility of Soils*, In press.

Six, J., Conant, R. T., Paul, E. A., & Paustian, K. (2002). Stabilization mechanisms of soil organic matter : Implications for C-saturation of soils. *Plant and Soil*, *241*, 155–176.

Skinner, C., Gattinger, A., Muller, A., Mäder, P., Flieβbach, A., Stolze, M., … Niggli, U. (2014). Greenhouse gas fluxes from agricultural soils under organic and non-organic management - A global meta-analysis. *Science of the Total Environment*, *468-469*, 553–563.

Solomon, S., Qin, D., Manning, M., Alley, R. B., Berntsen, T., Bindoff, N. L., Chen, Z., et al. (2007). Technical Summary. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, et al. (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 19–91). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Smith, P. (2012). Agricultural greenhouse gas mitigation potential globally, in Europe and in the UK: what have we learnt in the last 20 years? *Global Change Biology*, *18*, 35–43.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., Kumar, P., McCarl, B., et al. (2007). Agriculture. In B. Metz, O. R. Davidson, P. R. Bosch, R. Dave, & L. A. Meyer (Eds.), *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 497–540). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Snyder, C. S., Bruulsema, T. W., Jensen, T. L., & Fixen, P. E. (2009). Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agriculture, Ecosystems & Environment*, *133*, 247–266.

Song, Y. N., Zhang, F. S., Marschner, P., Fan, F. L., Gao, H. M., Bao, X. G., … 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.

Sorensen, P. (2001). Short-term nitrogen transformations in soil amended with animal manure. *Soil Biology and Biochemistry*, *33*, 1211–1216.

Spiertz, J. H. J. (2010). Nitrogen, sustainable agriculture and food security. A review. *Agronomy for Sustainable Development*, *30*, 43–55.

Spohn, M., & Giani, L. (2011). Impacts of land use change on soil aggregation and aggregate stabilizing compounds as dependent on time. *Soil Biology and Biochemistry*, *43*(5), 1081–1088.

Studdert, G., & Echeverría, H. E. (2000). Crop rotations and nitrogen fertilization to manage soil organic carbon dynamics. *Soil Science Society of America Journal*, *64*, 1496–1503.

Tracy, B. F., & Frank, D. A. (1998). Herbivore influence on soil microbial biomass and nitrogen mineralization in a northern grassland ecosystem : Yellowstone National Park. *Oecologia*, *114,* 556–562.

Trehan, S. P. (1996). Immobilization of $^{15}NH_4^+$ in three soils by chemical and biological processes. *Soil Biology & Biochemistry*, *28*(8), 1021–1027.

Trenberth, K. E., Jones, P. D., Ambenje, P., Bojariu, R., Easterling, D., Tank, A. K., … Zhai, P. (2007). Observations: Surface and atmospheric climate. In S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, … H. L. Miller (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 235–336). Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Treseder, K. K. (2008). Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology letters*, *11*, 1111–1120.

Undie, U. L., Uwah, D. F., & Attoe, E. E. (2012). Effect of intercropping and crop arrangement on yield and productivity of late season maize/soybean mixtures in the humid environment of south southern Nigeria. *Journal of Agricultural Science*, *4*(4), 37–50.

Vachon, K. (2008). *Soil carbon and nitrogen dynamics and greenhouse gas mitigation in intercrop agroecosystems in Balcarce, Argentina*. [Master's thesis]. University of Waterloo.

Vachon, K., & Oelbermann, M. (2011). Crop residue input and decomposition in a temperate maize-soybean intercrop system. *Soil Science*, *176*, 157–163.

Vandermeer, J. (1989). *The Ecology of Intercropping*. Cambridge: Cambridge University Press.

Verhulst, N., Govaerts, B., Verachtert, E., Castellanos-Navarrete, A., Mezzalama, M., Wall, P. C., … Sayre, K. D. (2010). Conservation agriculture, improving soil quality for sustainable production systems? In R. Lal & B. A. Stewart (Eds.), *Advances in Soil Science: Food Security and Soil Quality* (pp. 137–208). Boca Raton: CRC Press.

Vermeulen, S. J., Aggarwal, P. K., Ainslie, A., Angelone, C., Campbell, B. M., Challinor, A. J., … Wollenberg, E. (2012). Options for support to agriculture and food security under climate change. *Environmental Science & Policy, 15*, 136–144.

Vervaet, H., Boeckx, P., Boko, A. M. C., Van Cleemput, O., & Hofman, G. (2004). The role of gross and net N transformation processes and NH_4^+ and NO_3^- immobilization in controlling the mineral N pool of a temperate mixed deciduous forest soil. *Plant and Soil, 264*, 349–357.

Viglizzo, E. F., & Frank, F. C. (2006). Ecological interactions, feedbacks, thresholds and collapses in the Argentine Pampas in response to climate and farming during the last century. *Quaternary International*, *158*, 122–126.

Viglizzo, E. F., Frank, F., Bernardos, J., Buschiazzo, D. E., & Cabo, S. (2006). A rapid method for assessing the environmental performance of commercial farms in the Pampas of Argentina. *Environmental Monitoring and Assessment*, *117*, 109–34.

Viglizzo, E. F., Pordomingo, A J., Castro, M. G., & Lertora, F. A. (2003). Environmental assessment of agriculture at a regional scale in the Pampas of Argentina. *Environmental Monitoring and Assessment*, *87*(2), 169–95.

Voroney, R. P., Brookes, P. C., & Beyaert, R. P. (2008). Soil microbial biomass C, N, P, and S. In M. R. Carter & E. G. Gregorich (Eds.), *Soil Sampling and Methods of Analysis* (2nd ed., pp. 637–651). Boca Raton: Taylor & Francis Group.

Wander, M. (2004). Soil organic matter fractions and their relevance to soil function. In F. Magdoff & R. R. Weil (Eds.), *Soil Organic Matter in Sustainable Agriculture* (pp. 67–102). Boca Raton, FL: CRC Press.

Wang, Y., Tu, C., Cheng, L., Li, C., Gentry, L. F., Hoyt, G. D., … Hu, S. (2011). Long-term impact of farming practices on soil organic carbon and nitrogen pools and microbial biomass and activity. *Soil and Tillage Research*, *117*, 8–16.

Wardle, D. A. (1992). A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biological Reviews*, *67*, 321–358.

Weis, T. (2010). The accelerating biophysical contradictions of industrial capitalist agriculture. *Journal of Agrarian Change*, *10*(3), 315–341.

Willson, T. C., Paul, E. A., & Harwood, R. R. (2001). Biologically active soil organic matter fractions in sustainable cropping systems. *Applied Soil Ecology*, *16*, 63–76.

Wingeyer, A. B. (2007). The effect of residue C/N ratio on the turnover of N and C in various soil organic matter fractions (Master's thesis). University of Nebraska. *Theses, Dissertations, and Student Research in Agronomy and Horticulture*. Paper 42.

Zaman, M., Di, H. J., Cameron, K. C., & Frampton, C. M. (1999). Gross nitrogen mineralization and nitrification rates and their relationships to enzyme activities and the soil microbial biomass in soils treated with dairy shed effluent and ammonium fertilizer at different water potentials. *Biology and Fertility of Soils*, *29*, 178–186.

Appendix A – SMB-N (2007) and N_F (2012) results

Table A.1 - SMB-N (μg g^{-1} soil) in maize and soybean sole crops and 1:2 and 2:3 intercrops, 2007. Standard errors are given in parentheses.

Depth (cm)	Treatment			
	Soybean	**Maize**	**1:2**	**2:3**
0-10	40.12 (13.90) ^{A,a}	50.10 (17.36) ^{A,a}	36.71 (12.72) ^{A,a}	52.93 (18.34) ^{A,a}
10-20	41.26 (14.29) ^{A,a}	33.63 (11.65) ^{A,a}	29.46 (10.21) ^{A,a}	72.07 (24.97) ^{A,a}
20-40	52.42 (18.16) ^{A,a}	49.98 (21.21) ^{A,a}	21.18 (7.34) ^{A,a}	42.22 (17.91) ^{A,a}
40-80	21.42 (7.42) ^{A,a}	16.42 (5.69) ^{A,a}	32.93 (19.76) ^{A,a}	16.39 (5.68) ^{A,a}
0-80 mean	**36.92 (6.40) ^{A}**	**34.29 (6.30) ^{A}**	**29.47 (6.25) ^{A}**	**40.31 (7.41) ^{A}**

Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$. Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$.

Table A.2 – N_F (μg g^{-1} soil) in maize and soybean sole crops and 1:2 and 2:3 intercrops, 2012. Standard errors are given in parentheses.

Depth (cm)	Treatment			
	Soybean	**Maize**	**1:2**	**2:3**
0-10	58.38 (9.09) ^{A,a}	68.76 (9.87) ^{A,a}	49.34 (8.36) ^{A,a}	59.51 (10.16) ^{A,a}
10-20	54.65 (8.80) ^{A,a}	45.25 (8.00) ^{A,ab}	49.89 (7.82) ^{A,a}	47.91 (7.66) ^{A,a}
20-40	36.24 (7.16) ^{A,ac}	31.03 (6.63) ^{A,b}	34.94 (7.78) ^{A,ac}	36.29 (7.17) ^{A,ac}
40-60	22.21 (5.61) ^{A,bc}	20.87 (5.44) ^{A,bc}	22.25 (5.61) ^{A,bc}	21.90 (5.18) ^{A,bc}
60-80	14.95 (4.60) ^{A,b}	12.68 (4.24) ^{A,c}	17.35 (4.96) ^{A,bc}	13.62 (4.09) ^{A,b}
0-80 mean	**35.13 (4.72) ^{A}**	**32.99 (4.57) ^{A}**	**33.36 (4.65) ^{A}**	**33.71 (4.56) ^{A}**

N_F = total weight of extractable N in the fumigated sample.

Values followed by the same upper case letters, comparing treatments within each depth, are not significantly different at $p<0.05$. Values followed by the same lower case letters, comparing differences between depths within each treatment, are not significantly different at $p<0.05$.

Appendix B – ^{15}N and NH_4^+ results used to calculate nitrogen transformation rates

Table B.1 – NH_4^+ concentration and ^{15}N (% exc.) measured in isotope pool dilution experiment, 2007.

Plot	Crop	NH_4^+ (g kg^{-1})		^{15}N (% exc. NH_4^+)	
		t = 1 day	t = 4 days	t = 1 day	t = 4 days
1	Soybean	4.338	4.301	5.341	4.515
		17.821	4.617	7.141	4.231
2	Maize	6.272	9.973	5.615	1.795
		7.271	4.374	5.508	4.814
3	2:3	12.027	10.642	5.058	2.932
		12.895	6.239	5.234	5.741
6	2:3	11.515	5.784	5.836	6.138
		12.043	12.765	6.522	6.504
7	Soybean	6.998	3.363	4.983	3.488
		7.337	5.918	5.477	5.563
8	Maize	10.611	5.752	6.901	5.007
		10.009	3.004	6.672	4.667
9	Maize	7.147	9.192	6.604	1.748
		7.375	5.775	6.864	4.631
10	Soybean	9.193	5.077	6.319	4.895
		8.215	5.721	6.295	4.203
12	2:3	8.936	6.287	7.267	5.492
		8.550	6.920	5.744	5.947

Table B.2 – NH_4^+ concentration and ^{15}N (% exc.) measured in isotope pool dilution experiment, 2012.

Plot*	Crop	NH_4^+ (g kg^{-1})		^{15}N (% exc. NH_4^+)	
		t = 1 day	t = 4 days	t = 1 day	t = 4 days
1	Soybean	5.733	1.581	5.211	1.489
		6.108	3.843	5.544	4.233
		5.839	3.216	5.178	3.862
2	Maize	8.791	0.736	5.456	1.654
		7.019	2.036	4.598	3.564
		7.386	1.107	6.215	1.583
3	2:3	5.145	0.531	5.099	0.856
		5.766	1.435	5.375	1.501
		9.038	2.000	6.095	1.943
4	1:2	5.237	0.890	5.879	1.147
		5.453	0.553	3.705	1.953
		6.365	2.552	9.366	4.119
5	1:2	5.580	1.704	4.947	2.172
		6.331	0.927	5.710	1.577
		6.172	1.370	5.103	1.969
6	2:3	6.953	5.127	5.793	0.523
		6.636	0.844	4.638	0.715
		7.540	2.088	5.948	1.719
7	Soybean	7.040	1.997	6.060	3.297
		6.343	0.377	5.930	0.795
		7.735	0.954	5.356	2.293
9	Maize	7.408	1.723	5.356	3.231
		9.189	3.730	5.524	4.288
		8.203	12.119	6.101	1.348
10	Soybean	14.270	3.349	2.775	4.487
		5.210	1.001	4.534	1.802
		6.188	0.913	5.359	2.463
11	1:2	5.487	0.593	4.981	1.550
		9.483	0.624	6.311	1.658
		6.749	2.847	5.671	3.885
12	2:3	6.401	1.377	4.924	2.544
		9.813	0.991	6.221	1.865
		6.839	0.692	5.189	1.350

* Plot 8 results were not included, as an error occurred in the analysis.