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Nitrogen transfer in the intercropping system between organic cherry tomato and legumes in succession to green corn

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ABSTRACT

SALGADO, G. C. **Nitrogen transfer in the intercropping system between organic cherry tomato and legumes in succession to green corn**. 2022. 145 p. Tese (Doutorado em Ciências) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2022.

The main study aim was to investigate the N transfer from different legumes to cherry tomato in the intercropping system under residual straw of the previous green corn crop using the ^{15}N natural abundance method. There also were evaluated yield and nutrients concentration of the plants, soil fertilizer, cost and profitability of this organic system. The experiment was in a randomized complete block design with five replications and eight treatments: monocrop of cherry tomato adding the residual green corn crop residue (straw) as a mulch, a monocrop of cherry tomato without straw, cherry tomatoes intercropped with jack bean, sun hemp, dwarf velvet bean, mung bean, white lupine or cowpea bean grown as green manures, in two consecutive cycles. In 2011, the number and weight of the total and the marketable fruits of tomatoes were 70% - 88% higher than in 2012, and the number of damaged fruits was 12 % lower in 2011 than in 2012. The different treatments had no effect on the yield of the green corn in 2012 and 2013. There was no difference in BNF between legumes at 100 days after sowing, independent of the year. The BNF was responsible for more than half of the N accumulated in the legumes. The N of legumes was transferred to cherry tomato in similar quantities, and the leaves and fruits of cherry tomato received more N transfer than shoots. It was shown that N transfer increases with the growth/development of cherry tomato. The legume in an intercropping system with cherry tomato cultivated in the succession of green corn does not provide sufficient nitrogen to supply the green corn demand. In relation to green corn straw, the single exponential decay function fitted with the decrease of the decomposition time in the dry matter remaining of green corn straw in 2011 and 2012. In the same way, the exponential decay function fitted with the decrease of the decomposition time of the C and N release from the mass decomposition of green corn straw. However, the residual green corn straw under cherry tomato with different legumes in an intercropping system did not increase the straw decomposition rate and the C and N release from straw decomposition. The $\delta^{13}C$ decreased and the $\delta^{15}N$ tended to increase in the green corn straw during the decomposition time. The total C and N content and the C and N stock of the soil increased over the years, especially in 2013. The soil C:N ratio also increased over the year, the C accumulated was higher than the N accumulated, increasing the N immobilization in the system. The soil organic matter reduced over the years, although the total C and N increased, suggesting that the organic C and N decreased, and the inorganic C and N increased in the soil. This agricultural system reduced the soil fertility, although the N and C total stock in the soil increased over the year. Furthermore, it showed high profitability under the conditions in which this study was conducted.

Keywords: Green manure. Biological nitrogen fixation. ^{15}N natural abundance. Organic agriculture. Soil fertility.

RESUMO

SALGADO, G. C. **Transferência de nitrogênio no cultivo intercalar de minitomateiro orgânico e leguminosas em sucessão ao milho-verde.** 2022. 145 p. Tese (Doutorado em Ciências) - Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, 2022.

O objetivo principal foi avaliar a transferência de N de diferentes leguminosas (Fabaceae) para o minitomateiro em sistema de consórcio sobre a palha residual do milho-verde utilizando o método de abundância natural ^{15}N. Também foi avaliado a produtividade e o teor de nutrientes das plantas, fertilidade do solo e o custo e a rentabilidade desse sistema orgânico. O desenho experimental foi de blocos ao acaso com cinco repetições e oitos tratamentos: minitomateiro solteiro com palha residual de milho verde, minitomateiro solteiro sem palha, minitomateiro em consórcio com feijão-de-porco, crotalária-juncea, mucuna-anã, feijão-mungo, tremoço-branco, feijão-caupí, em dois anos consecutivos. Em 2011, o número e peso total de frutos comerciais de minitomateiro foram 70%-80% maior que em 2012, e o número de frutos danificados foi 12% menor em 2011 do que em 2012. Os diferentes tratamentos não afetaram a produtividade do milho no ano de 2012 e 2013. Não houve diferença entre a fixação biológica de nitrogênio (FBN) entre os legumes aos 100 dias após a semeadura, independente do ano. A FBN foi responsável por mais da metade do N acumulado pelas leguminosas. O N das leguminosas foi transferido para o minitomateiro em proporções similares, e as folhas e frutos do minitomateiro receberam mais N transferido que os brotos. A transferências de N aumentou com o crescimento e desenvolvimento do minitomateiro. A leguminosa em consórcio com o minitomateiro cultivado em sucessão ao milho verde não foi suficiente para suprir a demanda de N do milho. Em relação a palha de milho, a função exponencial decrescente simples ajustou-se com a redução do tempo de decomposição para a matéria seca remanescente da palha em 2011 e 2012. Assim como, a função exponencial decrescente simples ajustou-se com a redução do tempo de decomposição para a liberação de N e C da massa de decomposição da palha de milho. A palha de milho sobre o consórcio de minitomateiro e leguminosas (fonte de N) não aumentou a taxa de decomposição e a liberação de C e N da decomposição da palha. O $\delta^{13}C$ diminuiu e o $\delta^{15}N$ teve uma tendência a aumentar na palha de milho durante o tempo de decomposição. A concentração total de C e N e o estoque de C - no solo aumentou com os anos, especialmente em 2013. A relação C: N do solo também aumentou com os anos, sendo que o C acumulado foi maior que o N acumulado no solo, ou seja, houve um aumento da imobilização de N no sistema. A matéria orgânica do solo reduziu com os anos, apesar do estoque de C e N terem aumentado, sugerindo que o C e N orgânico diminuíram e o C e N inorgânico aumentaram no solo. Este sistema agrícola reduziu a fertilidade do solo, apesar do estoque de N e C no solo aumentarem com os anos. Além disso, ele apresentou alta rentabilidade sob as condições em que o estudo foi conduzido.

Palavras-chave: Adubação verde. Fixação biológica de nitrogênio. Abundância natural de ^{15}N. Agricultura orgânica. Fertilidade do solo.

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SUMMARY

1 INTRODUCTION

The world's population will reach 9.1 billion people in 2050 and will need to increase 70% of agricultural production in the same period to feed the population, according to estimate of FAO (2009). Increased concerns over depleting natural resources accompany the recognized need for increased food production, the competition for land with urban development, climate change, and environmental preservation, pressuring agriculture to produce more with less land and resources (FAO 2009; BHAN; BEHERA, 2014).

Organic agriculture systems propose to improve the sustainability of agriculture by fostering crop diversity, increasing soil fertility, enhancing the carbon soil, reducing pesticide pollution risk beyond improving biological pest and disease control (BEDOUSSAC et al., 2015; MDITSHWA et al., 2017; REGANOLD; WACHTER, 2016). This agriculture has been based on principles to maintain or improve the soil, plant, and human health and sustain the ecological system (IFOAM, 2020). Therefore, practices such as green manure, no-till, intercropping, crop rotation, and biological pest control are usually used to optimize soil, nutrients, and water and minimize the environmental impact (SCIALABBA; MLLER-LINDENLAUF, 2010). In recent decades, there has been a steady growth of organic agriculture, reaching 72.3 million hectares worldwide in 2019, and have grown more than 555% in 20 years. In addition, the market got more than 106 billion dollars in organic food and drink (WILLER et al., 2021).

Considering its importance, further studies on plant management and nutrition in organic agriculture have been necessary. Nitrogen (N) can be a limiting factor for increased productivity in organic agriculture. Low N availability and other nutrients from organic fertilizers might justify the lower productivity of organic agriculture compared to conventional agriculture (SACCO et al., 2015; SEUFERT; RAMANKUTTY; FOLEY, 2012). N is the most important nutrient for plants and is required in large quantities. The N uptake of tomatoes and green corn are approximately 137 kg N ha^{-1} (BADR; ABOU-HUSSEIN; EL-TOHAMY, 2016; TEI; BENINCASA; GUIDUCCI, 2002) and 125 kg N ha^{-1} (KHAN et al., 2018; SINGH et al., 2015), respectively.

The N availability from organic fertilizers such as manure is a consequence of the mineralization activity from the soil (SACCO et al., 2015). However, the rate of soil mineralization is highly variable because it depends on the temperature, humidity, aeration, type of soil, and source of N. Moreover, the mineralization rate must occur in sync with the culture demand for N such that N deficiency and losses of this nutrient do not happen through leaching, volatilization, denitrification (ABBASI; KHIZAR, 2012; BERRY et al., 2002; MIKKELSEN; ROSENDAHL; JAKOBSEN, 2008; PANG; LETEY, 2000).

The legumes' green manure is used as a source of N and other nutrients in an intercropping or crop rotation in organic system. The legumes have a biological nitrogen fixation capacity that can avoid the competition between legumes and other crops for N uptake, increasing the soil N concentration, which will be available for the following crop (DUCHENE; VIAN; CELETTE, 2017). Biological nitrogen fixation (BNF) by legumes (rhizobium symbiosis) has received attention as a source of N that can replace a portion of synthetic N fertilizer in a crop rotation or intercropping system. This method can be used in organic farming and is environmentally friendly (SANT'ANNA et al., 2018). According to Ambrosano et al. (2005), sugarcane (leaves + stalk) absorbed an equivalent to 11.5 kg ha^{-1} of N from *Crotalaria juncea* planted before the sugarcane in the crop rotation system. In an intercropping system, the N transfer from sun hemp (legume) to guinea grass or switchgrass (non-legumes) was 43% and 51%, respectively (ASHWORTH et al., 2015).

Intercropping system provides an interesting option in this scenario because two or more crops can be grown in the same area increasing the use efficiency of land and environmental resources such as water, light, and nutrients (BEDOUSSAC et al., 2015; LITHOURGIDIS et al., 2011). Cover crops and green manure were used for millennia in intercropping and crop rotation systems to maintain or improve the soil fertility and crop yields, to control pests, diseases, and weeds. In addition, to promote nutrient cycling, being a source of N (legumes), and providing extra income for the farmers (grains) (DUCHENE; VIAN; CELETTE, 2017; GITARI et al., 2019; LITHOURGIDIS et al., 2011; MENDONÇA et al., 2017; MOSJIDIS; WEHTJE, 2011; YU et al., 2016). For legumes in the intercropping system, the knowledge base on belowground interaction (roots) is fundamental for understanding the interaction between plants under intercrop and potential N supply to companion and subsequent crops (WICHERN et al., 2007a, 2008; ZANG et al., 2015). The intercropping benefits the development of different types of roots and changes their distribution and architecture beyond affecting the rhizodeposition. The rhizodeposition releases organic and inorganic compounds in the rhizosphere such as enzymes, exudate, ions and represents an essential flow of carbon (C) into the soil (MARSCHNER, 2012; PAUSCH et al., 2013; DUCHENE; VIAN; CELETTE, 2017). Taschen et al. (2017) demonstrated that intercropping between legume and non-legume plants can modify the bacterial community in the rhizosphere compared to the same species in monoculture, suggesting a synergic interaction in the mixed rhizosphere plant.

Therefore, intercropping legumes with the main crops can increase the N use efficiency in the organic agriculture. The N transfer from legumes to non-legumes in a intercropping system can occur by leaf leaching, the release of ammonia gas, rhizodeposition, and via mycorrhiza in interconnected roots (PEOPLES et al., 2015; THILAKARATHNA et al., 2016; ZANG et al., 2015). Moreover, the use of legumes maintains or increases the total N in the soil, and can be available for the cultures in succession (AMBROSANO et al., 2005).

However, the N transfer between plants can vary depending on the seasons (RASMUSSEN et al., 2013), species/variety of legume and non-legume plants (ASHWORTH et al., 2015; MENDONÇA et al., 2017; SAKAI et al., 2011), and presence of mycorrhizae in the soil (PEOPLES et al., 2015). In addition, the N transfer can also be affected by the type of intercropping system (such as the distance between legumes and non-legumes plants) (RASMUSSEN et al., 2013; ZHANG et al., 2017), the use of organic or conventional agriculture (AMBROSANO et al., 2005; CHU; SHEN; CAO, 2004; SALGADO et al., 2020), and the type of soil. Hence, because numerous variables can affect the process of N transfer, it is important to study the best arrangements in terms of the species of legumes and the density of sowing to increase the transfer of N between plants.

Furthermore, the legumes residue is an important source of N and C in the soil. NOTARIS et al. (2020) showed that red clove and winter vetch, as green manure, deposited in the soil 134 and 72 kg C ha^{-1}, respectively, and 29 and 85 kg N ha^{-1}, respectively, and ~9 kg N ha^{-1} were provided by mineralization from the crop residues. The meta-analysis research predicted that the cover crops (legumes and non-legumes) practice could accumulate 12.7 Mg ha^{-1} of soil organic carbon (SOC) in 54 years, which correspond to an average C sequestration rate of 0.23 Mg ha^{-1} yr^{-1} (POEPLAU; DON, 2015).

However, the decomposition of legumes and crop residue is influenced by the edaphoclimatic condition (temperature, rainfall, and soil moisture) and residue quality such as N, C, polyphenol, and lignin concentration and their ratio. Abera et al. (2012) studied the C and N mineralization dynamic in different soil with two legume residues and showed that the soil under residue with high C: N ratio (40.6) had lower cumulative CO_2 flux and production of NH_4^+ and NO_3^-; thus, lower C and N loss. Moreover, the concentration of lignin and cellulose also influences litter decomposition; the plants with low lignin had lower decomposition rate than the plants with low cellulose and low N in their composition (TALBOT; TRESEDER, 2012).

Therefore, the C and N dynamics are interconnected within the ecosystem, their availabilities determine the microbial activity, and the mineralization/immobilization of N and

C stock change in the soil (MARSCHNER, 2012; NOTARIS et al., 2020). Moreover, maintaining or increasing soil C stock is also important to mitigate the effects of greenhouse gas because the food system was responsible for 6 to 13% of total anthropogenic CO_2 emissions in 2019 (IPCC 2020). Studies with the ^{13}C and ^{15}N stable isotopes technique are usually used to trace the C and N into the plant and soil to better understand the C and N cycle (CRAINE et al., 2015; GLEIXNER, 2013; GUARESCHI; PEREIRA; PERIN, 2014).

1.1.Hypothesis and objectives

The hypotheses of this study are given as follows:

1. Organic cherry tomatoes and legumes that are grown with the addition of green corn straw residue in the intercropping system are more productive than tomatoes grown as a single crop system;
2. The green corn that is grown in succession with the cherry tomatoes/legume intercropping systems also are more productive;
3. The N transfer occurs from legumes to cherry tomato;
4. This transfer varies according to the species companion of the legume;
5. The BNF (Biological Nitrogen Fixation) and amount of N input through BNF varies according to the legume species;
6. The N derived from the soil and legumes is sufficient to supply the N demand of green corn;
7. Organic system of cherry tomato intercropping with green manures in succession to green corn increases N and C total stock in the soil, maintaining or increasing the soil fertilizer;
8. The residual green corn straw under cherry tomato with different legumes (source of N) in an intercropping system increases the straw decomposition rate and the C and N release from straw decomposition;
9. The organic cherry tomato and green corn in the succession system is profitable;
10. The intercropping system between tomato and jack bean would increase chitinase, β-glucosidase, phosphatase activities, and hotspots areas.

The main objective was to investigate the transfer of N from different legumes to cherry tomato in the intercropping system under residual straw of the previous green corn crop using the ^{15}N natural abundance method.

The specific objectives of this study are given as follows:

1. To evaluate the productivity and nutrient concentration of cherry tomato/legume intercrops and the green corn grown in succession.
2. To evaluate the BNF of each legume tested and the yield of green corn cultivated in succession.
3. To investigate the temporal variation (2011/2012) in the N transfer to a cherry tomato, the biological nitrogen fixation of legumes, and the N concentration of green corn cultivated in the intercropping succession.
4. To analyze a soil fertilizer, C and N content, and natural abundance of ^{13}C and ^{15}N on the 0-15 cm of deep during three years of the system.
5. To evaluate the decomposition rate of residual green corn straw and C and N release from residual green corn straw decomposition under cherry tomato and different legumes in the intercropping system.
6. To examine the cost and profitability of organic cherry tomato and green corn in the succession system.
7. To evaluate the activity and spatial distribution of enzymes (Phosphatase, Chitinase, and β-Glucosidase) of single and intercropping tomato and jack bean using the zymography technique.

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2 Yield and nutrient concentration of organic cherry tomatoes and legumes grown in the intercropping systems in rotation with green corn

Abstract

This study evaluated the yield and nutrient concentration of organic cherry tomatoes and leguminous green manures grown in intercropping systems in rotation with green corn. The experiment was in a randomized complete block design with five replications and eight treatments: monocrop of cherry tomato adding the residual green corn crop residue (straw) as a mulch (control), a monocrop of cherry tomato without the addition of green corn crop residue (control), cherry tomatoes intercropped with jack bean, sun hemp, dwarf velvet bean, mung bean, white lupine or cowpea bean grown as green manures in two consecutive cycles. In this production system, evaluated between 2011 and 2013, green corn was cultivated from January to April, and the cherry tomatoes, with or without leguminous green manures, were grown from May/July to November/December. In 2011, the number and weight of the total and the marketable fruits of tomatoes were 70% - 88% higher than in 2012, and the number of damaged fruits was 12 % lower in 2011 than in 2012. In 2011, the weight of total and marketable fruits in the intercropping treatment with white lupine was lower than that of the controls and the intercropping with dwarf velvet and cowpea bean. The white lupine and sun hemp green manures produced the highest biomass dry weight, followed by the jack beans, cowpea beans, mung beans and dwarf velvet beans. The different treatments had no effect on the yield of the green corn independently of the year.

Keywords: Legumes; macronutrients; micronutrients; organic agriculture; *Solanum lycopersicum*

Produtividade e concentração de nutrientes de minitomateiro orgânico e leguminosas em sistemas consorciados e em sucessão com milho verde

Resumo

Este estudo avaliou a produtividade e a concentração de nutrientes de minitomateiro orgânico e leguminosas em sistema consorciados e em sucessão com milho verde. O desenho experimental foi de blocos ao acaso com cinco repetições e oitos tratamentos: minitomateiro solteiro com palha residual de milho-verde, minitomateiro solteiro sem palha residual de milho-verde, minitomateiro em consórcio com feijão-de-porco, crotalária-juncea, mucuna-anã, feijão-mungo, tremoço-branco, feijão-caupí. Neste sistema de produção, as plantas foram avaliadas entre 2011 e 2013, sendo que o milho-verde foi cultivado de janeiro a abril e o minitomateiro (com ou sem leguminosa), de maio/julho a novembro/dezembro. Em 2011, o número e peso total de frutos comerciais de minitomateiro foram 70%-80% maior que em 2012, e o número de frutos danificados foi 12% menor em 2011 do que em 2012. O peso total e comercial de frutos nos tratamentos consorciados com tremoço-branco foi menor que os tratamentos com minitomateiros solteiros (controles) e o minitomateiros com mucuna-anã e feijão-caupi em 2011. O tremoço-branco e crotalária-juncea produziu maior biomassa seca seguido pelo feijão-de-porco, feijão-caupí, feijão-mungo e mucuna-anã. Os diferentes tratamentos não afetaram a produtividade do milho independentemente do ano

Palavra-chave: legumes, macronutrientes, micronutrientes, agricultura orgânica, *Solanum lycopersicum*

2.1 Introduction

The world's population will reach 9.1 billion people in 2050, and will need to increase 70% of agricultural production in the same period to feed the population, according to estimate of FAO (2009). Increased concerns over depleting natural resources accompany the recognized need for increased food production, the competition for land with urban development, climate change, and environmental preservation, pressuring agriculture to produce more with less land and resources (FAO, 2009; BHAN; BEHERA, 2014).

Intercropping system provides an interesting option in this scenario because two or more crops can be grown in the same are increasing the use efficiency of land and environmental resources such as water, light, and nutrients (BEDOUSSAC et al., 2015; LITHOURGIDIS et al., 2011). Cover crops and green manure were used for millennia in intercropping and crop rotation systems to maintain or improve the soil fertility and crop yields, to control pests, diseases, and weeds. In addition, to promote nutrient cycling, being a source of N (legumes), and providing extra income for the farmers (grains) (DUCHENE; VIAN; CELETTE, 2017; GITARI et al., 2019; LITHOURGIDIS et al., 2011; MENDONÇA et al., 2017; MOSJIDIS; WEHTJE, 2011; YU et al., 2016).

Organic agriculture systems propose to improve the sustainability of agriculture by fostering crop diversity, increasing soil fertility, banning the use of synthetic fertilizers, insecticides, fungicides, and herbicides, and enhancing biological pest and disease control (BEDOUSSAC et al., 2015; JOUZI et al., 2017a; LI et al., 2017; MDITSHWA et al., 2017). In recent decades, there has been a steady growth of organic agriculture, reaching the total cultivated area of 69.8 million hectares in 181 countries in 2017, with a capacity to handle $ 97 billion (WILLE; LERNOUD 2019).

Considering its importance, further studies on plant management and nutrition in organic agriculture have been necessary. According to Seufert et al. (2012), and Ronga et al. (2017), the availability of nitrogen may be a limiting factor to increase yield in organic agriculture. Nitrogen and other nutrients from organic fertilizers, such as compost, are released into the soil due to mineralisation and need to be in sync with the crops' nutrient uptake (SACCO et al., 2015). The legumes green manure is used as the N source and other nutrients in an intercropping or crop rotation in organic system. The legumes have a capacity of biological nitrogen fixation that can avoid the competition between the legumes and other crops for N uptake and increase the soil N concentration, which will be available for the following crop (DUCHENE; VIAN; CELETTE, 2017).

According to Beudossac et al. (2015), there are three types of plant-plant interaction in the intercropping system: (a) competition, when one resource became limited, (b) complementary, when the plants did not compete for the same resource in the time and space, and (b) facilitation, when the modification of the environment is beneficial for one species at least. There is a balance between them influencing soil nutrients concentration, environmental condition, species companion (BEDOUSSAC et al., 2015; DUCHENE; VIAN; CELETTE, 2017). Thus, we hypothesised that (1) organic cherry tomatoes and legumes that are grown with the addition of green corn crop residue in intercropping systems would be more productive than tomatoes grown as a single crop system and (2) the green corn that is grown in rotation with the cherry tomatoes/legume intercropping systems would also be more productive. The study aimed to evaluate the cherry tomato/legume intercrops' productivity and nutrient concentrations and the green corn grown in succession.

2.2 Materials and methods

2.2.1 Characterization of the experimental area

The experiment was carried out between 2010 and 2013 in an agroecological experimental area in Piracicaba, state São Paulo, Brazil (540 m elevation, 22°43'S, 47°38'W). This area is not having certified for organic production because it is used just for organic agriculture research. Therefore, it follows organic agriculture principles and organic agriculture law in Brazil (BRASIL, 2011). The mean temperature and rainfall in the period of the experiment are in Figure 1.

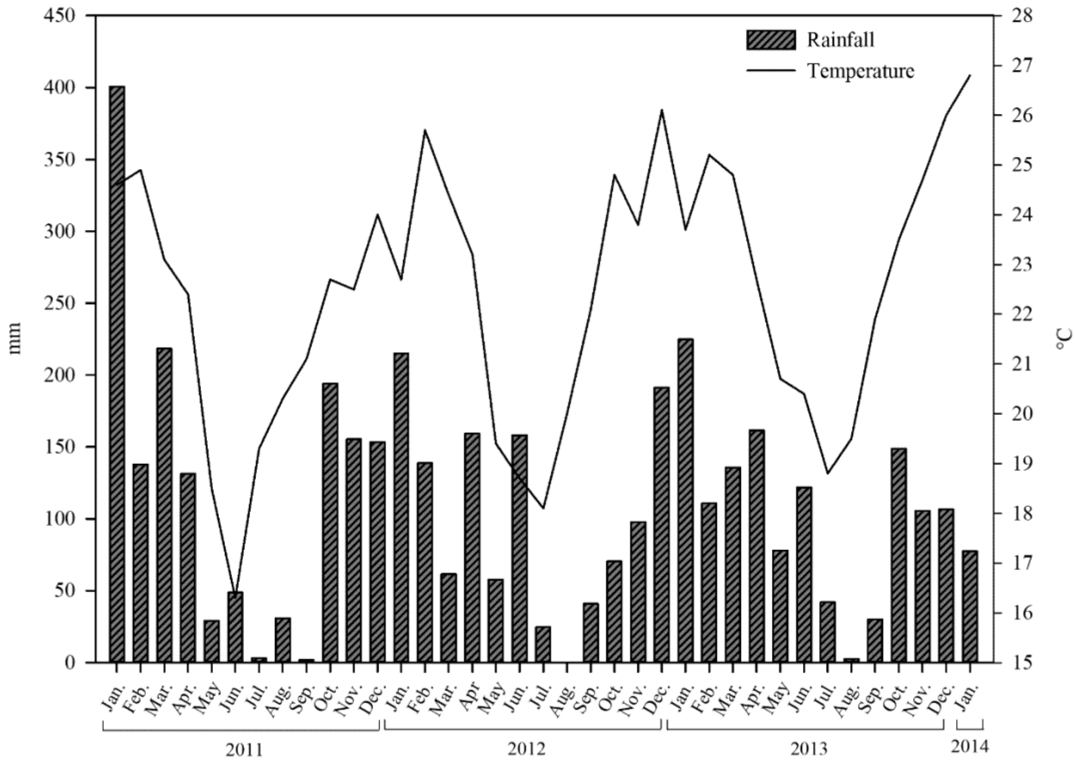

Figure 1. Mean monthly temperature and rainfall during the experiment

The soil of the experimental area is a Rhodic Kandiudox (USDA, 1999) with the follow physical and chemical characteristic (at a depth of 0 - 20 cm): 36% of clay; 23% of silt; 28% of fine sand, 13% of coarse sand, pH ($CaCl_2$): 6.0; organic matter (OM): 32 g dm $^{-3}$; N: 0.18%, P: 27 mg dm^{-3}; K: 6.4 mmolc dm^{-3}; Ca: 47 mmolc dm^{-3}; Mg: 15 mmolc dm^{-3}; H+Al: 24 mmolc dm^{-3}; Al: 0 mmolc dm^{-3}, S: 7.0 mg dm^{-3}; B: 0.21 mg dm^{-3}; Cu: 5 mg dm^{-3}; Fe: 46 mg dm^{-3}; Mn: 46 mg dm^{-3}; Zn: 6.4 mg dm^{-3}; base saturation (BS): 68.4 mmol$_c$ dm^{-3}; cation exchange capacity (CEC): 92.4 mmol$_c$ dm^{-3}; percentage of base saturation (V): 74%; aluminium saturation (m): 0%.

The experimental area had previously been used for pasture for approximately 60 years. In 2006 it was used for organic lettuce and beet, and in 2011 this experiment with the cherry tomato and legume intercropping system grown in rotation with green corn was established.

2.2.2 Experimental design

The experimente was in a randomized complete block design with eight treatments and five replications. The treatments were: (1) control, a cherry tomato monocrop with crop residue (straw) from the previous green corn crop, (2) control, a cherry tomato monocrop without crop residue (straw) from the previous green corn crop, (3) cherry tomato and jack bean in an intercropping system, (4) cherry tomato and sun hemp in an intercropping system, (5) cherry tomato and dwarf velvet bean in an intercropping system, (6) cherry tomato and mung bean in an intercropping system, (7) cherry tomato and white lupine in an intercropping system, (8) cherry tomato and cowpea bean in an intercropping system, in two consecutive cycles (Table 2). We conducted the agriculture system in no-till. The treatments with an intercropping system (3-8) were added crop residue (straw) from the previous green corn crop.

2.2.3 Field and Crop management

The experiment started with sowing of green corn in January 2011 and intercropping of tomato and legumes on straw green corn residue in May 2011. This succession of green corn/tomato+legumes was repeated in 2012 with the sowing of green corn in January and intercropping of tomato and legumes in July. The experiment finished with the last green corn culture in January 2013. Between May 2013 and January 2014, the experimental area was resting (Figure 2 and Table 1)

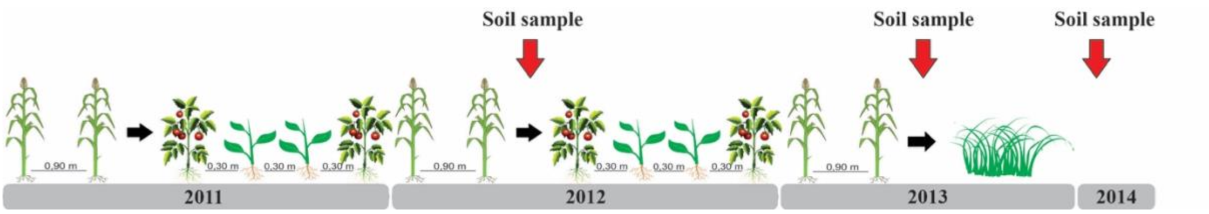

Figure 2. Illustration of succession system of green corn and cherry tomato/legumes with their respective spacing between the lines

The experimental area was prepared with a mouldboard plough and fertilizer with the following equivalent: (a) 25 Mg ha^{-1} of organic manure with concentrations of 50 g OM kg^{-1}, 1.5 g N kg^{-1}, 0.80 g P_2O_5 kg^{-1} (citric acid), 1.2 g K_2O kg^{-1}, 3.1 g Ca kg^{-1} , 0.6 g Mg kg^{-1} and 0.6 g S kg^{-1}; (b) 0.31 Mg ha^{-1} thermophosphate with concentrations of 50 kg P_2O_5 ha^{-1} , 56 kg Ca ha^{-1}, 21.85 kg Mg ha^{-1}, 0.31 kg B ha^{-1}, 0.16 kg Cu ha^{-1}, 0.47 kg Mn ha^{-1}, 31.20 kg Si ha^{-1}, and 1.72 kg Zn ha^{-1}; and (c) 0.1 Mg ha^{-1} of potassium sulphate with concentrations of 50 kg K_2O ha^{-1} and 15 kg S ha^{-1}. Subsequently, green corn was sowed in January 2011 (Table 1 and 2). Thermophosphate and potassium sulphate are natural fertilizers, originating from natural sources of P and K, obtained only through physical procedures; thus, they do not have a synthetic fertilizer (BRAZIL, 2011).

Table 1. Plant details and their planting density that were used in the research

Common names	**Scientific names**	**Variety**	**Spacing (m)**	**Planting density (seed m^{-1})**
Cherry tomatoes	*Solanum lycopersicum*	Access 21	0.9x0.6	-
Green corn	*Zean mays*	Cativerde 02	0.9	5
Jack bean	*Canavalia ensiformis* DC.	Common variety	0.3	5
Sun hemp	*Crotalaria juncea* L.	IAC-1	0.3	11
Dwarf velvet bean	*Mucuna deeringiana* (Bort)	Common variety	0.3	5
Mung bean	*Vigna radiata* (L.) Wilczek	Access 146-IAC	0.3	20
White lupine	*Lupinus albus* L.	Common variety	0.3	7
Cowpea bean	*Vigna unguiculata* (L.) Walp	Common variety	0.3	20

Table 2. Schedule of activities performed while conducting the experiment

Year / Month	**Activity**
2010	
November	Soil sample (0–0.2 m depth)
December	Soil fertility correction
2011	
January	Sowing green corn
April	Harvest green corn (R3 stage-immature maize crop)
April	Green corn straw formation
May	Soil preparation (digging) and application of fertilizer
May	Transplant cherry tomatoes and sow legumes (green manures)
July	Start harvest of cherry tomatoes
July	Shut samples of cherry tomato
August	Leave samples of cherry tomato
October	Fruit samples of cherry tomato
October e November	Legumes samples
November	Finish harvest cherry tomatoes
2012	
January	Sow green corn
March	Take leaf samples of green corn
May and June	Take green corn samples and harvest green corn (R3 stage-immature maize crop)
July	Green corn straw formation
July	Soil preparation (digging) and application fertilizer
July	Transplant cherry tomatoes and sow legumes (green manure)
August	Take shoot samples of cherry tomatoes
September	Start of harvest cherry tomatoes
October	Take leaf and fruit samples of cherry tomatoes
November e December	Take samples of legume
December	Finish harvest cherry tomatoes
2013	
January	Sow green corn
March	Take leaf samples of green corn
May	Take plants samples of green corn and harvest green corn.

The green corn plants were chopped by a Triton agricultural implement after the green corn harvest. Therefore, the equivalents of 6 Mg ha^{-1} of corn straw were on the ground in 2011 and 2012 for the subsequent no-till cherry tomato and legumes. The residual straw from the previous green corn crop was removed from the plot with single cherry tomato without straw. In 2012 and 2013, the green corn was not fertilized.

The cherry tomato and legumes were transplanted on the field and sowed on the same day. The cherry tomato seed used was Access 21 from the Agronomy Institute (IA). This variety has good productivity and has been used by selected organic farmers (AZEVEDO FILHO; MELO, 2001). The cherry tomato seedlings were produced in 128-cell expanded polystyrene trays in sprinkler-irrigated greenhouses. The seedlings were transplanted to the experimental area in pits (0.1 x 0.1 x 0.1 m) (Table 1). The pits were fertilized with 25 g of thermophosphate and 2.7 g of potassium sulphate, providing the equivalent of 4.4 g of P_2O_5, 4.5 g Ca, 1.8 g Mg, 2.5 g Si, 10 mg Cu, 30 mg B, 80 mg Mn, 14 mg Zn, 1.4 g K_2O, and 0.5 g S. A ribbon was tied under two stems of the cherry tomatoes, and pruning to the eighth raceme was performed at 120 days. Each plot contained two rows of cherry tomatoes with six plants (Table 1 and Figure 2). The legumes were sowed in two lines between the cherry tomato rows (Table 1 and Figure 2). There was no nitrogen fertilizer applied in the cherry tomato, and the source of N present in the area was the soil and green manure. The soil of the experiment has N bacteria fixation compatible with all legumes tested. Before this experiment, the other study and the pilot experiment were carried out in this soil, and verification of the presence of N bacteria fixation was performed (SAKAI et al., 2011). The cherry tomatoes were drip irrigated according to their water requirement in the field and the weed control was carried out by costal cutter.

2.2.4 Cherry tomato, legumes and green corn yields

The cherry tomatoes were harvested at the mature stage and were counted and weighted to determine the number of total fruits (NTF), weight of total fruit per plant (WTF) and the average weight of total fruit (AWTF). The fruits were classified into two groups marketable and damaged fruits for aim to determine the number of marketable and damaged fruits per plant (NMF, NDF), the weight of marketable and damaged fruit per plant (WMF, WDF), the average weight of marketable and damaged fruits (AWMF, AWDF). The fruits considered marketable were those with no physical damage (cut, cracks) or damage by pests and diseases.

To determine the yield (fresh above-ground biomass) of green manure, two linear meters of plants per plot were sampled and weighted. The dry biomass was determined sub-sampled 10% of the fresh material's weight that was dried at 65ºC in a forced circulation oven until reaching constant mass. Green manure dry matter biomass was calculated by the percentage of dry mass (ratio between dry mass and fresh mass) multiplied by the total weight of green manure in the plot, expressed in Mg $ha^{-1.}$

The yields of the green corn were determined as weight of ear with straw (WES), ear without straw (WEWS), marketable ear (WME) and damaged ear (WDE), and the number of total (NTE), marketable (NME) and damaged ear (NDE) of the green corn plant grown in succession to the cherry tomato and legumes in intercropping system. The ears considered marketable were those with well-formed ear with all grains, no physical damage (cut), damage by pests and diseases.

2.2.5 Macro and micronutrients

Cherry tomato shoot, leaves and fruit were sampled for macro and micronutrient analysis. The shoot was sampled 40 days after transplanting (DAT) (20 shoots $plot^{-1}$). The 20 leaves $plot^{-1}$ were sampled at 60 and 90 DAT in 2011 and 2012, respectively. The fruits were sampled when they were ripe (10 fruits $plot^{-1}$). All the aerial parts were sampled for the legumes, and only the pods were removed, if necessary. The green corn plants were sampled by sampling leaves (10 leaves $plot^{-1}$) at the reproductive growth stage R1 stage and by sampling aerial parts without the ears (10 plant $plot^{-1}$) at the reproductive growth stage R3. The samples were separately dried in an oven at 65ºC with forced air circulation until reaching constant mass. Subsequently, the samples were ground in a Wiley mill and taken to the laboratory to determine N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn, according to the methodology described by Malavolta, Vitti e Oliveira (1997). The accumulation of nutrients was determined for the legumes' aerial plant by determining each nutrient's concentration in the dry mass of the plant fraction and multiplying by the total dry biomass weight per plot, expressed as kg ha^{-1}.

2.2.6 Chemical analysis of cherry tomato quality

Chemical analyses assessed the quality of the fresh cherry tomato fruits to determine pH using a potentiometer following with method No. 981.12 (AOAC, 1997); soluble solids concentration (ºBrix) in refractometer according to method No. 932.12 (AOAC, 1997); titratable total acidity (% citric acid) by potentiometric titration to the second method No. 942.15 (AOAC ,1997); total sugars and reducing sugars (%) by the Eynon and Lane method described by Carvalho et al. (1990); ratio obtained by dividing the soluble solids content (ºBrix) by the value of the total titratable acidity (%).

2.2.7 Statistical analysis

Statistical analysis was performed with repeated measures, using the MIXED procedure of the SAS software (Statistical Analysis System, 9.3). After the descriptive and exploratory analysis of the data, analysis of variance (ANOVA) was applied, considering a mixed model for repeated measures in time, with the study factors treatment and year. Multiple comparisons were performed using the Tukey-Kramer test. The level of significance adopted for analysis of variance was 0.10 ($\alpha = 0.10$) (SALGADO et al., 2020).

2.3 Results

Cherry tomato yields

The statistical analyses showed that for the total number of tomato fruit (NTF), the number of marketable (NMF) or damaged (NFD) fruits, there were no significant interactions between year and treatment and no significant effects of the treatments (Table 3). However, the differences between 2011 and 2012 were significant ($p < 0.10$). In 2011, the NTF and NMF were 69 and 70% higher, and NDF was 12% lower than in 2012, respectively. The total weight (WTF) and weight of marketable (WMF) fruit also showed differences ($p < 0.10$) between the years, except in the intercropping treatment with cherry tomato and white lupine (Table 4). Overall, WTF and WMF were 71 and 88% higher in 2011 than in 2012, respectively. The weight of damaged fruit (WDF) was lower in 2011 than in 2012 (Table 4).

With regard to the average, weight of the total fruit (AWTF) and the average weight of the marketable fruit (AWMF), there were differences ($p < 0.10$) between the two years of cultivation for the controls (without and with straw) and for the treatments with jack bean and cowpea bean (Table 5). However, there were no differences between the years for the average weight of the damaged fruit (AWDF) (Table 5).

In 2012, there were no significant differences ($p < 0.10$) between the treatments for the parameters WTF, WMF, AWMF and AWDF (Table 4 and 5). However, in 2011, the WTF and WMF of the intercropping treatment of cherry tomatoes and white lupine were lower than the controls (with or without straw). (Table 4). In the same year, the AWTF in the treatments with intercropping of cherry tomatoes with white lupine or sun hemp was lower than that in the other treatments, except for the ones with cherry tomato intercropped with dwarf velvet bean or mung bean. In the treatment with white lupine, the AWMF was also lower than that for the other treatments, except for treatment with cherry tomatoes and sun hemp (Table 5).

Table 3. Number of total (NTF), marketable (NMF) and damaged (NDF) fruits of cherry tomatoes grown in and intercropping systems with legumes

Treatment	Number of total fruits (NTF)			Number of marketable fruits (NMF)			Number of damaged fruits (NDF)		
	2011	2012	Average	2011	2012	Average	2011	2012	Average
	---------- number plant^{-1} ----------								
Control without straw	156	87	122 A	139	71	105 A	17	16	17 A
Control with straw	152	107	129 A	135	87	111 A	17	20	18 A
Jack bean	145	101	123 A	129	82	106 A	16	19	17 A
Sun hemp	142	83	112 A	122	63	92 A	20	19	20 A
Dwarf velvet bean	160	102	131 A	142	81	112 A	18	21	19 A
Mung bean	134	90	112 A	119	72	96 A	15	18	16 A
White lupine	124	90	107 A	109	70	90 A	15	19	17 A
Cowpea bean	153	107	130 A	136	85	110 A	17	21	20 A
Average	147 a	87 b		129 a	76 b		17 b	19 a	
*CV (%)	14.43			15.51			16.94		

Notes: Means in rows followed by the same lowercase letter and means in columns followed by the same uppercase letter are not significantly different in accordance with the Tukey-Kramer test ($p < 0.10$).
*Coefficient of variation.

Table 4. Weight of total (WTF), marketable (WMF) and damaged (WDF) fruits of cherry tomato grown in intercropping systems with legumes

Treatment	Weight of total fruits (WTF)			Weight of marketable fruits (WMF) (1)			Weight of damaged fruits (WDF)		
	2011	2012	Average	2011	2012	Average	2011	2012	Average
	------------------ g $plant^{-1}$ ------------------								
Control without straw	1714 aA	784 bA	1249	1556 aA	657 bA	1106	158	127	143 A
Control with straw	1610 aA	988 bA	1299	1463 aAB	830 bA	1146	147	159	153 A
Jack bean	1499 aAB	908 bA	1203	1359 aABC	750 bA	1054	140	158	149 A
Sun hemp	1310 aAB	735 bA	1022	1160 aBC	579 bA	869	150	156	153 A
Dwarf velvet bean	1612 aA	931 bA	1271	1464 aAB	760 bA	1112	148	171	159 A
Mung bean	1335 aAB	816 bA	1076	1210 aABC	675 bA	943	125	141	133 A
White lupine	1123 aB	780 aA	952	1006 aC	631 aA	819	117	149	133 A
Cowpea bean	1600 aA	971 bA	1285	1447 aAB	791 bA	1119	153	179	166 A
Average	1475	864		1333	709		142 b	155 a	
*CV (%)	14.98			7.92			20.2		

Note: Means in rows followed by the same lowercase letter and means in columns followed by the same uppercase letter are not significantly different in accordance with the Tukey-Kramer test *(p* < 0.10).

(1) Statistics on transformed data for $\sqrt{(x)}$.

* Coefficient of variation of transformed data.

Table 5. Average mass of total, marketable and damaged fruits of cherry tomatoes grown in intercropping systems with legumes

Treatment	Average weight of total fruits (AWTF)			Average weight of marketable fruits (AWMF)			The average weight of damaged fruits (AWDF)		
	2011	2012	Average	2011	2012	Average	2011	2012	Average
	---------- g fruit^{-1} ----------								
Control without straw	11.0 aA	9.0 bA	10.0	11.2 aA	9.3 bA	10.2	9.2 aA	8.0 aA	8.6
Control with straw	10.6 aAB	9.2 bA	9.9	10.9 aAB	9.5 bA	10.2	8.8 aAB	8.1 aA	8.5
Jack bean	10.4 aAB	9.0 bA	9.7	10.6 aAB	9.2 bA	9.9	8.9 aAB	8.3 aA	8.6
Sun hemp	9.2 aCD	8.9 aA	9.1	9.6 aCD	9.1 aA	9.3	7.6 aB	8.1 aA	7.8
Dwarf velvet bean	10.1 aABC	9.1 aA	9.6	10.3 aBC	9.4 aA	9.8	8.3 aAB	8.2 aA	8.3
Mung bean	9.9 aBCD	9.0 aA	9.5	10.1 aBC	9.3 aA	9.7	8.2 aAB	7.9 aA	8.0
White lupine	9.0 aD	8.7 aA	8.9	9.2 aD	9.0 aA	9.1	7.7 aB	7.7 aA	7.7
Cowpea bean	10.4 aAB	9.1 bA	9.7	10.6 aAB	9.4 bA	10.0	8.8 aAB	7.9 aA	8.4
Average	9.0	8.0		9.2	8.2		7.5	7.1	
*CV (%)	5.37			4.99			9.90		

Notes: Means in rows followed by the same lowercase letter and means in columns followed by the same uppercase letter are not significantly different in accordance with the Tukey-Kramer test ($p < 0.10$).
*Coefficient of variation.

In 2011, the concentration of manganese (Mn) in the cherry tomato shoot was lower in the treatments with sun hemp, jack bean and cowpea bean than in the control without straw (Table 6), and the concentration of Ca in the leaf of the cherry tomatoes grown with dwarf velvet beans was lower than that in control with straw or the intercropping treatments with sun hemp or white lupine (Table 7). In 2011, the concentration of Mn in the leaves of cherry tomatoes in the same treatment (intercrop with dwarf velvet bean) was also lower than for the cherry tomatoes intercropped with sun hemp (Table 7).

Table 6. Concentrations of copper (Cu) and manganese (Mn) in the shoot of cherry tomato grown in intercropping system with legumes

Treatment	Cu			Mn (2)		
	2011	2012	Average	2011	2012	Average
	-------------- mg kg^{-1} ---------------			--------------- mg kg^{-1} ---------------		
Control without straw	22.6 aA	17.6 bA	20.1	72.8 aA	41.8 bA	57.3
Control with straw	20.0 aAB	16.3 aA	18.1	54.2 aAB	35.4 bA	44.8
Jack bean	19.0 aAB	17.1 aA	18.1	40.3 aB	43.9 aA	42.1
Sun hemp	18.0 aB	16.5 aA	17.3	46.1 aB	42.1 aA	44.1
Dwarf velvet bean	19.5 aAB	16.3 aA	17.9	52.6 aAB	35.9 bA	44.2
Mung bean	19.0 aAB	16.2aA	17.6	50.3 aAB	37.3 aA	43.8
White lupine	20.6 aAB	16.1 bA	18.4	57.2 aAB	36.6 bA	46.9
Cowpea bean	19.6 aAB	17.2 aA	18.4	44.7 aB	36.5 aA	40.6
Average	19.8	16.7		52.3	38.7	
*CV (%)	11.84					

Notes: Means in rows followed by the same lowercase letter and means in columns followed by the same uppercase letter are not significantly different in accordance with the Tukey-Kramer test ($p < 0.10$). (2) Statistics on transformed data for *log(x)*. *Coefficient of variation of transformed data.

Table 7. Calcium (Ca) and Manganese (Mn) concentration in the leaf of cherry tomato grown in intercropping system with legumes.

Treatment	Ca (2)			Mn (2)		
	2011	2012	Average	2011	2012	Average
	-------------- g kg^{-1} ---------------			--------------- mg kg^{-1} ---------------		
Control without straw	15.6 aAB	10.8 bA	13.2	49.4 aAB	25.8 bA	37.6
Control with straw	16.7 aA	11.7 bA	14.2	49.5 aAB	29.1 bA	39.3
Jack bean	13.8 aAB	12.7 aA	13.3	39.4 aAB	28.8 aA	34.1
Sun hemp	17.3 aA	11.5 bA	14.4	51.6 aA	26.9 bA	39.2
Dwarf velvet bean	12.2 aB	11.8 aA	12.0	36.5 aB	29.0 aA	32.7
Mung bean	13.9 aAB	11.1 aA	12.5	48.5 aAB	28.1 bA	38.3
White lupine	16.5 aA	12.7 aA	14.6	47.2 aAB	30.3 bA	38.8
Cowpea bean	15.2 aAB	12.2 aA	13.7	43.9 aAB	30.6 aA	37.3
Average	15.2	11.8		45.8	28.6	
*CV (%)	5.16			4.14		

Notes: Means in rows followed by the same lowercase letter and means in columns followed by the same uppercase letter are not significantly different in accordance with the Tukey-Kramer test ($p<0.10$). (2) Statistics on transformed data for *log(x)*. *Coefficient of variation of transformed data.

The interactions between year and treatment were not significant for P, K, Mg, Zn, and Fe concentrations. For these nutrients, the overall mean concentrations in the leaf, fruit and shoot of the cherry tomatoes were different ($p < 0.10$) between the two years (Figure 2). In the leaf, the Mg, K, Zn and Fe concentrations were higher in 2011 than in 2012 (Figure 3A and B), and in the shoots, the concentrations of P, K, Mg, Fe and Zn were also higher in 2011 (Figure 3C and D). In the fruit, however, only the concentrations of Cu, Fe and Zn were higher in 2011 (Figure 3 E and F, whereas the concentrations of P and Cu in the leaf and Ca, Mg and Mn in the fruit were higher in 2012 compared with that in 2011 (Figure 3). However, these differences were probably due to a concentration effect since the cherry tomatoes had lower productivity and smaller fruits in 2012.

With regards to the chemical parameters of the tomato fruit, there were no significant interactions between treatment and year ($p > 0.10$) and no significant differences between the treatments or the years ($p > 0.10$). The overall means of chemical parameters of cherry tomato fruit were 4.1 for pH, 6.1 for Brix of soluble solids concentration, 0.4% titratable total acidity, 17.8 for the ratio between soluble solids content and titratable acidity, 1.5% total sugars and 1.0% of reducing sugars.

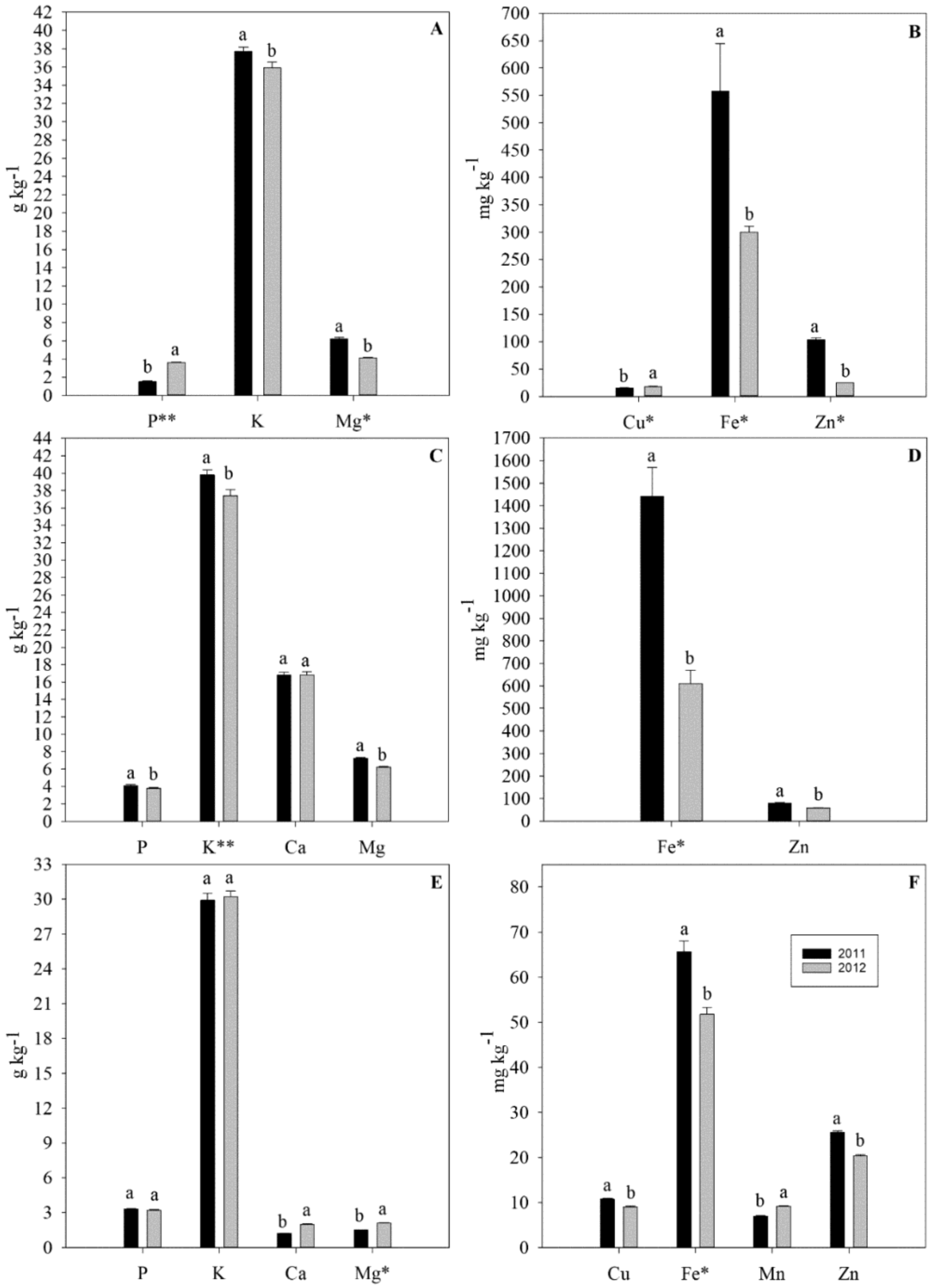

Figure 3. Concentration of macronutrients phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg); and micronutrients copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in the leaf (A and B), shoot (C and D) and fruit (E and F) of cherry tomato. Means followed by the same lowercase letter do not differ (Tukey test, $p < 0.10$)

* Statistics for transformed data for *log(x).*

** Statistics for transformed data for $\sqrt{(x)}$.

Legumes

The differences ($p < 0.10$) between the years in the yields of the leguminous green manures (fresh mass) grown in the intercropping systems with cherry tomatoes were only significant for the jack beans and cowpea bean, for which the yields were 160% and 630% higher in 2012 compared with that in 2011, respectively (Table 8). However, these increases in the fresh biomass weight in 2012 were only reflected in a significant increase in the dry biomass weight of the cowpea beans (Table 8). The highest yield of fresh biomass of the legumes (based on the average yields) was that of white lupine (30.2 Mg ha^{-1}), followed by jack bean (19.6 Mg ha^{-1}), cowpea bean (18.8 Mg ha^{-1}), sun hemp (14.9 Mg ha^{-1}), dwarf velvet bean (3.82 Mg ha^{-1}) and mung beans (2.9 Mg ha^{-1}) (Table 8).

The accumulation of macro- and micro-nutrients by the legumes varied according to the species and the dry mass yield. Jack beans, sun hemp, white lupine and cowpea bean were the legumes that accumulated the most macro- and micro-nutrients, and they were also the species that produced the highest dry mass (Table 8 and 9). On average, all the green manures accumulated macro-nutrients in the following decreasing order K > Ca > Mg > P. However, the accumulation of micro-nutrients varied between the species, with the jack beans, dwarf velvet bean and cowpea beans accumulating in decreasing order Fe > Mn> Zn> Cu, a similar order of accumulation to that of sun hemp and mung bean Fe> Zn > Mn> Cu, whereas for, the white lupine the order was Mn > Fe > Zn > Cu (Table 10). The P, Mg, Fe and Mn accumulated in the white lupine plant was lower in 2011 than in 2012 (Table 9 and 10).

Table 8. Fresh mass and dry mass of legumes grown in intercropping systems with cherry tomatoes

Treatments	Fresh weight [(1)]			Dry weight [(2)]		
	2011	2012	Average	2011	2012	Average
	---------- Mg ha^{-1} ----------					
Jack bean	10.9 bBC	28.3 aA	19.6	6.7 aB	7.6 aB	7.1
Sun hemp	17.5 aAB	12.4 aB	14.9	16.1 aA	8.9 bB	12.5
Dwarf velvet bean	3.3 aCD	4.3 aC	3.8	2.8 aC	1.5 aC	2.2
Mung bean	2.9 aD	2.9 aBC	2.9	2.4 aC	2.5 aC	2.4
White lupine	22.2 aA	38.3 aA	30.2	19.2 aA	16.7 aA	17.9
Cowpea bean	4.5 bCD	33.0 aA	18.8	2.7 bC	6.6 aB	4.6
Average	10.2	19.9		8.3	7.3	
*CV (%)	24.54			18.30		

Notes: Means in rows followed by the same lowercase letter and means in columns followed by the same uppercase letter are not significantly different in accordance with the Tukey-Kramer test ($p < 0.10$). [(1)] Statistics on transformed data for $\sqrt{(x)}$. [(2)] Statistics on transformed data for *log(x)*. *Coefficient of variation of transformed data.

Table 9. Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) accumulated in the aerial part of the legumes grown in intercropping systems with cherry tomatoes

Treatments	P [(1)]			K [(1)]			Ca [(1)]			Mg [(1)]		
	2011	2012	Avg.	2011	2012	Avg.	2011	2012	Avg.	2011	2012	Avg.
	kg ha^{-1}											
Jack bean	29.4 aA	21.4 aA	25.4	222.4 aA	213.2 aB	217.8	162.5 aA	115.8 aA	139.2	34.0 aA	31.6 aAB	32.8
Sun hemp	31.7 aA	20.4 aA	26.1	347.5 aA	192.2 bB	269.8	86.3 aB	84.8 aA	85.5	39.6 aA	36.1 aA	37.9
Dwarf velvet bean	8.5 aB	3.9 aB	6.3	66.5 aB	27.4 aC	46.9	57.9 aBC	20.3 aB	39.1	14.3 aB	5.9 aC	10.1
Mung bean	5.1 aB	7.2 aB	6.1	67.2 aB	64.6 aC	65.9	20.6 aC	29.3 aB	25.0	8.3 aB	13.2 aBC	10.8
White lupine	5.5 bB	28.8 aA	17.1	339.8 aA	430.7 aA	385.3	92.4 aAB	100.2 aA	96.3	22.6 bAB	46.0 aA	34.3
Cowpea bean	6.5 bB	23.9 aA	15.2	88.1 bB	233.8 aB	160.9	28.7 bC	112.9 aA	70.8	12.2 bB	38.0 aA	25.1
Average	14.5	17.50		188.6	193.7		74.7	77.22		21.8	28.47	
*CV (%)	22.23			21.38			22.60			23.69		

Notes: Means in rows followed by the same lowercase letter and means in columns followed by the same uppercase letter are not significantly different in accordance with the Tukey-Kramer test ($p < 0.10$).

(1) Statistics on transformed data for $\sqrt{(x)}$.

*Coefficient of variation of data transformed.

Avg. - average

Table 10. Copper (Cu), iron (Fe), manganese (Mn) e zinc (Zn) accumulated in the aerial part of the legumes grown in intercropping systems with cherry tomatoes

Treatments	Cu (1)			Fe (1)			Mn (2)			Zn (2)		
	2011	2012	#Avg.	2011	2012	Avg.	2011	2012	Avg.	2011	2012	Avg.
	-- g ha^{-1}--											
Jack bean	212.0 aA	79.4 bA	145.71	6865.8 aA	5059.5 aAB	5962.7	434.9 aB	364.0 aB	399.4	394.8 aAB	330.9 aB	362.8
Sun hemp	182.1 aAB	52.6 bA	117.33	6312.8 aA	3794.8 aBC	5053.8	450.9 aB	297.6 aB	374.3	574.9 aA	294.8 bBC	434.9
Dwarf velvet bean	149.1 aABC	8.6 bA	78.8	4241.4 aAB	302.5 bD	2004.4	243.6 aB	248.2 aB	245.9	189.2 aBC	76.6 aD	132.9
Mung bean	70.3 aC	32.2 aA	51.2	1533.8 aBC	1347.3 aCD	1443.5	82.1 aB	87.5 aB	84.8	79.8 aC	110.2 aCD	95.0
White lupine	73.1 aBC	94.5 aA	83.8	2774.7 bABC	9030.5 aA	5902.6	12843 bA	41780.0 aA	27311.0	383.4 aAB	657.8 aA	520.6
Cowpea bean	100.9 aBC	57.1 aA	79.0	1396.4 aC	2665.5 aBC	2273.0	123.8 aB	509.1 aB	316.4	174.1 aC	76.6 aAB	275.4
Average	131.2	54.1		3854.2	3700.02		2363.02	7214.8		299.4	307.8	
*CV (%)	27.96			23.44			10.83			23.45		

Notes: Means in rows followed by the same lowercase letter and means in columns followed by the same uppercase letter are not significantly different in accordance with the Tukey-Kramer test
($p < 0.10$).
(1) Statistics on transformed data for $\sqrt(x)$.
(2) Statistics on transformed data for *log(x)*.
*Coefficient of variation of transformed data.
Avg. - average

Green corn

There were no significant interactions ($p > 0.10$) between year and treatments for any of the variables analysed for the green corn yield and there were no significant effects of the treatments on the above-ground biomass dry weight of the green corn (Table 11). The productivity of green corn was 4.1, 2.5 and 1.7 Mg ha^{-1} for the weight of ears with straw (WES), the weight of ears without straw (WEWS) and marketable weight of ears (WME), respectively (Figure 4A) and for these variables, there were no differences between treatments or the years. However, the number of total ears (NTE) and the number of marketable ears (NME) were approximately 19 and 30% higher in 2012 compared to 2013 (Figure 4B). The weight of damaged ears (WDE) was lower in 2012 compared to 2013 (Figure 4A). The number of damaged ears (NDE) did not differ between the years (Figure 4A).

There were no significant interactions between year and treatment for the concentration and accumulation of macro- and micro-nutrients in the green corn ($p > 0.10$). The average concentrations of P, K, Ca, Mg, Cu, Fe, Mn, and Zn were 3.1g kg^{-1}, 23.4 g kg^{-1}, 3.7 g kg^{-1}, 2.4 g kg^{-1}, 5.4 mg kg^{-1}, 253.4 mg kg^{-1}, 35.0 mg kg^{-1} and 39.2 mg kg^{-1}, respectively. The macro- and micro-nutrient accumulation in the above-ground parts of the green corn (without ears) showed differences ($p < 0.10$) between the years (Figure 5), with less accumulation of P, K, Ca, Mg, Mn and Zn in the green corn in 2013 compared with that in 2012 (Figure 5A and B). The accumulation of Cu and Fe in the green corn did not differ ($p > 0.10$) between the years (Figure 5B and C). The lower accumulation of macronutrients by the green corn in 2013 may have affected the dry mass production of the green corn and, consequently, the yield in term of NET and NEC in 2012 (Figure 4 and 5)

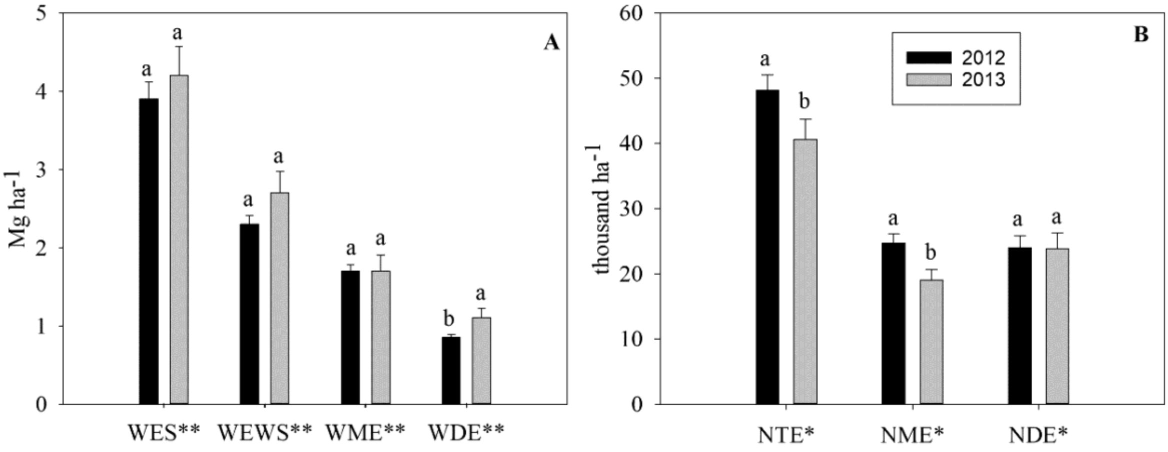

Figure 4. (A) Weight of ear with straw (WES), Weight of ear without straw (WEWS), Weight of marketable ear (WME), Weight of damaged ear (WDE), (B) number of total (NTE), marketable (NME) and damaged ears (NDE) of green corn ears cultivated in succession of cherry tomato and legumes in intercropping system. The means followed by the same lowercase letter do not differ by the Tukey-Kramer test ($p<0.10$). (1) Statistic on transformed data for $\sqrt{(x)}$. (2) Statistic on transformed data for log(x)

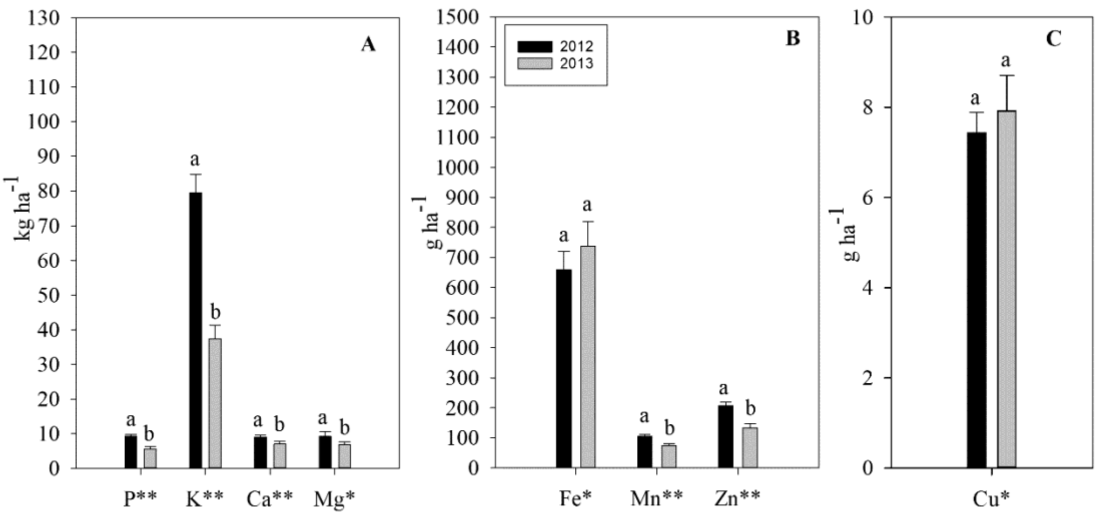

Figure 5. The macro and micronutrients accumulated in the aerial part without ears of green corn plants. (A) macronutrients: P-phosphorus, K-potassium, Ca- calcium, Mg-magnesium; (B) e (C) micronutrients: Fe-iron, Mn-manganese, Zn-zinc e Cu-cooper. The means followed by the same lowercase letter do not differ by the Tukey-Kramer test ($p<0.10$). (1) Statistics on transformed data for $\sqrt{(x)}$. (2) Statistics on transformed data for log(x)

Table 11. Dry weight of above ground biomass (without ears) of the green corn crop

Treatment	Dry weight [1]		
	---------- kg ha^{-1} ----------		
	2012	2013	Average
Control without straw	3127.44	2239.90	2683.67 A
Control with straw	3686.04	2017.74	2851.89 A
Jack bean	2990.48	2190.44	2590.46 A
Sun hemp	3363.30	2602.64	2982.97 A
Dwarf velvet bean	2591.08	1850.82	2220.95 A
Mung bean	3390.96	2996.78	3193.87 A
White lupine	2899.56	2128.20	2513.88 A
Cowpea bean	3410.58	2128.38	2769.48 A
Average	3182.43 a	2269.36 b	
*CV(%)	21.83		

Notes: Means in rows followed by the same lowercase letter and means in columns followed by the same uppercase letter are not significantly different in accordance with the Tukey-Kramer test ($p < 0.10$). [1]Statistic on transformed data for √(x). *Coefficient of variation of data transformed.

2.4. Discussion

Overall, the cherry tomato yields were higher, and there were fewer damaged fruits in the first year of cultivation (2011) than in the second year (2012) (Tables 3 and 4). The lower tomato yields in 2012 may have been due to immobilisation of nutrients in the soil by the addition of the green corn residue, coupled with a reduction of available nutrients by the successive cultivation of the green corn (unfertilised in 2012) between the two crops of cherry tomatoes. In the present study, the C:N ratio of the green corn straw was relatively high, approximately 32:1, and the decomposition of material of this nature has been reported to immobilize large amounts of N in the soil. Consequently, this may have reduced the availability of nitrogen for the cherry tomatoes grown under the straw mulch (SILVA et al., 2008; CARMEIS FILHO et al., 2014; MAESTRELO et al., 2014). According to Cucu et al. (2014) and Said-Pullicino et al. (2014), rice straw with a C:N ratio of 60:1 immobilised approximately 16 and 23% of N fertilizer applied to the soil.

Furthermore, the repeated sequence of green corn followed by cherry tomato/legumes intercropping appeared to have increased the problem with diseases in the second cycle of the cherry tomatoes, e.g. incidence of late blight (*Phytophthora infestans*) (data not presented), which may have affected the yield. Ambrosano et al. (2018) reported similar results in studies where organic cherry tomato/legume intercrop systems were grown through the residue of a previous green corn crop under greenhouse conditions and in which significant problems with diseases were also observed in the second growing cycle.

In 2011, the white lupine was thought to have posed competition with the cherry tomatoes for natural resources like light and nutrients, leading to lower tomato yields (WTF and WMF) (DUCHENE et al. 2017). A similar observation was also made, though to a lesser extent, in the treatment with cherry tomatoes intercropped with sun hemp (Tables 4 and 5). In 2011, the concentrations of Mn in the cherry tomato shoot were lower in the treatments with sun hemp, jack bean and cowpea compared to control without straw (Table 7), and this may have been an indication of competition at the beginning of the development of the cherry tomatoes. In 2011, the results of the legume yields were corroborated by the cherry tomato yields, with the white lupine and sun hemp producing greater dry biomass yields than the other legumes, and thus likely competing with the tomatoes for resources (Table 8). However, in 2012, there were no indications of competition between the white lupine and the cherry tomatoes. It appeared that in the first cropping cycle (in 2011), when the conditions for the development of both cultures within the intercropping systems were optimal, resulted in competition between the cherry tomato and the sun hemp or the white lupine. However, in the second year (2012), when the conditions were less optimal with a greater incidence of disease in the cherry tomatoes and the soil nutrients being immobilised during the decomposition of the green corn straw, there was less competition between the crops.

Even though the dwarf velvet beans were one of the legumes with the lowest dry matter yields and accumulation of Ca (Tables 8 and 9), the concentration of Ca in the leaf of the cherry tomatoes grown with dwarf velvet beans were lower than that for the tomatoes grown with, for example, the sun hemp (in 2011, Table 7).

The lower concentrations of some nutrients in the tomato leaf, shoot and fruit in 2012 may related to the lower productivity of the cherry tomato in this year (Figure 3). The immobilisation of nutrients during the decomposition of the green corn straw and the greater incidence of disease in 2012 may have affected the absorption of nutrients by the cherry tomatoes and, consequently, led to lower productivity (BABU et al. 2015).

The concentrations of nutrients in the tomato leaf and fruit were, on average, 15.1 and 22.3 g N kg^{-1}; 2.52 and 3.26 g kg^{-1} P; 36.8 and 30.0 g kg^{-1} K; 13.5 and 1.6 g kg^{-1} Ca; 5.2 and 1.8 g kg^{-1} Mg; 16.6 and 9.9 mg kg^{-1} Cu; 428.9 and 58.7 mg kg^{-1} Fe; 37.2 and 8.0 mg kg^{-1} Mn; 64.0 and 23.0 mg kg^{-1} Zn. For some of these nutrients, the concentrations were close to, or within, the ranges reported for these nutrients in tomatoes in the literature (BORGOGNONE et al., 2013; KUMAR et al., 2015; COLE et al., 2016; AMBROSANO et al., 2018). However, the mean concentration of Ca in the cherry tomato leaf was below the concentrations found in the literature, indicating a minimum of 24 g kg^{-1}. In 2011, the Fe concentration in the tomato leaves

(557.7 mg kg^{-1}; Figure 3B) was above that considered adequate for cherry tomatoes, reported as 300 mg kg^{-1}. However, no visual symptoms of nutrient deficiency or toxicity were diagnosed in the leaf or fruit of the cherry tomato plants. The concentration of nutrients in the plant tissue provides information about the nutritional status of the plants and consequently gives a general indication of whether the soil and the cropping system have provided sufficient nutrients for the growth and development of the crops. Competition between the plants in the intercropping system can occur in soils with low nutrient availability, which can be reflected in the nutrient concentrations in the plant tissue (DUCHENE et al., 2017).

According to Ambrosano et al. (2018), there were no indications of competition between the legumes and the cherry tomatoes in terms of the productivity or the nutrient concentrations in the plant tissue. These authors evaluated the productivity of intercropping systems with cherry tomatoes and different legumes (jack beans, sun hemp, dwarf velvet bean, mung bean, white lupine and cowpea) grown in greenhouse conditions. In another study, El-Gaid et al. (2014) investigated an intercropping system with tomatoes and common beans under field conditions, and no interspecific competition was observed in terms of the productivity of the tomatoes (number and mass of fruits). In intercropping systems, the balance between competition and complementarity between the species can vary in time and space, and depends on different factors such as nutrient availability, edaphoclimatic conditions, agricultural management and on the species and cultivars selected (BROOKER et al., 2015; BEDOUSSAC et al., 2015; DUCHENE et al., 2017; YU et al., 2016).

In this study reported here, the productivity of the sun hemp and jack bean were higher than that reported in the literature, indicating dry yields of 11.2 and 6.2 Mg ha^{-1}, respectively, when grown in monoculture in the summer (MANGARAVITE et al., 2014; FOSTER et al., 2017). However, compared with the work of Ambrosano et al. (2014), sun hemp (62.1 Mg ha^{-1}) grown in monoculture in the summer had much higher productivity in terms of the dry matter yield than that recorded in the present study.

In intercropping systems, the growth and the yield of the legume grown as green manures are important for effective control of weeds and for mobilising nutrients from different soil layers to make them available for subsequent crops. By occupying the space available between the rows of the main crop, the intercropped legume can provide effective competition with the weeds for space, light, nutrients, and green manures with fast-growing characteristics, such as sun hemp, that has been reported to have significant potential for suppressing weeds (MOSJIDIS; WEHTJE, 2011; SILBERG et al., 2019).

The values for the nutrients accumulated in the legumes in the present study (Tables 9 and 10) were in agreement with those reported by Ambrosano et al. (2010) and Mangaravite et al. (2014). The accumulation of Fe in legumes was, as expected, high, as this element is essential in the process of biological nitrogen fixation (BNF). Fe is incorporated into many symbiotic proteins; for example, the key to BNF is the nitrogenase enzyme complex formed by two iron proteins, one of which is the FeMo protein, containing two metals in the centre (MARSCHNER, 2012; BREAR et al., 2013). The white lupine accumulated large amounts of Mn (average, 27,311 g ha^{-1}), which is a known characteristic of this species, with cluster roots with large surface areas and high secretion activity of carboxylases that acidify the rhizosphere and solubilise Fe and Mn (PAGE et al., 2006; MARSCHNER, 2012).

When grown as green manures, legumes can provide an important source of nutrients and besides providing nitrogen through biological nitrogen fixation, they can increase the availability and cycling of nutrients by mobilising different chemical forms of nutrients in the soil and by taking up nutrients from different soil layers (TALGRE et al., 2012; DUCHENE et al., 2017). According to Ambrosano et al. (2011), about 20% of the N fixed by the sun hemp was reused by the following crop of sugar cane (*Saccharum* spp. cv. IAC-87-3396) grown in succession. Also, part of the N and other nutrients accumulated in legumes remain in the soil in the organic form, making them available for crops over successive years.

The jack bean was one of the legumes highlighted as promising option as a green manure for this cropping system, due to its relatively high yield and accumulation of nutrients and because there were no indications of competition between the cherry tomatoes and the jack beans in the intercropping system tested here. Legumes used as green manures in intercropping systems need to provide a balance in terms of dry weight yield and nutrient accumulation to provide effective weed control and improve the N concentration in the soil, without competing with the main crop (TREVISAN et al., 2017).

In this study, the yields of the green corn in terms of the weight of ears with straw (WES), without straw (WEWS) and the weight of marketable ears (WME) was lower than that reported in other studies, such as by Moreira et al. (2010). The low yields were probably linked to the lack of specific fertilization for the green corn. However, the concentration of Ca, Mg, P, K, Mn, and Zn in the green corn leaves were within the range considered appropriate for this crop (Table 10) (VITOSH et al., 1995; MALAVOLTA; VITTI; OLIVEIRA, 1997; GOTT et al., 2014). In 2012, the concentration of Cu in the above-ground part of the green corn (without ears) was below the minimum adequate concentration; however, this did not appear to have influenced the number and weight of the ears, compared to that recorded in 2013

(Table 11, Figure 4). In 2013, the Fe concentration was above the maximum concentration considered adequate for green corn. However, in the two years of cultivation of the green corn, no visual symptoms of nutrient toxicity or deficiency were observed. The reduced accumulation of nutrients may be related to the lower availability of nutrients in the soil due to the absence of specific fertilization for the green corn and the gradual removal of nutrients from the soil with the successive crops. This result may indicate that the use of leguminous green manures in the intercropping system as the only source of nutrients was insufficient to supply the nutritional demand of successive crops of green corn.

2.5 Conclusion

In the first year (2011), the weights of total and marketable tomato fruit (weight of total yield and of individual fruit) were lower in the treatments where cherry tomatoes were intercropped with white lupine or sun hemp, compared with the controls, indicating interspecific competition between the cherry tomatoes and these legumes. Although there were no indications of competition between the crops in 2012, it was recommended that white lupine and the sun hemp should be used as intercrop green manures with some caution, due to the high dry biomass yields produced in both years by these green manures.

The jack beans were highlighted as a promising green manure for this cropping system due to the high yield and nutrients accumulated by this legume and as there were no indications of competition between the jack beans and the cherry tomatoes.

No treatment affected the yield of green corn grown in the rotation, but the cropping system by itself was not thought to have supplied sufficient nutrients to meet the demand of the successive green corn crops.

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3 N transfer and biological N fixation in an intercropping system between legumes and organic cherry tomatoes in succession to green corn

Abstract

The intercropping of the main crops with legumes can increase the efficiency of N use in organic agriculture through nitrogen transfer. This study investigated the transfer of N from different legumes to cherry tomato in the intercropping system under residual straw of the previous green corn crop using the 15N natural abundance method. We also investigated the temporal variation in nitrogen transfer to a cherry tomato, the biological nitrogen fixation (BNF) of legumes and the N concentration of green corn cultivated in the intercrop succession. The experimental design was a complete randomized block with eight treatments and five replications, described as follows: (a) two controls consisting of a monocrop of cherry tomato with or without residual straw from the previous green corn crop, (b) cherry tomato and jack bean, (c) cherry tomato and sun hemp, (d) cherry tomato and velvet bean-dwarf, (e) cherry tomato and mung bean, (f) cherry tomato and white lupine, and (g) cherry tomato and cowpea bean in an intercropping system. There was no difference in BNF between legumes at 100 days after sowing, independent of the year. The BNF was responsible for more than half of the N accumulated in the legumes. The N of legumes was transferred to cherry tomato in similar quantities, and the leaves and fruits of cherry tomato received more N transfer than shoots. It was shown that N transfer increases with the growth/development of cherry tomato. The intercropping system with legumes did not affect the ^{15}N natural abundance of leaves and the aboveground biomass of green corn cultivated in succession. The legume in an intercropping system with cherry tomato cultivated in the succession of green corn does not provide sufficient nitrogen to supply the green corn demand.

Keywords: green manure, ^{15}N natural abundance, N concentration

Transferência de N e fixação biológica de nitrogênio em sistema consorciados entre leguminosas e minitomateiro em sucessão ao milho verde

Resumo

O consórcio entre culturas principais com leguminosas pode aumentar a eficiência de uso de N na agricultura orgânica através da transferência de nitrogênio. O objetivo do estudo foi investigar a transferência de N de diferentes leguminosas (Fabaceae) para o minitomateiro em sistema consorciados sob palha residual de milho verde utilizando o método de abundância natural de ^{15}N em dois anos consecutivos. Assim como, também foi avaliado a variação temporal na transferência de N para o minitomateiro, a fixação biológica de nitrogênio dos legumes e a concentração de N de milho verde cultivado em sucessão. O desenho experimental foi de blocos ao acaso com cinco repetições e oitos tratamentos: minitomateiro solteiro com palha residual de milho verde, minitomateiro solteiro sem palha residual de milho, minitomateiro em consorcio com feijão-de-porco, crotalária-juncea, mucuna-anã, feijão-mungo, tremoço-branco, feijão-caupi. Não houve diferença entre a fixação biológica de nitrogênio (FBN) entre os legumes aos 100 dias após a semeadura, independente do ano. A FBN foi responsável por mais da metade do N acumulado pelas leguminosas. O N das leguminosas foi transferido para o minitomateiro em proporções similares, e as folhas e frutos do tomate receberam mais N transferido que os brotos. A transferências de N aumentou com o crescimento e desenvolvimento do minitomateiro. O sistema consorciado de tomate com leguminosas não afetou a abundância natural de ^{15}N das folhas e da parte aérea do milho verde cultivado em sucessão. O legume em consórcio com o minitomateiro cultivado em sucessão ao milho verde não foi suficiente para suprir a demanda de N do milho.

Palavras-chaves: adubação verde, teor de N, abundância natural de 15N

3.1 Introduction

Biological nitrogen fixation (BNF) by legumes- Fabaceae (rhizobium symbiosis) has received attention as a source of nitrogen (N) that can replace a portion of synthetic N fertilizer in a rotation or intercropping system. This method can be used in organic farming and is environmentally friendly (SANT'ANNA et al., 2018). According to (AMBROSANO et al., 2005), sugarcane (leaves + stalk) absorbed an equivalent of 11.5 kg ha^{-1} of N of *Crotalaria juncea* (sun hemp) planted before the sugarcane in the rotation system. The intercropping system between sun hemp and guinea grass or switchgrass has shown legume to non-legume transfers of N of 43 and 51%, respectively (ASHWORTH et al., 2015).

Nitrogen (N) is an essential nutrient for plants and is required in large quantities. The N uptakes of tomatoes and green corn are approximately 137 kg ha^{-1} (BADR; ABOU-HUSSEIN; EL-TOHAMY, 2016; TEI; BENINCASA; GUIDUCCI, 2002) and 125 kg ha^{-1} (KHAN et al., 2018; SINGH et al., 2015), respectively. Nitrogen can be a limiting factor for increased productivity in organic agriculture. Low N availability and other nutrients from organic fertilizers might justify the lower productivity of organic agriculture than conventional agriculture (SACCO et al., 2015; SEUFERT; RAMANKUTTY; FOLEY, 2012). The availability of N from organic fertilizers such as manure is a consequence of the mineralization activity of the soil (SACCO et al., 2015). However, the rate of soil mineralization is highly variable because it depends on the temperature, humidity, aeration, type of soil, and source of N. Moreover, the mineralization rate must occur in sync with the culture demand for N such that N deficiency and losses of this nutrient do not occur through leaching, volatilization, or denitrification (ABBASI; KHIZAR, 2012; BERRY P.M. et al., 2002; MIKKELSEN; HARTZ, 2008; PANG; LETEY, 2000).

Therefore, intercropping the main crops with legumes can increase N use in organic agriculture. A portion of N from legumes can be transferred to non-legumes in a intercropping system through leaf leaching, the release of ammonia gas, exudation of compounds with N from root nodules, and transfers of interconnected roots via mycorrhiza (PEOPLES et al., 2015; THILAKARATHNA et al., 2016; ZANG et al., 2015). In addition, the use of legumes maintains or increases the total N present in the soil, which can be made available for the crop in succession (AMBROSANO et al., 2005). However, the quantities of N transfer between plants are variable, depending on the seasons (RASMUSSEN et al., 2013), the species/variety of legume and non-legume plants (SAKAI et al., 2011; CHU; CHEN; CAO, 2004), the presence of mycorrhizae in the soil (PEOPLES et al., 2015), the type of intercropping system (such as the distance between legumes and non-legumes plants) (RASMUSSEN et al., 2013; ZHANG et al., 2017), the use of organic or conventional agriculture, and the type of soil. Hence, because numerous variables can affect N transfer, it is essential to study the best arrangements in terms of the species of legumes and the density of sowing to increase the transfer of N between plants.

The hypotheses of this study are given as follows: (1) N transfer occurs from legumes to cherry tomato; (2) this transfer varies according to the species companion of the legume; (3) the BNF and amount of N input through BNF varies according to the legume species; (4) the N derived from the soil and legumes is sufficient to supply the N demand of green corn. Our aim was (a) to investigate the transfer of N from different legumes to cherry tomato in the intercropping system under residual straw of the previous green corn crop using the ^{15}N natural abundance method, and (b) evaluate the BNF of each legume tested and (c) the N concentration and ^{15}N natural abundance of green corn cultivated in succession. We also investigated the temporal variation (2011/2012) in N transfer to the cherry tomato and the BNF of legume.

3.2 Materials and Method

3.2.1 Characterisation of the area experimental

The same topic 2.2.1

3.2.2 Experimental design

The same topic 2.2.2

3.2.3 Field and Crop management

The same topic 2.2.3

3.2.4 ^{15}N natural abundance ($\delta^{15}N$ ‰)

The part of the plant samples that was destination of micro and macronutrients (Topic 2.2.5) also taken to the Stable Isotopic laboratory for analysis ^{15}N natural abundance ($\delta^{15}N$ ‰). The samples were transported to the laboratory for determination of total nitrogen (%) and natural abundance of ^{15}N ($\delta^{15}N$ ‰). The analysis was performed using an isotope ratio mass spectrometer containing an automatic N analyser connected to a mass spectrometer (IRMS) – N 20-20 ANCA GSL (Automatic Nitrogen and Carbon Analyzer, Gas, Solid and Liquid - SERCON) (BARRIE; PROSSER, 1996). The natural abundance of ^{15}N was expressed by standard formula:

$$\delta^{15}N‰ = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] * 10^3 \quad (1)$$

Where the R_{sample} and $R_{standard}$ are the ratio between $^{15}N/^{14}N$ of the sample and the atmosphere, respectively.

3.2.5 Biological nitrogen fixation (BNF)

The percentage of N was derived from the atmosphere (%Ndfix) using the ^{15}N natural abundance method (SHEARER et al., 1983).

$$\%Ndfix = \frac{\delta^{15}N_{non-legume} - \delta^{15}N_{legume}}{\delta^{15}N_{non-legume} - \beta} * 100 \quad (2)$$

where $\delta^{15}N_{legume}$ is the aboveground biomass of legume plants, $\delta^{15}N_{non-legume}$ is the mean of the maize leaf and the leaf of the cherry tomato in the monocrop that was growing in the same soil, and β is the $\delta^{15}N$ of leaves of white lupine (-1,16 ‰) and vignia (-1,48 ‰) grown hydroponically without soil (BODDEY et al., 2000; NGULUU et al., 2002). The $\delta^{15}N$ of white lupine was used to calculate the %Ndfix of the sun hemp and white lupine, and the $\delta^{15}N$ of vignia was used to calculate %Ndfix of the other legumes tested in this research.

The accumulation of N was determined for the aboveground biomass of the legume using the product of the N concentration and the dry mass of the plant fraction, expressed in kg ha^{-1}. The nitrogen derived from biological nitrogen fixation (Ndf) was determined the N accumulation by the percentage of BNF. Subsequently, the N derived from soil (Nds) was determined for the subtraction of Ndf by the N accumulated in all aboveground biomass of legumes.

3.2.6 Nitrogen transfer from legume to cherry tomato

The calculation of N transfer from legume to cherry tomato was performed with the formula:

$$\%\ N\ transfer = \left(1 - \frac{\delta^{15}N‰_{(m)}}{\delta^{15}N‰_{(p)}}\right) * 100 \quad (3)$$

where %N transfer denotes the proportion of cherry tomato nitrogen derived from the legume, $\delta^{15}N$ ‰$_{(p)}$ is the monocrop of cherry tomato (reference plant), and $\delta^{15}N$ ‰$_{(m)}$ is the cherry tomato intercropped with the legume. O $\delta^{15}N_{(p)}$, on average, was +12,25‰ for the shoot and leaf and +13,54 ‰ for the fruit (Table 12). The reference plant is the leaf and the fruit of the same cherry tomato that was grown in the same soil in monocrop but one year before this experiment (SALGADO et al., 2020). The N concentration was also determined by mass spectrometry analysis.

Table 12. Natural abundance ^{15}N of leaf and fruit of reference plant of cherry tomato

	Leaf	Fruit
Repetitions	--------------- $\delta^{15}N$‰ (δar) ----------------	
1	+12.52	+13.78
2	+11.4	+12.95
3	+14.26	+15.91
4	+9.35	+9.39
5	+13.71	+15.81
Means	+12.25	+13.57

Note: The table taken from the work of Salgado et al. (2020).

3.2.7 Statistical analyses

Statistical analysis with repeated measures was performed using the MIXED procedure in SAS software (STATISTICAL ANALYSIS SYSTEM, 9.3). The Tukey-Kramer test was applied for comparisons between treatment means, and the F test was applied for comparisons between year means. The Dunnett test was used to contrast the effects of each treatment of cherry tomato with the additional treatment (reference plant) and each legume treatment with the additional treatment (non-legume plant) for ^{15}N natural abundance ($\delta^{15}N$ ‰). The level of significance adopted for analysis of variance was $p < 0.10$.

3.3 Results

Biological Nitrogen Fixation (BNF)

A difference was observed in the ^{15}N natural abundance between all legumes and non-legumes, except for the dwarf velvet bean at 40 days after sowing (DAS) in 2011 and 2012, and white lupine at 100 DAS in 2012 (Table 13). Therefore, the dwarf velvet bean at 40 DAS in 2011 and 2012 and the white lupine at 100 DAS in 2012 did not have biological nitrogen fixation (BFN) (Table 14). The legumes had a ^{15}N natural abundance in 2011 between +1.03 and +5.20 at 40 DAS and between +0.49 and +3.32 at 100 DAS, and in 2012, this value was between +0.83 and +5.55 at 40 DAS and between +0.59 and +6.32 at 100 DAS (Table 13). In general, the smaller ^{15}N natural abundance, the higher the BNF.

Table 13. ^{15}N natural abundance of leaves 40 days after sowing (DAS) and aerial part without grain 100 DAS of legumes in intercropping system with cherry tomatoes

Treatments	40 DAS (1)			100 DAS (1)		
	2011	2012	Average	2011	2012	Average
	$\delta^{15}N‰$ (δair)					
Jack bean	+5.20 aA*	+3.91 aA**	+4.55	+3.32 aA*	-1.17 bB**	+1.08
Sun hemp	+2.33 aBC**	+1.24 aAB**	+1.78	+1.43 aA**	+0.53 aB**	+0.98
Dwarf velvet bean	+5.83 aA^ns	+5.55 aA^ns	+5.69	+0.49 aA**	-0.63 aB**	-0.07
Mung bean	+4.36 aAB**	+2.77 aAB**	+3.56	+2.06 aA**	-0.59 aB**	+0.73
White lupine	+1.03 aC**	+0.83 aB**	+0.93	+1.57 aA**	+6.32 aA^ns	+3.94
Cowpea bean	+4.31 aAB**	+1.74 aAB**	+3.03	+1.76 aA**	+0.19 aB**	+0.98
Average	+3.84	+2.67		+1.77	+0.77	
Non-legume	+8.43	+8.43		+8.43	+8.43	
#CV (%)	15.15			20.13		

Note: the means followed by the same lowercase letter in the lines do not differ by the F-test ($p < 0.10$) and means followed by the same uppercase letter in the columns do not differ by the Tukey-Kramer test ($p < 0.10$). (1)Statistics on transformed data for √(x). *It differs from non-legume by the Dunnet test at 1%. **It differs from non-legume by the Dunnet test at 5%. ns Not significant. # Coefficient of variation of data transformed.

Table 14. Biological nitrogen fixation of legumes at 40 days after sowing (DAS) and 100 DAS in intercropping system with cherry tomatoes

Treatments	40 DAS			100 DAS		
	2011	2012	Average	2011	2012	Average
	%					
Jack bean	32.6 aC	45.4 aB	39.0	51.6	95.0	73.3 A
Sun hemp	63.6 aAB	75.2 aA	69.4	73.0	82.6	77.8 A
Dwarf velvet bean	-	-		80.2	80.9	80.5 A
Mung bean	41.0 aBC	59.4 aAB	50.2	64.4	85.2	74.8 A
White lupine	77.0 aA	79.2 aA	78.1	79.3*	-	
Cowpea bean	41.6 aBC	67.6 aAB	54.6	67.2	83.2	75.2 A
Average	51.2	65.4		67.3 b	85.4 a	
#CV (%)	24.01			20.39		

Note: the means followed by the same lowercase letter in the lines do not differ by the F-test ($p < 0.10$) and means followed by the same uppercase letter in the columns do not differ by the Tukey-Kramer test ($p < 0.10$). #Coefficient of variation of transformed data.
* It does not include in the statistic because there was not BNF in the second year (2012).

The sun hemp and white lupine showed the highest BNF followed by cowpea bean, mung bean and jack bean at 40 DAS in 2011 and 2012 (Table 14). However, there was no difference in BNF between legumes at 100 DAS, independent of the year (Table 14). In the first year (2011), the BFN was less than in 2012 at 100 DAS, on average (Table 14). There was no difference of BNF ($p > 0.10$) between 40 and 100 DAS in 2011 for any legumes (Figure 6). In 2012, only the jack bean and mung bean showed a statistically increased BFN from 40 DAS to 100 DAS (Figure 6). However, in terms of numerical observation, the BFN in 100 DAS tends to be higher than that at 40 DAS (Figure 6).

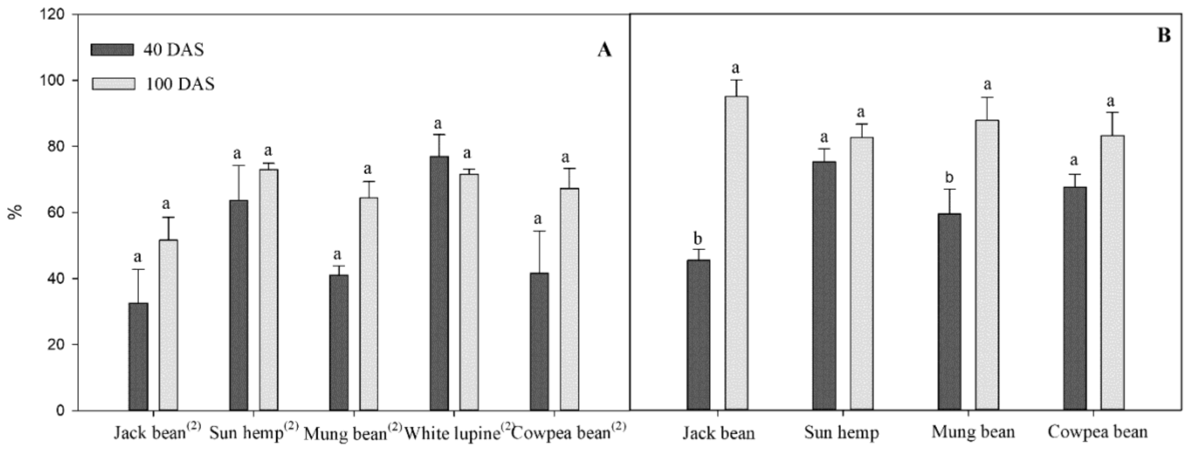

Figure 6. Biological nitrogen fixation (BNF) of legumes at 40 days after sowing (DAS) and 100 DAS in intercropping with cherry tomato in (A) 2011 and (B) 2012. The means followed by the same lowercase letter in the lines do not differ by the Tukey-Kramer test ($p < 0.10$).[(2)] Statistics on transformed data for log(x).

The highest total N accumulation of legumes was shown by jack bean followed by sun hemp, white lupine, cowpea bean, mung bean, and velvet bean-dwarf at 100 DAS, on average (Table 15). A difference in total N accumulation was observed between years only for velvet bean-dwarf and cowpea bean (Table 15). The velvet bean-dwarf had less total N accumulation in 2012 than in 2011. However, the seeds showed low emergence, not displaying all the potential of this species. The cowpea bean showed more total N accumulation in 2012 than in 2011 (Table 15). Consequently, in this same year, the cowpea bean had higher BNF (Table 14).

The jack bean and cowpea bean showed a difference in N accumulation derived from biological nitrogen fixation (Ndf) between years (Table 15). The jack bean and cowpea bean had higher Ndf in 2012 than in 2011. Nevertheless, only jack bean had lower N accumulation derived from soil (Nds) in 2012 than in 2011 (Table 15). The highest Ndf was shown by jack bean followed by sun hemp, cowpea bean, velvet bean-dwarf, and mung bean, on average (Table 15). Additionally, the highest Nds was found in white lupinee followed by jack bean, sun hemp, cowpea bean, velvet bean-dwarf and mung bean, on average (Table 15).

Table 15. Nitrogen accumulates total and the N accumulate derived from biological nitrogen fixation (Ndf) and nitrogen derived from soil (Nds) at 100 DAS

Treatments	N accumulate (1)			Ndf (1)			Nds (2)		
	2011	2012	Average	2011	2012	Average	2011	2012	Average
	---------- kg ha^{-1} ----------								
Jack bean	180.5 aA	243.9 aA	212.2	94.0 bAB	233.9 aA	164.0	86.6 aA	10.0 bCD	48.3
Sun hemp	186.7 aA	160.6 aA	173.6	135.7 aA	128.6 aBC	132.2	51.0 aAB	31.9 aAB	41.4
Dwarf velvet bean	89.2 aABC	27.9 bC	58.5	71.6 aAB	27.2 aD	49.4	17.6 aAB	0.3 aD	9.0
Mung bean	31.9 aC	49.8 aBC	40.9	21.0 aB	45.6 aCD	33.3	10.9 aB	3.5 aCD	7.2
White lupine	106.4 aAB	147.4 aAB	126.9	66.6*	-		39.9 bAB	147.4 aA	93.6
Cowpea bean	64.0 bBC	182.3 aA	123.1	41.5 bB	148.7 aB	95.1	22.5 aAB	33.6 aBC	28.1
Average	109.8	135.3		72.8	126.8		38.1	37.8	
#CV (%)	25.22			24.21			40.41		

Note: the means followed by the same lowercase letter in the lines and means followed by the same uppercase letter in the columns do not differ by the Tukey-Kramer test ($p < 0.10$).
(1) Statistics on transformed data for √(x).
(2) Statistics on transformed data for log(x).
Coefficient of variation of transformed data.
* It does not include in the statistic, because there was not BNF in the second year (2012).

Cherry tomato and transfer of nitrogen

No interactions were noted between treatment and year for the variables the natural abundance of ^{15}N, % transfer from legumes to cherry tomato and N concentration of shoots, leaves, and fruits of cherry tomato (Tables 16 and 17 and Figure 7).

The natural abundance ^{15}N showed a difference between the reference plant and each treatment of cherry tomato and legumes in the intercropping system ($p < 0.01$ or 0.05) that had shown N transfer from legume to cherry tomato (Table 16). The smaller the ^{15}N natural abundance of cherry tomato compared to the reference plant, the higher the N transfer from legume to cherry tomato. The natural abundance of ^{15}N of the cherry tomato fruits in 2011 showed more depletion in ^{15}N than in 2012 (Table 16). Therefore, more N transfer occurred from legumes to cherry tomato fruits in 2011 than in 2012, on average (Figure 7). The BNF was lower and the N transfer to cherry tomato was higher in 2011 than in 2012 (Table 14 and Figure 7).

Furthermore, the shoots had less N transfer than the leaves and fruits in both years (Figure 7), but the BNF showed no difference ($p < 0.1$) between 40 and 100 DAS in 2011 and 2012. The fruits received less N transfer than the leaves in 2012, but the same did not occur in 2011 (Figure 7). Consequently, the N concentration in 2012 was lower than in 2011, as well as for the shoots. However, the N concentration of leaves was higher in 2011 than in 2012 (Table 17).

Table 16. The natural abundance of ^{15}N of shoots, leaves and fruits of cherry tomato organic in intercropping system with legumes

Treatments	Shoots			Leaves			Fruits		
	2011	2012	Average	2011	2012	Average	2011	2012	Average
	$\delta^{15}N$‰ (δ air)								
Control without straw	+8.82 **	+9.68 *	+9.25 A	+8.13 **	+7.07 **	+7.60 A	+8.66 **	+8.70 **	+8.68 A
Control with straw	+8.45 **	+8.99 **	+8.72 A	+7.77 **	+7.27 **	+7.52 A	+8.59 **	+9.71 **	+9.15 A
Jack bean	+9.18 **	+8.66 **	+8.92 A	+7.20 **	+6.58 **	+6.89 A	+8.64 **	+9.45 **	+9.05 A
Sun hemp	+8.77 **	+9.51 **	+9.14 A	+6.66 **	+6.8 **	+6.78 A	+8.54 **	+8.85 **	+8.70 A
Dwarf velvet bean	+9.16 **	+9.11 **	+9.13 A	+6.69 **	+7.04 **	+6.87 A	+8.46 **	+9.13 **	+8.79 A
Mung bean	+8.34 **	+9.26 **	+8.80 A	+7.28 **	+7.31 **	+7.30 A	+8.32 **	+8.69 **	+8.50 A
White lupine	+8.91 **	+8.70 **	+8.81 A	+7.30 **	+6.99 **	+7.15 A	+8.23 **	+9.74 **	+8.98 A
Cowpea bean	+9.10 **	+9.50 **	+9.30 A	+6.98 **	+6.98 **	+6.98 A	+9.01 **	+9.71 **	+9.36 A
Average	+8.84 a	+9.18 a		+7.25 a	+7.02 a		+8.56 b	+9.25 a	
Reference plants	+12.25	+12.25		+12.25	+12.25		+13.57	+13.57	
#CV (%)	12.02			13.32			11.74		

Note: the means followed by the same lowercase letter in the lines do not differ by the F-test ($p < 0.10$) and means followed by the same uppercase letter in the columns do not differ by the Tukey-Kramer test ($p<0.10$).
*Different from reference plant by the Dunnet test at 5%.
** Different from reference plant by the Dunnet test at 1%.
^{ns} Not significant.
Coefficient of variation

Table 17. N concentration of shoots, leaves and fruits of cherry tomato in intercropping system with legumes in the field

Treatments	Shoots			Leaves			Fruits		
	2011	2012	Average	2011	2012	Average	2011	2012	Average
	------	------	------	------	g kg^{-1}	------	------	------	------
Control without straw	38.3	34.0	36.2A	15.1	49.0	32.0A	24.4	20.6	22.5A
Control with straw	38.8	33.9	36.3A	14.8	46.9	30.8A	24.6	20.1	22.4A
Jack bean	36.0	32.4	34.2A	15.0	50.5	32.7A	24.1	20.3	22.2A
Sun hemp	37.8	29.9	33.9A	14.3	47.0	30.7A	23.0	20.8	21.9A
Dwarf velvet bean	35.1	34.7	34.9A	11.6	50.4	31.0A	25.0	21.7	23.3A
Mung bean	36.6	32.6	34.6A	14.8	48.8	31.8A	25.2	20.3	22.7A
White lupine	38.4	33.3	35.9A	16.4	49.4	32.9A	23.8	20.3	22.0A
Cowpea bean	35.2	34.0	34.6A	12.8	50.3	31.6A	24.1	19.2	21.7A
Average	37.0a	33.1b		14.4b	49.0a		24.2a	20.4b	
#CV (%)	13.64			13.44			8.19		

Note: the means followed by the same lowercase letter in the lines do not differ by the F-test ($p < 0.10$) and means followed by the same uppercase letter in the columns do not differ by the Tukey-Kramer test ($p < 0.10$).

Coefficient of variation of transformed data.

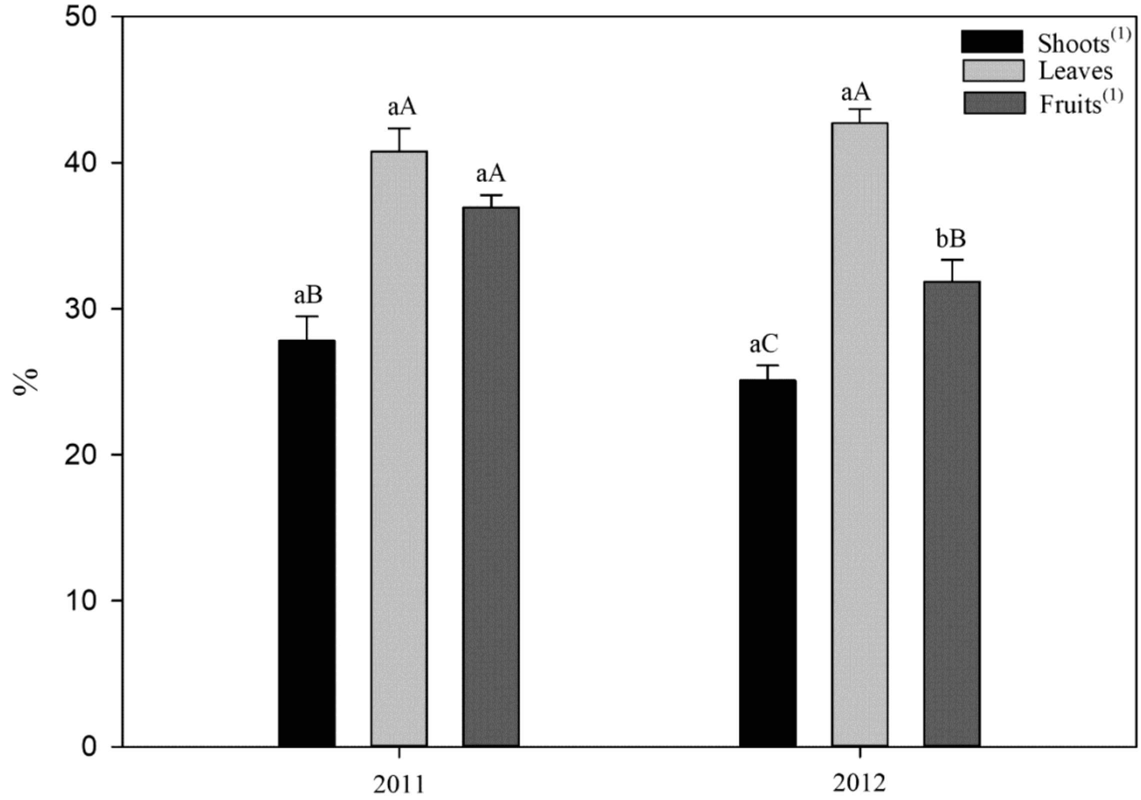

Figure 7. %N transfer from legumes to a cherry tomato in intercropping system. The means followed by lowercase letters show differences between the years by the F-test ($p < 0.10$), and the means followed by uppercase letters show the difference between the parts of the plants (shoots, leaves and fruit) by the Tukey-Kramer test ($p < 0.10$). [(1)] Statistics on transformed data for $\sqrt{(x)}$ for years

Green corn

There was no interaction between treatments and year for ^{15}N natural abundance, N concentration and N accumulated of leaves of green corn (Table 18 and Figure 8). The N concentration of leaves was less in 2013 than in 2012 (Table 18). Consequently, N accumulated was also less in 2013 than in 2012 (Table 18). The aboveground biomass of green corn in the succession of dwarf velvet bean and cherry tomato in the intercropping system showed more N accumulated than the control with straw (monocrop of a cherry tomato), on average (Table 18). The ^{15}N natural abundance of the leaves and aboveground biomass did not show a difference between treatments (Figure 8). The leaves in 2012 showed more depletion in ^{15}N than in 2013 (Figure 8).

Table 18. N concentration of leaves and aerial part and N accumulated of aerial part of green corn

Treatments	Leaves			Aerial Part		
	N concentration			N accumulated (1)		
	2012	2013	Average	2012	2013	Average
	---------------- g kg^{-1} -------------			------------------- kg ha^{-1} ---------------		
Control without straw	14.2	9.5	11.9 A	15.25	11.93	13.60 AB
Control with straw	15.5	9.9	12.7 A	14.62	9.40	12.00 B
Jack bean	14.1	11.1	12.6 A	16.17	9.59	12.90 AB
Sun hemp	15.6	10.9	13.3 A	22.75	13.48	18.10 AB
Dwarf velvet bean	15.9	9.6	12.7 A	26.50	16.95	21.70 A
Mung bean	15.6	8.9	12.2 A	20.43	8.52	14.50 AB
White lupine	15.6	11.0	13.3 A	22.56	17.17	19.90 AB
Cowpea bean	15.3	11.4	13.3 A	20.28	5.67	13.00 AB
Average	15.2 a	10.3 b		19.82 a	11.59 b	
#CV (%)	13.90			22.07		

Note: The means followed by the same lowercase letter in the lines do not differ by the F-test ($p < 0.10$) and means followed by the same uppercase letter in the columns do not differ by the Tukey-Kramer test ($p < 0.10$). (1) Statistics on transformed data for $\sqrt{(x)}$. # Coefficient of variation of transformed data.

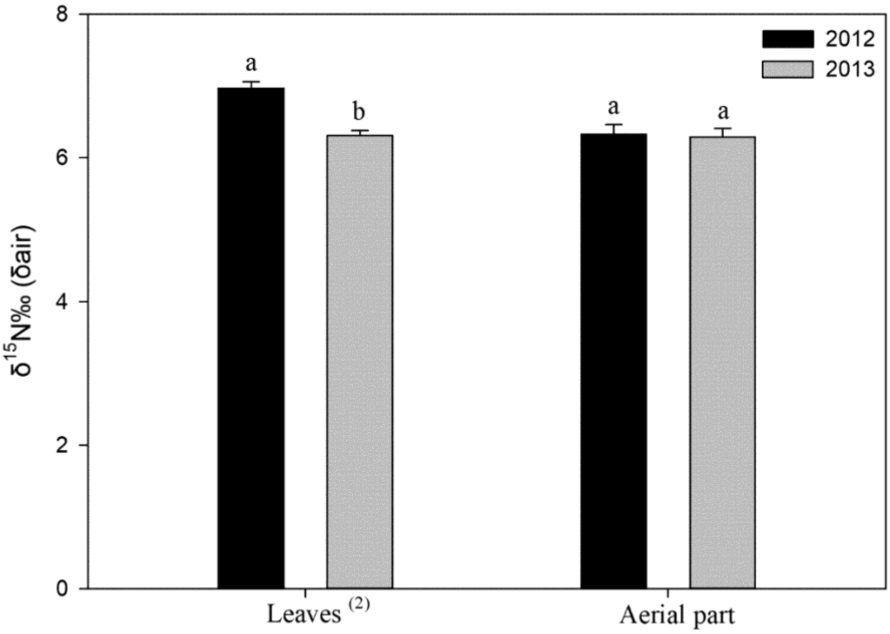

Figure 8. ^{15}N natural abundance of leaves and aboveground biomass without ears of green corn. The means followed by the same lowercase letter do not differ by the F-test ($p < 0.10$). (2) Statistics on transformed data for *log(x)*

3.4 Discussion

Biological nitrogen fixation was responsible for more than half of N accumulated in the legumes (Tables 14 and 15), and the values were higher or similar to values in other studies (MENDONÇA et al., 2017; POLANIA et al., 2016; SAIA et al., 2016; SANT'ANNA et al., 2018). However, the BNF was less in 2011 than 2012 at 100 DAS (Table 14). This result could be due to a reduction of the N available in the soil. The utilization of corn straw (C:N ratio was 69:1) under the soil can occur because of immobilization of the N (CARMEIS FILHO et al., 2014; CUCU et al., 2014; MAESTRELO et al., 2014; SAID-PULLICINO et al., 2014), and the succession of the green corn without N fertilizer probably reduced the N available in the soil and forced the legumes to make an effort to increase BFN. Studies have shown a higher BFN of legumes under less fertilizer or without N fertilizer (CHU; SHEN; CAO, 2004; FAN et al., 2006). In chickpea and bitter vetch, Romanyà and Casals (2020) verified that in soil that has less N available, the N from BNF is higher than N from the soil.

The dry matter yield of legume was 17.9 Mg ha^{-1} for white lupine, 12.5 Mg ha^{-1} for sun hemp, 7.1 Mg ha^{-1} for jack bean, 4.6 Mg ha^{-1} for cowpea bean, 2.2 Mg ha^{-1} for mung bean, and 2.4 Mg ha^{-1} for dwarf velvet bean. Although jack bean did not have a higher dry matter yield, it accumulated higher Ndf (164 kg ha^{-1}) than the other legumes, mainly in 2012 (Table 15). The sun hemp (132.1 kg ha^{-1}) and cowpea bean (95,1 kg ha^{-1}) also accumulated a large quantify of N from BNF (Table 15). Sant'Anna et al. (2018) reported average Ndf values for jack bean, velvet bean and sun hemp of 210, 133, and 80 kg ha^{-1}, respectively. The BNF differs depending on species, edaphoclimatic condition and agronomy management parameters such as temperature, rainfall, soil fertility, fertilizer and sowing time (ENRICO et al., 2020; MCCAULEY et al., 2012; PEOPLES et al., 2009).

In this study, the transfer of N from legume to cherry tomato in the field (Figure 7) showed results similar to those obtained by Salgado et al. (2020) in a greenhouse. Tang et al. (2018) verified approximately 15% of N transfer from broad bean (*Vicia faba* L.) to garlic. Clermont-Dauphin et al. (2016) demonstrated N transfer varying from 39 to 46% from the legume (*Pueraria phaseoloides*) to the rubber tree leaf. Moreover, another study with coffee and different legumes showed that 11.5 to 21.8 g kg^{-1} of N in the leaves of coffee came from the N of legumes (MENDONÇA et al., 2017). According to the same authors, *Cajanus cajan* and coffee in an intercropping system showed more N transferred to the coffee.

The N of legumes could be transferred to non-legume plants in an intercropping system as a portion of the N supply of these non-legume plants. The quantities of N transfer are variable depending on the seasons (RASMUSSEN et al., 2013), the species/variety of legume and non-legume plants (ASHWORTH et al., 2015; MENDONÇA et al., 2017; SAKAI et al., 2011), the presence of mycorrhizae in the soil (PEOPLES et al., 2015), the type of intercropping system (such as the distance between legume and non-legume plants) (RASMUSSEN et al., 2013; ZHANG et al., 2017), and fertilizer application (CHU; SHEN; CAO, 2004).

The fruits and shoots of cherry tomato received less N transfer from legumes in 2012 than in 2011, although BNF was higher in 2012 (Table 14 and Figure 7). The same result occurred in the study by Salgado et al. (2020) in which cherry tomato leaf had less N transferred in the second year of cultivation in a greenhouse. The lower N transfer and N concentration might have been caused by immobilization of the N in the soil, a problem with cherry tomato diseases, and interspecific competition between cherry tomato and green manure.

Corn straw that has a high C:N ratio under the soil can cause immobilization of N, as previously reported by Carmeis Filho et al. (2014), Cucu et al. (2014), Maestrelo et al. (2014), and Said-Pullicino et al. (2014). Additionally, the C:N ratio of the soil that increased between the cultivation years (2011/2013) from 8:1 to 10:1 showed increased carbon in the soil, corroborating with this theory. Moreover, the repetition of the cherry tomato crop increases the problem with diseases such as late blight (*Phytophthora infestans*), which affects the leaves of cherry tomato and consequently affects their photosynthetic capacity and reduces N absorption. (AMBROSANO et al., 2018; SALGADO et al., 2020). If the cherry tomato was debilitated because of the disease in 2012, this could have facilitated competition for a portion of the legume with the cherry tomato for space, water, and nutrients, although the legume produced a similar dry mass in 2011/12 in general (8.3 and 73 Mg ha^{-1}).

The leaves and fruits received more N transfer than the shoots (Figure 7). It was shown that the N transfer increases with the growth/development of the cherry tomato. This observation can be likely associated with the increasing necessity of N supply for a portion of cherry tomato. Furthermore, as the legume grows and develops, it also increases the N accumulation from BNF, the relationship between the roots (legume and non-legume) and the interaction between mycorrhizae and roots, which consequently increase the possibility of N transfer to cherry tomato.

Although, the cherry tomato had a higher N concentration in 2012 than in 2011, the yield was reduced by 41% in 2012 (864 g plant^{-1} of fruits) compared with that in 2011 (1475 g plant^{-1}). The cherry tomato showed less growth in 2012, and this high N concentration is probably the result of concentration of this nutrient in less dry mass. However, we did not observe symptoms of N deficiency independent of the year, and thus we concluded that the soil and the legumes provided sufficient N for the cherry tomato.

This study did not show a difference in N transfer ($p > 0.10$) for the treatments (Figure 7). Thus, the N of legumes was transferred to cherry tomato in similar quantities. However, the absence of the capacity of N transfer of legumes to non-legume might have been masked for one possible N contamination between the plots. As discussed in the study of Salgado et al. (2020), the proximity of the plot might have allowed the N from one plot with jack bean to have contaminated the other plot with velvet bean-dwarf treatment, for example, because N is highly mobile and can be transferred between plants for many pathways and over long-distance (>1.5 m) such as ammonia gas leaves, roots exudates, and mycorrhizae (PAULA et al., 2015; PEOPLES et al., 2015; RASMUSSEN et al., 2013). In future studies with nitrogen transfer in the field, it will be important to take care to randomize the plot and use greater distances between the plots or even barriers to avoid cross-contamination.

The green corn in 2013 had less N concentration in the leaves and aboveground biomass and less N accumulated in the aboveground biomass (Table 18). Despite the cultivation of legumes in the intercropping system before green corn, the absence of specific fertilization for green corn might have caused a reduction of the N available in the soil and consequently reduced the absorption by green corn. Furthermore, the N concentration of the leaves was less than recommended for the corn (VITOSH et al., 1995; GOTT et al., 2014; MALAVOLTA; VITTI; OLIVEIRA, 1997), and the N accumulated in the aboveground biomass was less than that found by Singh et al. (2015) and Suthar et al. (2012). The legume cultivated in the succession of green corn does not provide sufficient N to supply the green corn demand.

The intercropping system with legumes did not affect the ^{15}N natural abundance of the leaves and aboveground biomass of green corn cultivated in succession (Figure 8). The leaves in 2013 had less ^{15}N natural abundance ($\delta^{15}N$) (Figure 8). The leaves reflect the $\delta^{15}N$ of N available in the soil, and consequently, the $\delta^{15}N$ of the plant can be altered due to mycorrhizae dependence, the N forms absorbed and the depth of acquisition within the soil profile (CRAINE et al., 2015). Therefore, it is possible that the reduction in N available in the soil for green corn had increased mycorrhizae dependence. The plants infected by mycorrhizae displayed $\delta^{15}N$ reduced by 2‰ compared that in non-infected plants (CRAINE et al., 2015; HÖGBERG et al., 2011).

3.5 Conclusion

The BNF was responsible for more than half of the N accumulated in the legumes.

The N of legumes was transferred to cherry tomato in similar quantities, and the leaves and fruits of cherry tomato received more N transfer than shoots. It was shown that N transfer increases with the growth/development of cherry tomato.

The intercropping system with legumes did not affect the ^{15}N natural abundance of leaves and the aboveground biomass of green corn cultivated in succession. The legume in an intercropping system with cherry tomato cultivated in the succession of green corn does not provide sufficient nitrogen to supply the green corn demand.

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4 Decomposition and release of N and C from the residual green corn straw in organic cherry tomato intercropping with legumes

Abstract

The residual crop straw contains different macro and micronutrients essential for maintaining or increasing soil fertility and plant growth that are important for sustainable agriculture. The aim of this study was to evaluate the decomposition rate of residual green corn straw and the release of C and N from the residual green corn straw decomposition under cherry tomato and different legumes in intercropping system. The treatments were straw green corn under control, a cherry tomato monocrop, cherry tomato intercropping with jack bean, sun hemp, dwarf velvet bean, mung bean, white lupine or cowpea bean, in two consecutive years. The single exponential decay function fitted with the decrease of the decomposition time in the dry matter remaining of green corn straw in 2011 and 2012. In the same way, the exponential decay function fitted with the decrease of the decomposition time of the C and N release from the mass decomposition of green corn straw. However, the residual green corn straw under cherry tomato with different legumes (source of N) in an intercropping system did not increase the straw decomposition rate and the C and N release from straw decomposition. The C: N ratio has been a gradual decline over the days of decomposition, considering the maintenance of the C concentration and the increase of N concentration during the decomposition time, independently of treatment. The $\delta^{13}C$ decreased and the $\delta^{15}N$ tended to increase in the maize straw during the decomposition time.

Keywords: litter, green manures, $\delta^{13}C$ and $\delta^{15}N$ of straw

Decomposição e liberação de N e C da palha residual de milho em sistema de consórcio com minitomateiro e leguminosas

Resumo

A palha residual de milho contém nutrientes essenciais para manter ou aumentar a fertilidade do solo e para o crescimento das plantas, os quais são importantes para a sustentabilidade da agricultura. O objetivo foi avaliar a taxa de decomposição da palha residual de milho sobre o minitomateiro e diferentes leguminosas em sistemas consorciados. Os tratamentos foram a palha residual de milho sobre: minitomateiro solteiro, minitomateiro em consorcio com feijão-de-porco, crotalária-juncea, mucuna-anã, feijão-mungo, tremoço-branco, feijão-caupi em dois anos consecutivos. A função exponencial decrescente simples ajustou-se com a diminuição do tempo de decomposição para a matéria seca remanescente da palha em 2011 e 2012. Assim como, a função exponencial decrescente simples ajustou-se com a redução do tempo de decomposição para a liberação de N e C da massa de decomposição da palha de milho. A palha de milho sobre o consorcio de minitomateiro e leguminosas (fonte de N) não aumentou a taxa de decomposição e a liberação de C e N da decomposição da palha. A relação C: N tendenciou a reduzir com o tempo de decomposição, considerando que a concentração de C se manteve e a concentração de N aumento durante este tempo independentemente do tratamento. O $\delta^{13}C$ diminuiu e o $\delta^{15}N$ teve uma tendência a aumentar na palha de milho durante o tempo de decomposição.

Palavras-chave: palha de milho, adubação verde, $\delta^{13}C$ e $\delta^{15}N$ da palha

4.1. Introduction

The residual crop straw contains different macro and micronutrients essential for maintaining or increasing soil fertility and plant growth that are important for sustainable agriculture (SUN et al., 2021). Their decomposition is responsible for nutrient cycling, adding organic carbon to the soil, consequently forming humic substances (BERG; MCCLAUGHERTY, 2014). According to Sun et al. (2021) the maize straw application for two years increased the soil organic content and porosity, and decreased the bulk density promoting the soil aggregation. Furthermore, the residual straw applied to the soil plus fertilization in the long-term increased the decomposition of straw, consequently increased the biomass of bacteria and fungi, and resulted in improved soil fertility (ZHAO et al., 2019).

The straw application under the soil is a widely used practice in the organic agriculture system, enhancing the soil carbon, soil fertility, nutrients cycling, and minimizing the impact on the environment (REGANOLD; WACHTER, 2016; SCIALABBA; MLLER-LINDENLAUF, 2010). However, it is important that the nutrient release from straw decomposition be rapid for plant uptake, mainly the nitrogen, beyond reducing the N immobilization into the soil. The N availability can be a limiting factor for increasing the yield in this system (JOUZI et al., 2017b; SEUFERT; RAMANKUTTY; FOLEY, 2012) due to N and other nutrients being released into the soil through the mineralization from organic fertilizers, such as composts, green manures, residual crop. In addition, this needs to be in synchrony with the nutrient uptake of the crops (SACCO et al., 2015).

The decomposition rate of crop residue is influenced by different factors such as temperature, rainfall, soil type, fertilization, and residue quality (WANG et al., 2012). Chen et al. (2014) showed that the straw decomposition rate in the treatments with straw and N mineral was higher compared to the treatments with only straw. Another study observed that the straw decomposition rate increased by 17% in the treatments of manure fertilizer compared to the treatments without fertilizer (SUN et al., 2021). According to Torres et al. (2015), the decomposition rate of maize straw was higher in the treatments with sun hemp (legume) residue because the legume has the biological nitrogen fixation capacity, it consequently increased the N availability in the system, and resulted in a greater decomposition rate.

The leguminous are often used as the source of N, and they can be grown either in intercropping systems or in rotation with other crops (SALGADO et al., 2021). Based on that, we hypothesized that the residual green corn straw under cherry tomato with different legumes (source of N) in an intercropping system increases the straw decomposition rate and the release of C and N from the straw decomposition. The aim of this study was to evaluate the decomposition rate of residual green corn straw and the release of C and N from the residual green corn straw decomposition under cherry tomato and different legumes in intercropping system.

4.2 Materials and Methods

4.2.1 Characterisation of the experimental area

The same topic 2.2.1

4.2.2 Experimental design

The same topic 2.2.2

4.2.3 Field and Crop management

The same topic 2.2.3

4.2.4. Decomposition rate and release of Carbon and Nitrogen from the residual green corn straw

The decomposition of straw was evaluated in a bag of mesh nylon with a 2 mm opening and 0.2 x 0.2 m of dimension (ANDERSON; INGRAM, 1996). Each plot received ten bags that contained 100 g of straw bag^{-1} in 2011 and 70 g of straw bag^{-1} in 2012. It corresponds to 6 Mg ha^{-1} of residual green corn straw applied under the soil. The bags were randomly distributed in the plots after planting the cherry tomato and legumes in the middle of between the rows in 2011 and 2012. The bags were sampled at 15, 30, 60, 90, and 120 days after being placed under the soil. Two bags were sampled randomly at each time.

The straws in the bags sampled were separately dried on an oven 65°C with forced air circulation until they reached constant mass, then they were weighted to determine the remaining dry mass (DM). The decomposition calculation was determined by the difference between the dry mass of straw applied to start (0 days) and the dry mass of the remaining straw. The decomposition results were corrected using the ash content method. One grama of each straw bag was sampled and incinerated in the muffle oven 550°C for four hours (HUE; EVANS, 1996). This correction is necessary to exclude the straw contamination by the soil.

Subsequently, the samples were ground in the Wiley mill and taken to the laboratory for analysis. There was determined the total nitrogen (N), total carbon (C), the natural abundance of ^{15}N ($\delta^{15}N$ ‰), and the natural abundance of ^{13}C ($\delta^{13}C$ ‰) for straw samples using the sample preparation procedure described by Trivelin et al. (1973). The analysis was performed by an isotope ratio mass spectrometer containing an automatic N and C analyzer connected with a mass spectrometer (IRMS) – N and C 20-20 ANCA GSL (Automatic Nitrogen and Carbon Analyzer, Gas, Solid and Liquid – SERCON) (BARRIE; PROSSER, 1996).

The accumulation of C and N in the straw was determined using C concentration and the dry mass of each sample, expressed in Mg ha^{-1} or kg ha^{-1}.

The decomposition and nutrients release from decomposition was adjusted to the math model by Olson (1963) (Equation 4). There was determined the decomposition constant (k) by Equation 4 and the half lifetime ($t_{1/2}$, required time in days for decomposed 50% of DM initial) by Equation 5 (Olson 1963).

$$y = Xo.e^{-kt} \tag{4}$$

$$t_{1/2} = \frac{0.693}{k} \tag{5}$$

Where:

The y is DM or nutrient remaining (Mg ha^{-1} or kg ha^{-1}) after the time (t) in days; the Xo is the DM or nutrient remaining initial (Mg ha^{-1} or kg ha^{-1}); k is the constant of decomposition.

4.2.5. Statistics Analysis

The R software was used for statistical analysis with repeated measures (Version 1.3.1093) to determine the variance and covariance matrix structure. The significant interactions were broken down according to the factors involved. The averages of the main effects and interactions were compared using the Tukey test. The level of significance adopted for the analysis of variance was $p < 0.05$. The decomposition of straw and the nutrients released during the time was adjusted by the math model. The measure data and the simulates data were compared by statistic test following modeling efficiency (EF), coefficient of determination (CD), root mean square error (RMSE) with $RMSE_{95\%}$ corresponding to the 95% of the confidence interval of measurements, error relative (E) with $E_{95\%}$ corresponding to the 95% of the confidence interval of measurements and mean difference (M) (SMITH et al., 1997).

4.3. Results

There were no differences ($p > 0.05$) among the treatments and no interaction between treatments and time for dry mass remaining (DM) (2011), nitrogen and carbon concentration (2011 and 2012), C accumulated (2011), N accumulated (2011) and ratio C: N (2011 and 2012) (Table 19 and Figure 9).

The DM and the C and N accumulated reduced during the decomposition time in 2011 (Table 19). The C concentration was maintained during the time, and the N concentration has been slightly increased during the time (Figure 9A). Therefore, the C: N ratio has been a gradual decline over the days of decomposition in the same year. Likewise, the C: N ratio in 2012 decreased over the time, considering the maintenance of the C concentration and the increase of N concentration during the decomposition time (Figure 9B).

Table 19. Dry mass remaining and Carbon (C) accumulated in 2011, Nitrogen (N) accumulated in 2011 and 2012 of green corn straw during the decomposition time (days)

	Dry Mass	N accumulated		C accumulated
Days	2011	2011	2012	2011
	--Mg ha^{-1}--	-------------- kg ha^{-1} --------------		--- Mg ha^{-1} ---
0	6.0 A	66.6 A	33.4 B	2.31 A
15	3.9 B	44.7 B	40.9 A	1.48 B
30	3.7 BC	43.9 B	40.0 A	1.43 BC
60	3.6 CD	42.3 BC	33.3 B	1.36 C
90	3.4 DE	42.2 BC	30.7 BC	1.32 CD
120	3.2 E	38.7 C	29.6 C	1.22 D
*CV (%)	10.9	14.1	15.6	10.7

Note: The means in columns followed by the different uppercase letters are significantly different under the Tukey (at $p < 0.05$). *Coefficient of variation.

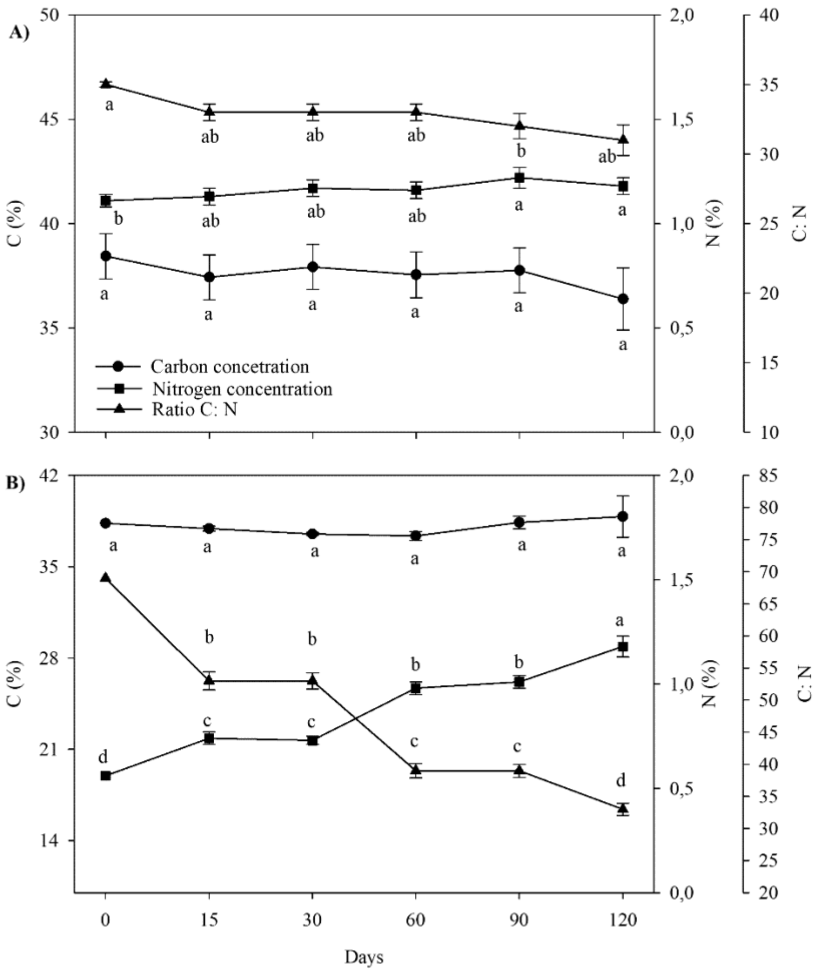

Figure 9. Carbon concentration, Nitrogen concentration, and C: N ratio of green corn straw in 2011 (A) and 2012 (B) under cherry tomato or cherry tomato and legume in an intercropping system during the time of decomposition (days). Note: The means followed by the different lowercase letters are significantly different between the time (days) under the Tukey ($p < 0.05$)

The DM remaining in 2012 showed an interaction ($p < 0.05$) between treatments and time of decomposition (Table 20). The treatments of cherry tomatoes in monocropping had higher DM remaining of straw than cherry tomatoes and dwarf velvet bean in the intercropping system at 30 days of decomposition time (DDT). Similarly, the monocrop treatment had higher DM remaining than cherry tomatoes and legumes in the intercropping system at 60 DDT (except jack bean treatment). However, this difference of treatments did not repeat at 90 and 120 days.

Regarding C accumulated, there was also showed interaction ($p < 0.05$) between treatments and time of decomposition (Table 21). The cherry tomatoes in monocropping had higher C accumulated in the DM remaining of straw than cherry tomatoes and dwarf velvet bean in the intercropping system at 30 DDT. In the same way, N accumulated in the straw of monocrop treatment was higher than jack bean, dwarf velvet bean, white lupine, and cowpea bean treatments at 60 DDT. However, this treatments difference did not repeat at 90 and 120 days, comparable to the results of DM remaining in 2012. In general, the DM and the C and N accumulated of straw decreased over decomposition time in 2012 (Tables 20, 21, and 22).

Table 20. The dry mass remaining in 2012 during the decomposition time in the cherry tomatoes monocrop and cherry tomatoes and legumes in intercropping system

Treatments	Decomposition Time					
	0	15	30	60	90	120
	------------------------------------ Mg ha^{-1} -----------------------------------					
Monocrop	6.0 Aa	5.8 Aa	5.5 Aa	4.1 Ab	3.2 Ac	2.9 Ac
Jack bean	6.0 Aa	5.5 Aab	5.4 Ab	3.4 ABc	3.0 Ac	2.3 Ad
Sun hemp	6.0 Aa	5.5 Aab	5.3 Ab	3.2 Bc	3.0 Acd	2.4 Ad
Dwarf velvet bean	6.0 Aa	5.3 Ab	4.4 Bc	3.0 Bd	3.0 Ad	2.5 Ad
Mung bean	6.0 Aa	5.5 Aab	5.0 ABb	3.6 Bc	3.0 Acd	2.5 Ad
White lupine	6.0 Aa	5.5 Aa	5.4 Aa	3.3 Bb	3.0 Ab	2.8 Ab
Cowpea bean	6.0 Aa	5.4 Aab	5.2 Ab	3.2 Bc	3.0 Ac	2.3 Ad
Average	6.0	5.5	5.2	3.4	3.0	2.5
CV (%)	8.0					

Notes: Means in rows followed by the different lowercase letters and means in columns followed by the different uppercase letters are significantly different under the Tukey test (at $p < 0.05$). *Coefficient of variation

Table 21. Carbon (C) accumulated in the dry mass remaining of green corn straw in 2012 during the decomposition time in the cherry tomatoes monocrop and cherry tomatoes and legumes in intercropping system

Treatments	Decomposition Time					
	0	15	30	60	90	120
	------------------------------------ Mg ha^{-1} --					
Monocrop	2.3 Aa	2.2 Aab	2.0 Ab	1.5 Ac	1.2 Ad	1.1 Ad
Jack bean	2.3 Aa	2.2 Aab	2.0 Ab	1.2 Bc	1.1 Acd	0.9 Ad
Sun hemp	2.3 Aa	2.1 Aab	2.0 Ab	1.2 Bc	1.1 Ac	0.9 Ad
Dwarf velvet bean	2.3 Aa	2.0 Ab	1.7 Bc	1.2 Bd	1.2 Ade	1.0 Ae
Mung bean	2.3 Aa	2.1 Aab	1.9 ABb	1.4 ABc	1.2 Acd	1.0 Ad
White Lupine	2.3 Aa	2.1 Aab	2.0 Ab	1.2 Bc	1.2 Ac	1.1 Ac
Cowpea bean	2.3 Aa	2.1 Ab	2.0 Ab	1.2 Bc	1.2 Ac	0.8 Ad
Average	2.3	2.1	1.9	1.3	1.2	1.0
*CV (%)	8.1					

Notes: Means in rows followed by the different lowercase letters and means in columns followed by the different uppercase letters are significantly different under the Tukey test (at $p < 0.05$). *Coefficient of variation

The single exponential decay function fitted with the decrease of the decomposition time in the DM remaining of green corn straw in 2011 and 2012 (Figure 10A and Table 22). In the same way, the exponential decay function fitted with the decrease of the decomposition time in the C and N release from the mass decomposition of straw green corn (Figure 10B and C and Table 22).

The statistics for the simulation model procedure for kinetic of mass decomposition, and C and N release from mass decomposition, were similar in each year (Table 23). The modeling efficient (EF) was a positive value, and the coefficient of determination (CD) had a value above the value 1 for all data tested in the model. Although the model simulated produced a high total error as measured by RMSE, which was outside the 95% confidence interval of the measurements ($RMSE_{95\%}$), the relative error (E) was within the 95% confidence interval of the measurements ($E_{95\%}$), corroborated by the t value that was within two-tailed 2.5% t value. The straw half-life of the mass decomposition was higher in 2011 than in 2012, and the decomposition rate was high in 2012 on average. The same happened with the half-life and decomposition rate of Kinect of C release from mass decomposition (Table 22). Furthermore, the release of N from the mass decomposition was higher in 2011 compared to 2012, on average. In 2011, 13% of the N accumulated and 45% of the C accumulated in the green corn straw at 120 days were released. In 2012, 24% of the N accumulated and 62% of the C accumulated in the straw at 120 days were released (Figure 10C).

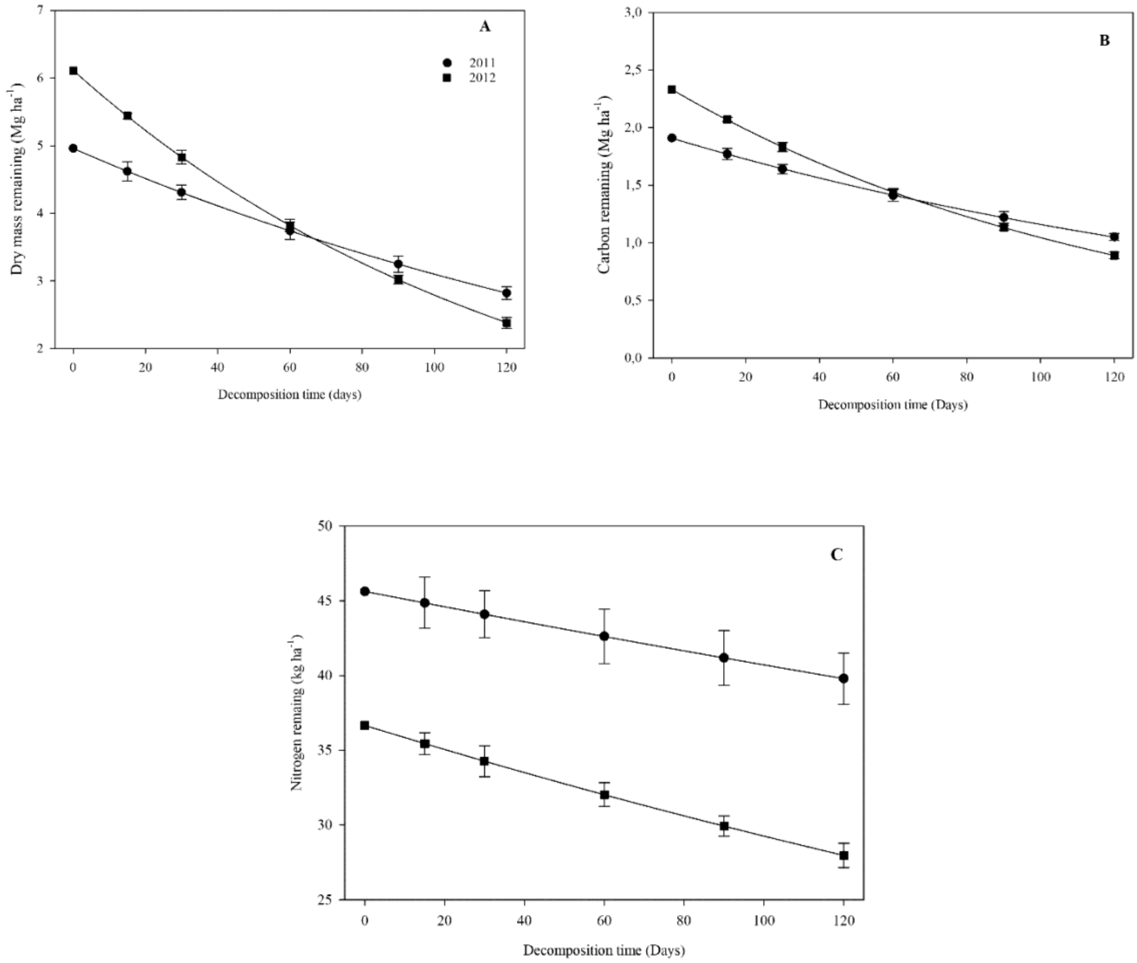

Figure 10. Kinetic of the mass decomposition (A), C release from mass decomposition (B), N release from the mass decomposition of green corn straw in 2011 and 2012 under cherry tomato or cherry tomato and legume in an intercropping system

Table 22. Parameters of kinetic of the mass decomposition, C release from mass decomposition, N release from the mass decomposition of green corn straw in 2011 and 2012

Kinetic	Coefficient equation			
	Year	yo	k	$t_{1/2}$
		Mg ha^{-1}		days
The mass decomposition	2011	4.96	0.005	139
	2012	6.11	0.008	87
C release from decomposition	2011	1.93	0.005	139
	2012	2.33	0.008	87
		kg ha^{-1}		
N release from decomposition	2011	45.63	0.001	608
	2012	36.67	0.002	307

Table 23. Statistic tests for evaluating the precision of model to simulate the value measured for kinetic of mass decomposition, C and N release from mass decomposition in 2011 and 2012

Statistic test	Kinetic					
	The mass decomposition		C release from decomposition		N release from decomposition	
	2011	2012	2011	2012	2011	2012
r	0.77	0.98	0.77	0.98	0.71	0.84
EF	0.99	1.00	0.99	1.00	0.97	0.99
CD	1.57	1.02	1.42	1.01	5.10	1.43
RMSE	6.3	2.3	6.3	2.5	7.6	2.7
$RMSE_{95\%}$	2.3	1.4	2.3	1.4	2.8	2.1
E	-0.23	-0.03	0.02	0.05	0.9	-0.06
$E_{95\%}$	±2.85	±1.74	±0.95	±0.60	±	±0.72
M	0.0002	0.0003	0.003	0.001	0.59	-0.001
t	0.002	0.007	0.08	0.06	0.41	-0.004
t table	± 2.02					

Note: r – coefficient of correlation, EF- modeling efficient; CD - coefficient of determination, RMSE - root mean square error, $RMSE_{95\%}$ corresponding to the 95% of the confidence interval of measurements; E - error relative; $E_{95\%}$ corresponding to the 95% of the confidence interval of measurements; M - mean difference; t statistic, t table - Student´s t distribution (n-2, 95%).

There was no difference among treatments and no interaction between treatments and decomposition time for the natural abundance of ^{13}C (Table 24). The $\delta^{13}C$ has been a slight depleted-^{13}C over the time of decomposition while comparing the 0 day with 120 day (Table 24).

However, there was an interaction between treatments and time for the natural abundance of ^{15}N ($\delta^{15}N$) in 2011 ($p < 0.05$) and 2012 ($p < 0.10$) (Tables 25 and 26). In an intercropping system, the green corn straw under cherry tomato with jack bean had lower $\delta^{15}N$ than cherry tomato with dwarf velvet bean, mung bean, and cowpea bean at 60 DDT in 2011 (Table 25). In the treatment with jack bean, the straw had depleted-^{15}N at 90 DDT compared to 15, 30, and 120 DDT in the same year. Nevertheless, the straw under treatment with dwarf velvet bean had depleted-^{15}N at 30 DDT compared to 90 DDT. In 2012, the straw under cherry tomato and mung bean in an intercropping system had lower $\delta^{15}N$ compared to monocrop, dwarf velvet bean, white lupine, and cowpea bean system with cherry tomato at 30 DDT (Table 26). In the same year, the cherry tomato in an intercropping with jack bean showed depleted-^{15}N of straw at 30 DDT compared to 0 DDT. Similarly, the straw under treatment with sun hemp and mung bean had depleted-^{15}N at 30 DDT compared to others decomposition time. However, on average, the green corn straw has slightly riched-^{15}N while comparing the $\delta^{15}N$ between 0 and 120 days in 2011, numerically (Tables 25 and 26).

Table 24. The natural abundance of ^{13}C of green corn straw under the cherry tomato or cherry tomato and legume in an intercropping system over the decomposition time

Decomposition time	Natural Abundance of ^{13}C	
	2011	2012
	--------- $\delta^{13}C$ ‰ --------	
0	-11.87 A	-12.01 A
15	-12.26 BC	-13.47 C
30	-12.21 AB	-14.01 D
60	-12.23 AB	-13.06 BC
90	-12.60 C	-12.85 B
120	-12.33 BC	-13.67 BC
*CV(%)	6.11	9.09

Note: Means in columns followed by the different uppercase letters are significantly different under the Tukey test (at $p < 0.05$). *Coefficient of variation of transformed data by x+21.

Table 25

Table 25. The natural abundance of ^{15}N of green corn straw under the cherry tomato or cherry tomato and legumes in an intercropping system over the decomposition time in 2011

Treatments	Decomposition Time					
	0	15	30	60	90	120
	-- $\delta^{15}N$ ‰ (air) --					
Monocrop	+8.54 Aa	+9.32Aa	+8.63 Aa	+8.87 Aa	+8.38 ABa	+9.22 Aa
Jack bean	+8.54 Abc	+9.38 Aab	+9.28 Aab	+8.78 Aabc	+7.90 Bc	+9.87Aa
Sun hemp	+8.54 Aa	+8.85 Aa	+9.28 Aa	+8.48 Aa	+8.93 ABa	+9.32 Aa
Dwarf velvet bean	+8.54 Aab	+9.20 Aab	+8.27 Ab	+9.00 Aab	+9.74 Aa	+9.09 Aab
Mung bean	+8.54 Aa	+9.46 Aa	+9.02 Aa	+9.63 Aa	+9.80 Aa	+9.47 Aa
White lupine	+8.54 Aa	+9.03 Aa	+8.96 Aa	+9.00 Aa	+8.61 ABa	+9.63 Aa
Cowpea bean	+8.54 Aa	+8.74 Aa	+9.48 Aa	+8.86 Aa	+9.54 Aa	+9.52 Aa
Average	+8.54	+9.14	+8.99	+9.95	+8.99	+9.45
*CV (%)	7.60					

Notes: Means in rows followed by the different lowercase letters and means in columns followed by the different uppercase letters are significantly different under the Tukey test (at $p < 0.05$). *Coefficient of variation.

Table 26. The natural abundance of ^{15}N of green corn straw under the cherry tomato or cherry tomato and legumes in an intercropping system over the decomposition time in 2012

Treatments	Decomposition Time					
	0	15	30	60	90	120
	-- $\delta^{15}N$ ‰ (air) ---					
Monocrop	+5.77 Aa	+5.41 Aa	+5.12 Aa	+5.24 Aa	+5.64 Aa	+5.58 Aa
Jack bean	+5.77 Aa	+5.27 Aab	+4.87 ABb	+5.36 Aab	+5.67 Aab	+5.43 Aab
Sun hemp	+5.77 Aa	+4.97 Aab	+4.87 ABb	+5.76 Aa	+5.36 Aab	+5.27 Aab
Dwarf velvet bean	+5.77 Aa	+5.35 Aa	+5.62 Aa	+5.97 Aa	+5.40 Aa	+5.73 Aa
Mung bean	+5.77 Aa	+5.12 Aa	+4.07 Bb	+5.75 Aa	+5.70 Aa	+5.79 Aa
White lupine	+5.77 Aa	+5.13 Aa	+5.09 Aa	+5.31 Aa	+5.76 Aa	+5.72 Aa
Cowpea bean	+5.77 Aa	+5.79 Aa	+5.29 Aa	+5.46 Aa	+5.62 Aa	+5.53 Aa
Average	+5.77	+5.58	+4.99	+5.55	+5.59	+5.58
*CV (%)	9.05					

Notes: Means in rows followed by the different lowercase letters and means in columns followed by the different uppercase letters are significantly different under the Tukey test (at $p < 0.10$). *Coefficient of variation

4.4. Discussion

The single exponential model is one of the models that better describe straw decomposition in mathematical and biological behavior (GUAN et al., 2020; WIDER; LANG, 2013). According to Olson (1963); Berg and Meentemeyer (2002), the model divides the decomposition proceeds into two phases: in the first, the soil microorganisms decompose the labile fraction of the straw mass (sugars, starches, and protein), and in the second phase the microorganisms decompose the more recalcitrant materials remain (lignin, cellulose), which was observed in this experiment (Figure 10).

The statistic test showed that the data simulated by the model described well the measured data (Table 23). Although the coefficient of correlation (r) in 2011 for the model was only 0.77 or 0.71, it indicts that 77% or 71% of the variability of the data could be explained by the model. The efficient modeling (EF) explains the data measured more than 97%. The coefficient of determination (CD) was higher than one, it indicts that the model describes the data measured better than the data measurements means (Table 23) (SMITH et al., 1997). A similar statistic was shown in 2012 (Table 23). The root-means-square error (RMSE) value was greater than $RMSE_{95\%,}$ indicating that the RMSE fell was outside the 95% confidence interval for the dataset in both years. However, the relative error (E) value was within 95% of confidence interval ($E_{95\%}$), and the t value was lower than the t critical, suggesting no bias in 2011 and 2012 (Table 26) (SMITH et al., 1997).

According to Berg and Meentemeyer (2002), the decomposition rate was between 0.1 and 0.2% day^{-1} of losing weight, which can decrease gradually. The current study showed a decomposition rate of 0.005 day^{-1} and 0.008 day^{-1} in 2011 and 2012, respectively, corresponding to the accumulated mass loss of 43 and 55% at 120 DDT (Table 22). This result corroborates to the study of Faust et al. (2019), in which the decomposition rate for green corn straw in the litterbags field was 0.007 day^{-1} in no-tillage soil under a mean annual temperature of 9°C and mean annual precipitation of 516 mm. Another study with green corn straw showed mass accumulated loss of 45% at 120 DDT with mean annual temperature of 9°C and mean annual precipitation of 516 mm (SUN et al., 2021).

However, the decomposition rate could change due to temperature, rainfall, soil type, and residue quality (WANG et al., 2012). The greater half-life of straw in 2011 than 2012 (Table 25) can be explained by the lower accumulated precipitation and mean temperature in 2011 that was 113 mm and 19°C against 234 mm and 22°Cin 2012 (Figure 1, section 2.2.1), although the straw had higher C:N ratio in 2012 (69) than 2011 (35) (Figure 9). Guan et al. (2020) showed that the wheat straw had 8.3 g kg^{-1} of N concentration, and the decomposition rate was two times higher than the green corn straw (11.9 g kg^{-1} of N concentration). In this case, the wheat straw was applied in the soil under high temperature and precipitation and the green corn in the opposite climate condition. In our study, the climate condition was more determinant for the difference in straw rate decomposition between 2011 and 2012 than the C: N ratio of the straw residue.

The C release rate from the mass decomposition has a similar pattern compared to the decomposition rate of the mass decomposition (Figure 10A and B). The C, N, and other nutrients released during the straw decomposition are regulated by soil microorganisms, balancing mineralization and immobilization processes (GUAN et al., 2020). The N had a lower release rate from the mass decomposition than C (Table 22). The soil microorganisms demand a greater N quantity to decompose an organic residue (HESSEN et al., 2004). According to Trinsoutrot et al. (2000), there was significant net N immobilization (18 mg N g^{-1} of C added) of mineral N from the soil during the period of rapid decomposition of the C residues added. These authors found that 35% of C and 23-27% of N were released from the residue decomposition at 168 days.

Nevertheless, the N concentration increased over the decomposition time (Figure 11). Innangi et al. (2015) found that the concentration of N and other nutrients (P, Fe, and Mn) increased during the mass loss of litter in two forests in Italy. The authors also found that the N and the lignin concentrations increased linearly with the accumulated mass loss and the explanation for that are not very clear. The suggestion is that the N is transported to the litter through fungal mycelia from soil and to a more decomposed litter layer (INNANGI et al., 2015; BERG; McCLAUGHERTY, 2014). Besides, the low-molecular N compounds repress the formation of ligninase, and the products of lignin degradation react with ammonia or amino acids to form recalcitrant complexes (BERG; MEENTEMEYER, 2002; BERG; McCLAUGHERTY, 2014). It can explain why the remaining materials at 120 DDT had a slower decomposition despite the low C: N ratio of the straw.

The treatments cherry tomato monocrop or cherry tomato with legumes in the intercropping system did not influence the decomposition rate of green corn straw, and the release of the C and N from straw decomposition. However, the DM remaining and the C accumulated in the DM remaining straw in 2012 at 60 DDT showed a higher mass loss in cherry tomato and legumes (Tables 20 and 21). Torres et al. (2015) observed a lower half-time of maize straw (46-38 days) under sun hemp (*Crotalaria juncea*) residue. The legumes had biological nitrogen fixation capacity and increased the N availability in the soil.

Nevertheless, the soil of this present study had a high N concentration (2 g kg^{-1}) and high fertility that can explain the nonresponse by the treatments.

Regarding the natural abundance of ^{13}C, the soil organic matter (SOM) slightly increased the $\delta^{13}C$ with the age, and the SOM decomposition was derivate from the intense fractionation during the decomposition (KRULL; BESTLAND; GATES, 2002). However, the litter in the initial stage of decomposition can decrease the $\delta^{13}C$ when increase the lignin concentration because the lignin (-20 ‰) is depleted in ^{13}C compared to the cellulose (-14 ‰) (BENNER et al., 1987). According to Preston et al. (2006), the more decomposed residues of wood (-26.6 ‰) had depleted-^{13}C compared to the less decomposed residues (-24.5 ‰), explained by the increase in the lignin concentration from 30 to 98%. Therefore, the decrease of the $\delta^{13}C$ in the green corn straw during the decomposition in the present study can be related to the increase of the lignin with the advance of decomposition (Table 24).

However, the green corn straw tended to increase the $\delta^{15}N$ during the decomposition time in 2011 (Tables 25 and 26). As the decomposition advanced, because of the fractionating processes by bacteria during the denitrification, nitrification, mineralization, the SOM was slightly enriched with ^{15}N (HOGBERG et al., 1996; SHILENKOVA; TIUNOV, 2013).

4.5. Conclusion

The residual green corn straw under cherry tomato with different legumes (source of N) in an intercropping system did not increase the straw decomposition rate and the release of the C and N from the straw decomposition.

The C: N ratio declined gradually over the days of the decomposition, considering the maintenance of the C concentration and the increase of the N concentration during the decomposition time, independently of treatment.

The $\delta^{13}C$ decreased in the green corn straw and the $\delta^{15}N$ tended to increase during the decomposition time.

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5 Soil carbon and nitrogen storage in an organic system of cherry tomato intercropping with green manures in succession to green corn

Abstract

The crop residues (legume or non-legume) increase the stock of C, N, and other nutrients in the soil, improving the soil fertility. The aim of this research was to analyze the soil fertility, C and N content, and the natural abundance of ^{13}C and ^{15}N at 0-15 cm soil depth during three years in an organic system of cherry tomato intercropping with green manures in succession to green corn. The experimental was in a randomized complete block design with eight treatments and five replications. The treatments were control, a cherry tomato monocrop with or without crop residue crop (straw) from the previous green corn crop, cherry tomato intercropping with jack bean, sun hemp, dwarf velvet bean, mung bean, white lupine or cowpea bean in two consecutive years. In this study, the legumes used as green manure had a lower C: N ratio than green corn. The total C and N content and the C and N stock of the soil increased over the years, especially in 2013. The soil C: N ratio also increased over the year, the C accumulated was higher than the N accumulated, increasing the N immobilization in the system. The soil organic matter reduced over the years, although the total C and N increased, suggesting that the organic C and N decreased and the inorganic C and N increased in the soil. This agricultural system reduced the soil fertility, although the N and C total stock in the soil increased over the year.

Keywords: soil organic matter; C: N ratio; soil fertility, N and C stock.

Estoque de carbono e nitrogênio do solo em sistema orgânico de minitomateiro consorciado com adubos verdes em sucessão ao milho verde

Resumo

Os resíduos de culturas (leguminosas ou não-leguminosas) aumenta o estoque de C e N no solo, além de outros nutrientes, consequentemente melhora a fertilidade do solo. O objetivo do estudo foi avaliar a fertilidade do solo, a concentração de C e N, e a abundância natural de ^{13}C e ^{15}N na profundidade de 0 a 15 cm durante três anos de sistema orgânico de cultivo de minitomateiro e adubos verdes em consórcio e milho verde em sucessão. O desenho experimental foi de blocos ao acaso com cinco repetições e oitos tratamentos: minitomateiro solteiro com palha residual de milho verde, minitomateiro solteiro sem palha residual de milho, minitomateiro em consorcio com feijão-de-porco, crotalária-juncea, mucuna-anã, feijão-mungo, tremoço-branco, feijão-caupi em dois anos consecutivos. As leguminosas utilizadas como adubos verdes tiveram uma relação C: N baixa se compara ao milho verde. A concentração total de C e N e o estoque de C em no solo aumentos com os anos, especialmente em 2013. A relação C: N do solo também aumentou com os anos, sendo que o C acumulado foi maior que o N acumulado no solo, ou seja, houve um aumento da imobilização de N no sistema. A matéria orgânica do solo reduziu com os anos, apesar do estoque de C e N terem aumentado, sugerindo que o C e N orgânico diminuíram e o C e N inorgânico aumentaram no solo. Este sistema agrícola reduziu a fertilidade do solo, apesar do estoque de N e C no solo aumentarem com os anos.

Palavras-chave: matéria orgânica do solo, relação C: N, fertilidade do solo, estoque de C e N

5.1 Introduction

The organic farmland was 72.3 million hectares worldwide in 2019, have grown more the 555% in 20 years, and its market reached more than 106 billion dollars in organic food and drink (Willer et al. 2021). This agriculture has been based on principles to maintain or enhance the soil, plant and human health and sustain the ecological system (IFOAM, 2020). Therefore, practices such as green manure, no-till, intercropping and rotating crops, biological pest control are usually used to optimize the use of soil, nutrients and water, and minimize the impact on the environment (SCIALABBA; MLLER-LINDENLAUF, 2010). According to Reganold and Wachter (2016), organic agriculture is environmentally friendly because enhance the soil carbon, soil fertility and faunal diversification. In addition, it can reduce pesticide pollution risk, nitrate leaching, and emission of nitrous oxide and ammonia, being more energy efficient.

The legume, as green manure or cover crops, is usually used in the organic system for providing the nitrogen (N) through the biological nitrogen fixation (BNF) (REGANOLD; WACHTER, 2016). Hence, the legumes residue is an important source of N and C in the soil. De Notaris et al. (2020) showed that red clove and winter vetch, as green manure, deposited in the soil 134 and 72 kg C ha^{-1}, respectively, and 29 and 85 kg N ha^{-1}, respectively, and ~9 kg N ha^{-1} were provided by mineralization from the crop residues. The meta-analysis research predicted that the cover crops (legumes and non-legumes) practice could accumulate 12.7 Mg ha^{-1} of soil organic carbon (SOC) in 54 years, which correspond to an average C sequestration rate of 0.23 Mg ha^{-1} yr^{-1} (POEPLAU; DON, 2015). Moreover, the green manure could improve the biological, physical, and chemical soil characteristics, improving the soil N concentration, the organic matter, pH, the microbial activity, and the organic and inorganic P, for example (GOGOI; BARUAH; MEENA, 2018; SINGH; SINGH; KHIND, 1992).

The crop residue (straw) also improves the C stock and other nutrients, consequently enhancing soil fertility. Studies showed the crop residue of wheat and maize in a rotation system added to the soil 4.5 Mg SOC ha^{-1} yr^{-1} (LI et al., 2019; LIU et al., 2019). According to Tian et al. (2019), the application of maize straw mixed or buried to the soil at depths of 10, 30, and 50 cm increased the SOC, the total soil nitrogen, and the C: N ratio in the soil profile.

However, the decomposition of legumes and crop residues are influenced by the edaphoclimatic condition (temperature, rainfall and soil moisture) and the residue quality (N, C, polyphenol, and lignin concentration and their ratio). Abera et al. (2012) studied the C and N mineralization dynamic in different soil with two legume residues and showed that the soil under residue with high C: N ratio (40.6) had lower cumulative CO_2 flux and production of NH_4^+ and NO_3^-; thus, lower C and N loss. Moreover, the concentration of lignin and cellulose also influences litter decomposition; the plants with low lignin had lower decomposition rate than the plants with low cellulose and low N in their composition (TALBOT; TRESEDER, 2012).

Therefore, the C and N dynamics are interconnected within the ecosystem, their availabilities determine the microbial activity, and the mineralization/immobilization of N and C stock change in the soil (MARSCHNER, 2012; NOTARIS et al., 2020). Moreover, maintaining or increasing soil C stock is also important to mitigate the gas greenhouse effect because the food system was responsible for 6 to 13% of total anthropogenic CO_2 emissions in 2019 (IPCC 2020). To better understand the C and N cycle, studies with the ^{13}C and ^{15}N stable isotopes technique are usually used to trace the C and N into the plant and soil (CRAINE et al., 2015; GLEIXNER, 2013; GUARESCHI; PEREIRA; PERIN, 2014). The $\delta^{13}C$ of soil carbon reflects the plant ^{13}C signature; thus it is possible to identify the natural vegetation alteration or the origin of the soil C when the C3 and C4 plants are in rotation (crop rotation) (CHEN et al., 2018; GUARESCHI; PEREIRA; PERIN, 2014). The mineralization, volatilization, and nitrification increase the $\delta^{15}N$ from the soil and reflect in the losses of soil organic matter (SOM) (GUARESCHI; PEREIRA; PERIN, 2014; HOGBERG et al., 1996).

This study hypothesized that in an organic system of cherry tomato intercropping with green manures in succession to green corn increase the total N and C stock in the soil, maintaining or increasing the soil fertility. The aim of this research was to analyze the soil fertility, C and N content, and natural abundance of ^{13}C and ^{15}N at 0-15 cm soil depth during three years with the cropping system.

5.2 Materials and Method

5.2.1 Characterisation of the area experimental

The same topic 2.2.1

5.2.2 Experimental design

The same topic 2.2.2

5.2.3 Field and Crop management

The same topic 2.2.3

5.2.4 Crop and soil sampling and analysis

The aerial part without ears of the green corn was sampled (10 plant plot^{-1}) at the R3 growth stage in 2012 and 2013. The green manure aerial part was sampled in two linear meters per plot without the pods 100 days after seeding (DAS). The samples have been dried at 65ºC in a forced circulation oven until a constant mass. Subsequently, the samples were ground in a Wiley mill and taken to the laboratory for analysis. The soil was sampled at depths of 0-5, 5-10, and 10-15 cm in 2012, 2013 and 2014 (Figure 2, section 2.2.3). The soil samples were air-dried and ground in a ball mill.

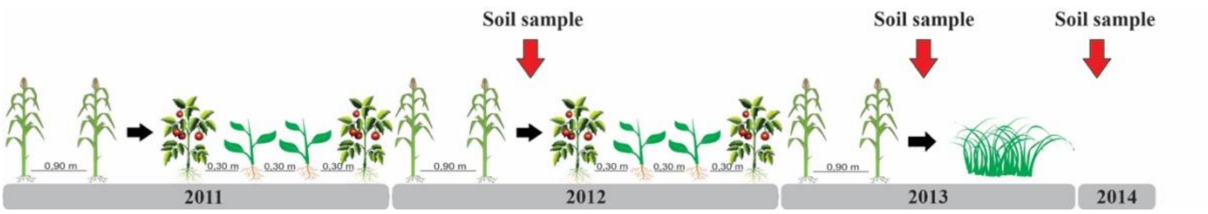

Figure 2. Illustration of succession system of green corn and cherry tomato/legumes with their respective spacing between the lines (section 2.2.3)

The plant and soil samples were prepared for the analyzes of total nitrogen (N), total carbon (C), the natural abundance of ^{15}N (δ^{15}N ‰) and the natural abundance of ^{13}C (δ^{13}C ‰) using the procedure described by Trivelin et al. (1973). The analyzes were performed by an isotope ratio mass spectrometer containing an automatic N and C analyzer connected with a mass spectrometer (IRMS) – N and C 20-20 ANCA GSL (Automatic Nitrogen and Carbon Analyzer, Gas, Solid and Liquid – SERCON) (BARRIE; PROSSER, 1996).

The accumulation of C for green manure and green corn was determined for the aboveground biomass using the C concentration and the dry mass of each plant fraction, expressed in kg ha^{-1}. The soil's total nitrogen stock and total carbon stock were calculated using the N and C concentration and the soil density, expressed in Mg ha^{-1}.

5.2.5 Statistical Analysis

Statistical analysis was performed with repeated measures, using the MIXED procedure of the SAS software (Statistical Analysis System, 9.3) to determine the structure of the matrix of the variance and covariance. The significant interactions were broken down according to the factors involved. The averages of the main effects and interactions were compared using the Tukey-Kramer test. The level of significance adopted for the analysis of variance was $p < 0.10$.

5.3 Results

C and N in the crops

The green corn produced 3.2 and 2.3 Mg ha^{-1} of dry weight (without ears) in 2012 and 2013, respectively. This crop showed the ^{13}C natural abundance ($\delta^{13}C$) between -12.37 and -13.59‰ and the ^{15}N natural abundance ($\delta^{15}N$) between +6.97 and +6.31‰ in 2012 and 2013 on average, respectively. There was no interaction between treatment and year, and there was no difference (p > 0.10) among treatments for C and N concentration, C: N ratio and C accumulated of green corn (Figure 11). The N concentration of green corn was lower in 2013 than in 2012. Although the C concentration did not differ among years, the C accumulated was lower in 2013 than in 2012. Consequently, the C: N ratio increased in 2013 compared to 2012 (Figure 11).

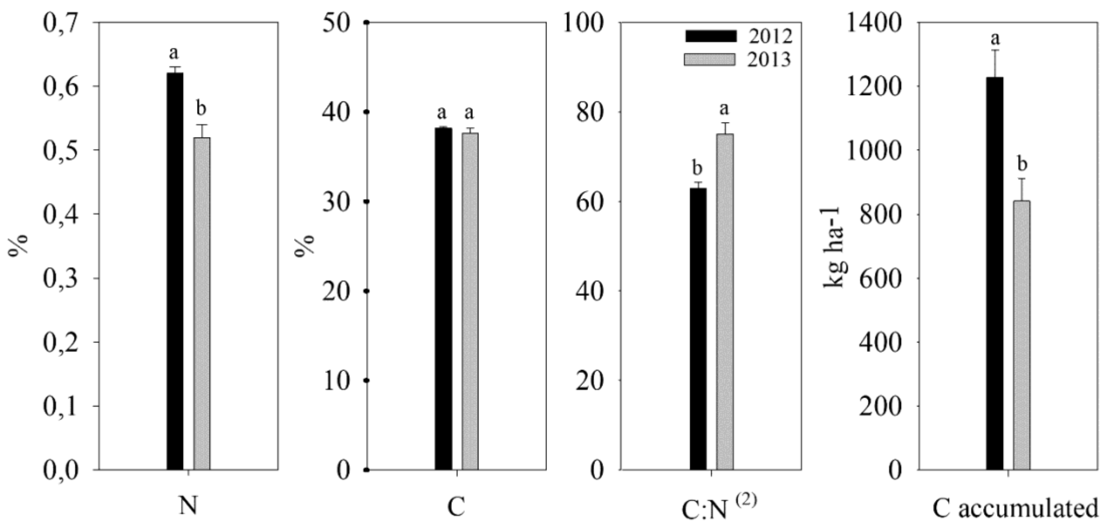

Figure 11. Nitrogen (N) and Carbon (C) concentration, C: N ratio, C accumulated of green corn aerial part without ears in the R3 stage. Note: the means followed by the same lowercase letter do not differ by the F-test (p<0.10). (2) Statistic on transformed data for log(x)

The green manure yielded on average 7.1, 12.5, 2.2, 2.4, 17.9 and 4.9 Mg ha^{-1} of dry weight for jack bean, sun hemp, dwarf velvet bean, mung bean, white lupine, and cowpea bean, respectively (Table 8, section 2.3). The ^{13}C natural abundance ($\delta^{13}C$) of green manure was between -26.62 and -29.23‰, and their ^{15}N natural abundance ($\delta^{15}N$) was +1.77 and +0.77‰ in 2012 and 2013 on average, respectively (Table 13, section 3.3.1).

There were no difference ($p > 0.1$) for the green manure C concentration among treatments, and it was lower in 2012 than in 2013 (Table 27). Despite that, the C accumulated did not show a difference among years. The white lupine had higher C accumulated than the dwarf velvet bean. The dwarf velvet bean and jack bean had the highest N concentration, followed by cowpea bean, sun hemp, mung bean and white lupine. The C: N ratio was highest in the white lupine (66), followed by mung bean (28), sun hemp (28), cowpea bean (16), jack bean (13), dwarf velvet bean (11) (Table 27).

Table 27. The concentration of Nitrogen (N) and Carbon (C), C: N ratio, and C accumulated of green manure intercropping with cherry tomatoes

Treatments	N			C			C: N [(2)]			C accumulated		
	---------------- % -----------------			---------------- % ---------------						------------- Kg ha^{-1} -------------		
	2011	2012	Average	2011	2012	Average	2011	2012	Average	2011	2012	Average
Jack bean	2.7 aA	3.2 aAB	3.0	38.1	38.9	38.5 A	14 aC	12 aD	13	2562	2625	2593 AB
Sun hemp	1.2 aCD	1.8 aC	1.5	36.1	41.2	38.7 A	31 aB	24 aB	28	5767	1333	3550 AB
Dwarf velvet bean	3.2 aA	3.9 aA	3.5	34.9	41.6	38.2 A	11 aC	11 aD	11	990	2080	1535 B
Mung bean	1.3 aC	1.8 aC	1.5	38.4	38.3	38.4 A	31 aB	25 aBC	28	905	2209	1557 AB
White lupine	0.6 aD	0.9 aD	0.7	38.3	40.9	39.6 A	74 aA	57 aA	66	7403	4132	5768 A
Cowpea bean	2.0 bB	2.9 aB	2.4	34.9	37.2	36.1 A	18 aC	13 aCD	16	1199	3661	2430 AB
Average	1.8	2.4		36.8 b	39.7 a		30	24		3138 a	2674 a	
[#] CV(%)	10.43			8.43			9.72			17.05		

Note: the means followed by the same lowercase letter in the lines do not differ by the F-test ($p < 0.10$), and means followed by the same uppercase letter in the columns do not differ by the Tukey-Kramer test ($p < 0.10$).

[(2)] Statistic on transformed data for log(x).

[#] Coefficient of variation of data transformed.

Fertility, N and C stock, and natural abundance of ^{13}C and ^{15}N of the soil

The soil pH, base saturation (BS), calcium (Ca), manganese (Mn) and zinc (Zn) were higher in 2013 than in 2012 and 2014 (Table 28). The soil organic matter (OM), phosphorus (P), sulfur (S) and iron (Fe) of the soil were higher in 2012 than in 2013 and 2014. The soil's potassium (K) and CEC were higher in 2012 and 2013 than in 2014. The boron (B) was the only soil nutrient that did not show a difference among the years, and the magnesium (Mg) and copper (Cu) were the only soil nutrient that increased over the years (Table 28).

There was an interaction between treatments and years just for H + Al and the sum of bases of the soil ($p < 0.10$) (Table 29). The soil under treatment with dwarf velvet bean showed higher H + Al than treatment with sun hemp and the controls in 2012. However, this difference did not happen in 2013 and 2014. On average, the H + Al of the soil significantly reduced in 2013 and 2014 compared to 2012 (Table 29). The sum of bases of the soil was higher in the treatment with sun hemp than the jack bean treatment only in 2013. The soil under treatment with sun hemp, white lupinee and the control with straw had the sum of bases higher in 2013 than in 2012 and 2014. In the other treatments, the sum of bases was higher in 2013 than in 2014 (Table 29).

The N concentration of the soil did not show a difference among the soil depths in 2012 and 2013 (Figure 12A). Therefore, in 2014, the soil N concentration was lower in the 10-15 cm than 0-5 cm depth. The soil N stock increased over the soil depths (Figure 12B). In the 5-15 cm depth, the N stock was higher in 2013 and 2014 than in 2012. The ^{15}N natural abundance was enriched with^{15}N in 2014 than 2012 on average (Figure 12C).

The soil C concentration was higher in 2013 than in 2012 and 2014 in the 0-5 and 10-15 cm soil depths (Figure 12D). The total C stock increased over the soil depths in the three years analyzed (Figure 12E). In 2013 and 2014, the C stock was higher than in 2012. The natural abundance of ^{13}C was between -17.53 and -18.89‰ (Figure 12F). The ^{13}C signature was less negative in 2013 and 2014 than in 2012.

The C: N ratio was higher in 2013 than in 2012 and 2014 in the 0-5 and 10-15 cm soil depths (Figure 12G). However, on average, the C: N ratio was higher in 2013 (11) and 2014 (10) than in 2012 (8).

Table 28. Chemical analysis of soil cultivated with cherry tomato and green manure in an intercropping system under straw residual of green corn. CEC- cations exchange capacity, BS – base saturation, OM- organic matter

Years	pH	CEC (2)	BS	OM	P (2)	S (2)	K (1)	Ca (2)	Mg	B (1)	Cu	Fe	Mn (2)	Zn
	$CaCl_2$ (0.01 mol L^{-1})	$mmol_c$ dm^{-3}	%	g kg^{-1}	-- mg dm^{-3} --		---- $mmol_c$ dm^{-3} ----			---------- mg dm^{-3} ----------				
2012	5.6 c	97.3 a	65.6 c	29.8 a	50.0 a	14.6 a	3.8 a	47.4 b	12.4 b	0.56 a	3.83 c	46.29 a	45.69 b	8.11 b
2013	6.3 a	96.9 a	84.2 a	26.0 b	18.7 b	5.9 b	4.0 a	64.4 a	15.9 b	0.62 a	4.74 b	35.35 c	50.87 a	9.80 a
2014	5.9 b	76.7 b	71.4 b	22.0 c	16.7 b	5.8 b	2.8 b	36.5 c	15.8 a	0.61 a	5.31 a	41.47 b	42.31 c	4.87 c
Average	5.9	90.3	73.7	25.9	36.7	8.8	3.6	49.4	14.7	0.58	4.63	41.04	46.28	7.59
# CV(%)	2	2.4	4.8	14.2	11.2	13.0	17.4	4.2	9.2	16.4	12.5	10.3	3.7	25.6

Note: the means followed by the same lowercase letter in the columns do not differ by Tukey-Kramer test (p<0.10).

(1) Statistic on transformed data for $\sqrt{x}$.

(2) Statistic on transformed data for log(x).

Coefficient of variation of data transformed.

Table 29. Chemical analysis of soil cultivated with cherry tomato and green manure in intercropping system under straw residual of green corn

Treatments	H+Al				Sum of bases			
	---------- $mmol_c$ dm^{-3} ----------							
	2012	2013	2014	Average	2012	2013	2014	Average
Control without straw	29.6 aB	15.5 bA	20.7 bA	22.0	70.5 abA	90.3 aAB	56.0 bA	72.2
Control with straw	29.3 aB	16.4 bA	22.3 abA	22.7	57.6 bA	90.8 aAB	56.3 bA	68.2
Jack bean	35.6 aAB	15.1 bA	21.1 bA	23.9	60.7 aA	77.7 aB	56.3 aA	64.9
Sun hemp	30.3 aB	15.1 bA	21.8 bA	22.4	69.5 bA	102.0 aA	51.7 bA	74.4
Dwarf velvet bean	37.8 aA	14.7 cA	22.0 bA	24.9	64.7 abA	84.5 aAB	54.9 bA	68.0
Mung bean	35.8 aAB	14.8 bA	21.6 bA	24.1	60.8 abA	83.2 aAB	57.6 bA	67.2
White lupine	32.4 aAB	15.7 bA	21.6 bA	23.2	59.4 bA	85.1 aAB	53.3 bA	65.9
Cowpea bean	32.6 aAB	15.4 bA	21.3 bA	23.1	71.5 abA	80.3 aAB	54.6 bA	68.
Average	32.9	15.3	21.6		64.3	86.7	55.1	
#CV(%)	14.50				16			

Note: the means followed by the same lowercase letter in the lines do not differ by the F-test ($p < 0.10$) and means followed by the same uppercase letter in the columns do not differ by the Tukey-Kramer test ($p < 0.10$).

#Coefficient of variation of data transformed.

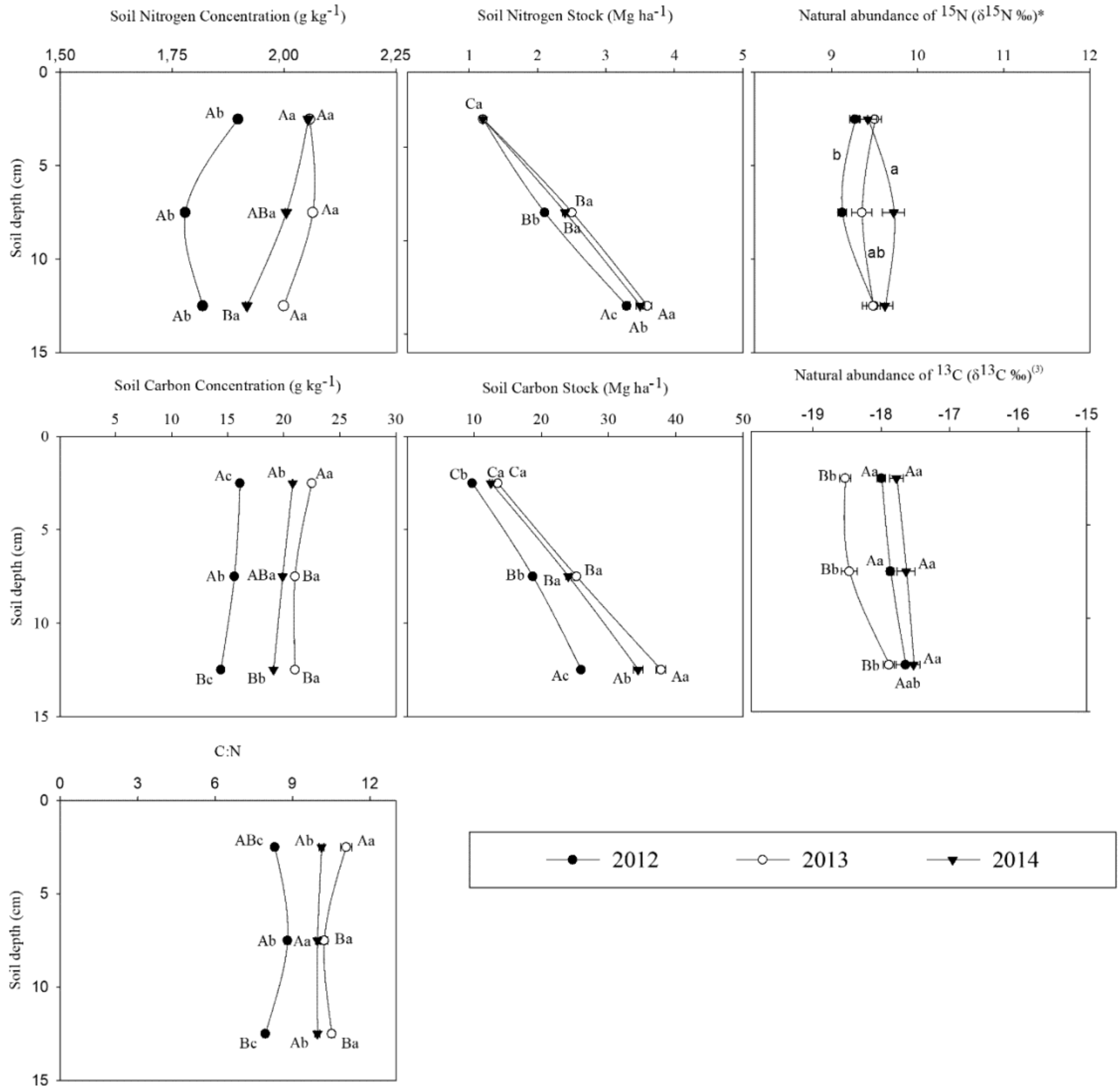

Figure 12. Nitrogen and Carbon concentration, total N and C stocks, natural abundance of 13C and 15N of the soil in the 0-15 cm of deep layer. Note: the means followed by the same lowercase letter among years and means followed by the same uppercase letter among deep layers do not differ by the Tukey-Kramer test ($p < 0.10$). (3) Statistic on transformed data for (X+21.0). * The years means followed by the same lowercase letter among years do not differ by the Tukey-Kramer test ($p < 0.10$)

5.4 Discussion

The legumes used as green manure in this study had lower C: N ratio compared to the green corn because they have had biological nitrogen fixation capacity that increased the N concentration (Table 17 and Figure 11) (SALGADO et al., 2020). The C concentration of both plants was similar. The residue of green manure and green corn provided C, N, and other nutrients for the soil (SALGADO et al., 2020), increasing the microbiota activity beyond protecting against erosion (ABERA; WOLDE-MESKEL; BAKKEN, 2012; DE NOTARIS et al., 2020). The green manure yielded more dry matter than green corn, consequently accumulated the largest C quantities. However, more than 50% of C in the legumes can release in the form of CO_2 by the soil biomass microorganism in the first four weeks of decomposition, and 15 to 20% of this C could remain as organic residue after four years (LADD; OADES; AMATO, 1981). This percentage can vary according to the soil type, temperature, rainfall, species, agriculture system, for example.

Nevertheless, the total C concentration and the C stock of the soil increased over the years, especially in 2013 (Figure 12). The green corn residue had a high C: N ratio that increased the C source in the system (Figure 11), increasing the soil microorganisms demands for N and other nutrients to decompose organic residues, immobilizing part of soil nutrients. Using two residues with high and low C: N ratios in the crop rotation system could be a good strategy because the N immobilization can mitigate the NO_3^- loss and offer the high C sequestration. Abera et al. (2012) studied the C and N mineralization dynamic in different soil with two legume residues and showed that the soil under residue with high C: N ratio (40.6) had lower cumulative CO_2 flux and production of NH_4^+ and NO_3^-; thus, lower C and N loss.

The isotope analysis of total C concentration suggests that C3 plant (green manure and cherry tomato) residues significantly contributed to the C in the soil. In 2013, after two cycles of green manure and cherry tomato and three cycles of green corn (C4 plant), the soil $\delta^{13}C$ was more negative than in 2012 and 2014 in the 0-10 cm soil depth (Figure 10F). The C3 plant had more ^{13}C-depleted than the C4 plant due to the discrimination $^{13}CO_2$ during the photosynthesis, reflecting the C3 or C4 pathway (CHRISTENSEN et al., 2011). The C_4 plants had the $\delta^{13}C$ between -16 and -9 ‰, and the C3 plants, between -33 and -22 ‰ (GUARESCHI; PEREIRA; PERIN, 2014). The $\delta^{13}C$ of soil carbon reflect the plant ^{13}C signature; thus it is possible to identify the origin of soil C when the cultivation of C3 and C4 plants are in rotation or the natural vegetation alteration (CHEN et al., 2018; GUARESCHI; PEREIRA; PERIN, 2014).

Although the cherry tomato was taken from the experimental area at its final harvest, the ^{13}C-depleted soil suggests that it contributed to the soil C fixation during its development (Figure 12F). Mancinelli et al. (2015) studied the effect of different mulching on the soil in tomato monocrop, theses authors verified an increase of total organic C in the soil among the years (1.1 to 1.6%) cultivated only with tomato without soil mulching. Another study in four different soil cultivated with C3 and C4 plants showed that the soil $\delta^{13}C$ under C3 vegetation had the ^{13}C signature between -26‰ and -21‰, and the soil under C4 vegetation had the $\delta^{13}C$ between -24‰ and -17‰ (WANG et al., 2010).

The soil nitrogen concentration and the N stock in the 5-15 cm soil depth increased over the years, mainly in 2013, independently of treatments (Figure 12A and B). This increase in the N from the soil can be related to the return of the N residues into the soil through decomposition and the N immobilization by soil microorganisms that reduce the N losses. The corn residues contain a high quantity of soluble C and intermediate available C, which gives a high capacity of N immobilization (HADAS et al., 2004). The soil C: N ratio increased over the year, showing that the C accumulation was higher than the N accumulation (Figure 12G), consequently increasing the N immobilization in the system. However, the soil was enriched with ^{15}N over the year, indicating a loss of N from the soil. The process of N loss from soil, such as mineralization, volatilization, nitrification, increase the $\delta^{15}N$ of the soil and reflect the loss of the soil organic matter (SOM) (GUARESCHI; PEREIRA; PERIN, 2014; HOGBERG et al., 1996).

Therefore, SOM reduced over the years, although the total C and N stock increased (Table 28 and Figure 12F), suggesting that the organic C and N decreased and the inorganic C and N increased in the soil. The organic C is the most component of SOM, followed by oxygen, hydrogen, nitrogen, sulfur and phosphorus (GLEIXNER, 2013), and its compartments are the "light" fraction, microbial biomass, dissolved organic matter, rhizodeposits, stable humic substance (SANDERMAN; AMUNDSON, 2014). The inorganic C is composed of plant residue, rain leaked, origin minerals materials. Thus, the soil total C increased due to the corn and green manure residues that will compose the SOM.

The SOM is formed by plant or animal residues through biological, physical, and chemical transformation process into organic compost that can intimately associate with soil minerals (LEHMANN; KLEBER, 2015). This transformation process includes decomposition, humidification, interaction with the mineral soil surface, and mineralization (GLEIXNER 2013). According to Janzen (2015), the residence time of SOM can be minutes or decades depending on the kind of humic substance, the physical protection (minerals) and microbial activity. The soil microbial biomass mediates the decomposition of organic C; therefore, the temperature increase, the oxygen availability, the soil pH and the nutrients influence this process (JANZEN, 2015; LEHMANN; KLEBER, 2015). The high demand for nutrients by the plants, especially the green corn that was not fertilized, can increase the competition for nutrients with microorganisms, accelerating the decomposition of SOM, in the current study.

The cation exchange capacity (CEC), P and S concentration in the soil reduced among the years due to this SOM reduction (Table 28). The SOM is necessary to generate negative charges for the weathered soil as the soil in the present experiment. Therefore, this succession system did not increase the fertility of the soil. However, the same nutrients increased in the soil in 2013 compared to 2012 such as Mn, Zn, Cu, Ca, and the Mg that also increased in 2014. The thermophosphate used in tomato fertilizer added these nutrients and can improve its availability in the soil. Studies with different termophosphate rates showed an increase in the Ca and Mg concentration in the soil, increasing the base saturation (DEMATTÊ et al., 2003; FAGERIA; SANTOS, 2008). The base saturation and the sum of base were higher in 2013, after two applications of thermophosphate (Table 29).

The soil pH was higher in 2013 and 2014, reducing the H + Al (Table 29). The thermosphosphate also affect the H + Al and the soil pH, increasing the pH and reducing the H + Al. The treatment with the dwarf velvet bean had higher H + Al than controls. The legume residues applied to the soil can change the soil pH due to the organic acids formed during the decomposition that can provide protons to the soil (SINGH, SINGH AND KHIND, 1992).

5.5 Conclusion

The organic system of cherry tomato intercropping with green manures in succession to green corn reduced the soil fertility because it reduced the SOM and consequently the S and P concentration, and the CEC beyond the reduced concentration of same other nutrients. Therefore, this system demands nutrients from the soil faster than the crop residues can release through decomposition.

However, the N and C total stock in the soil increased over the years showing the cropping system capacity to sequestration C and N that will compose the SOM.

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6 Economic analysis of organic cherry tomatoes and green corn grown in the succession systems

Abstract

Organic agriculture and its market have been increasing in recent decades; however, its growth has been limited by lack of information and knowledge. In Brazil, the absence of systematic official data (production and marketing) about organic agriculture is a limitation to the more robust growth of this sector. Therefore, the study aimed to examine the cost and profitability of organic cherry tomato and green corn in the succession system. The economic analysis was based on agricultural inputs used, the activities carried out, and how production was conducted in the experimental area. We started the analysis summing all expenses inherent to the production process with the base year 2020 for green corn and cherry tomato: operation (soil preparation, weed control, fertilization, sow, transplanting, irrigation, transporting, sprouts, harvest), inputs (fertilizers and seeds), administration (light, water, travel, tax, accounting, land lease). The yield of green corn and cherry tomato was expressed kg ha^{-1} and 2021 as the base year for marketing prices of these products. The cost and revenue accumulated in the two years of organic cherry tomato showed the highest net profit was CS, followed by CB, DVB, CWS, JB, MB, SH, and WL. The benefits/cost ratio was above 83% of organic cherry tomatoes. Furthermore, 77.27% of the operation cost of cherry tomato crop was inputs, 18.17% manual operation, 4.51% administrations (included tax), 0.05% mechanized operation. The cost and revenue accumulated in the two years of organic green corn showed the highest net profit was the green corn in succession to JB, followed by CB, SH, and WL, in which the benefits/cost ratio was above 50%. Moreover, approximately 66% of the total cost of the organic green corn crop was the operations, 24% inputs, 7% administrations (included tax), 4% post-harvest. The organic system with cherry tomato and legumes in an intercropping system and the green corn in succession was high profitability under the conditions in which this study was conducted.

Key words: green manure, profitably, operation cost

Análise econômica do minitomateiro e do milho verde orgânicos em sistemas de sucessão

Resumo

A agricultura orgânica e o seu mercado tiveram um grande crescimento nas recentes décadas, entretanto, este crescimento está limitado pela falta de informações e conhecimento. No Brasil, a ausência de dados sistemáticos oficiais (produção e mercado) sobre a agricultura orgânica é uma limitação para o crescimento mais robusto do setor. Portanto, o presente estudo objetivou avaliar o custo operacional e o lucro do minitomateiro orgânico e o milho verde em sistema de sucessão. A análise econômica foi baseada nos insumos, atividades e na forma que o experimento foi conduzido. Iniciou-se a análise reunindo todos os gastos inerente ao processo de produção com ano de base 2020 para o milho verde e minitomateiro: operações (preparação do solo, controle de plantas daninhas, fertilização, semeadura, transplante, irrigação, transporte, colheita, retiradas dos brotos), insumos (fertilizante e sementes), administração (energia, água, viagens, impostos, contabilidade, arrendamento). A produtividade do milho verde e minitomateiro foi expressa em kg ha^{-1}, e o ano base utilizado para a coleta de preços de mercado foi 2021. O custo e a receita acumulada nos dois anos do minitomateiro orgânicos mostraram maior lucro líquido nos tratamentos do CS, seguido pelo CB, DVB, CWS, JB, MB, SH e WL. A relação benefício/custo foi acima de 83% para esta cultura. Além disso, o insumo foi responsável por 77,27% do custo de operação, operação manual foi responsável por 18,17%, a administração (incluindo imposto) 4,41% e a operação mecanizada 0,05%. Em relação ao milho-verde, o custo e a receita acumulados nos dois anos mostraram um maior lucro líquido no milho-verde em sucessão ao JB, seguido pelo CB, SH e WL, em que a relação beneficio/custo foi acima de 50%. Além disso, aproximadamente, 66% do custo operacional desta cultura foi as operações realizadas, 24% os insumos, 7% a administração e 4% a pós-colheita. O sistema orgânico com minitomateiro e legumes em consórcio e o milho-verde em sucessão apresentou alta rentabilidade sobre as condições em que este estudo foi conduzido.

Palavras-chaves: Adubação verde, rentabilidade, custo de operação

6.1. Introduction

Agriculture is fundamental for the development of human civilization, supplying food and other products. However, it is related to environmental problems such as water pollution by nitrates, phosphates, and pesticides, loss of biodiversity, beyond it is a significant contributor to green gas emission (IPCC, 2020; FAO, 2002). Concerning environmental preservation has promoted alternative agriculture and more environmentally friendly practices such as organic agriculture, crop diversity, no-tillage, integrated pest, and disease control.

The organic agriculture system has been based on principles to maintain or enhance the health of soil, plant and human and sustain the ecological system through practices that maintain or increase soil fertility, reduce pesticide pollution risk and minimize the impact on the environment (SCIALABBA; MLLER-LINDENLAUF, 2010; IFOAM, 2020). This agriculture has been represented worldwide by 3.1 million organic farmers with 72.3 million hectares in 2019, 13% and 1.6% higher than in 2018, respectively (WILLER et al., 2021). Its global market got more than 106 billion dollars in organic food and drink (WILLER et al., 2021). The organic farmland in Brazil has been growth 2% per year between 2000 and 2017, reaching 1.3 million hectares in 2019 (FLORES, 2021; LIMA et al., 2020), and the organic market was growing 30% in 2020 compared to 2019, moving R$ 5.8 billion (ORGANIS, 2021).

Although organic agriculture and its market have been increasing in recent decades, its growth has been limited by lack of information and knowledge, governmental politics, inadequate technical infrastructure, low consumer spending power (CROWDER; REGANOLD, 2015; REGANOLD; WACHTER, 2016). In Brazil, the organic agriculture law that established the normative of production, process, distribution, packaging, identification and certification of organic products was created in 2003 (MUÑOZ et al., 2016). This law projected Brazil internationally as one of the countries that advanced the most in the production and marketing of organics, and its regulation boosted the organic sector and development of governmental politics and social movements (LIMA et al., 2020; SAMBUICHI et al., 2017).

Nevertheless, this country still lacks governmental technical support and investment and dissemination of research in this sector. Barbosa; Sousa (2012) showed that 10% of organic farmland had received technical support regularly, 15% occasionally, and 68% never received technical support in 2009. Furthermore, the absence of systematic official data (production and marketing) about organic agriculture is a limitation to the more robust growth of this sector, making it difficult to prepare strategic plans and dimension the production and demand for producers and companies (LIMA et al., 2020).

Therefore, research, information, and data are essential to the growth of the organic sector, such as cost and profitability analysis of production. This agriculture has a proposal to produce food healthier and environmentally friendly, that must also be profitable. According to Seufert; Ramankutty (2017), yield, cost of production, and prices received determined the relative profitability. Although organic yield usually has been lower than the yield of conventional agriculture (SEUFERT; RAMANKUTTY; FOLEY, 2012), the organic products have a premium price, more crops diversity, environmental footprint, and better united human health that turn organic agriculture more profitable than conventional agriculture (CROWDER; REGANOLD, 2015). Hence, the study aimed to examine the cost and profitability of organic cherry tomato and green corn in the succession system.

6.2. Materials and Methods

The economic analysis was based on agricultural inputs used, the activities carried out, and how production was conducted in the experimental area (Section 2.1). We started the analysis summing all expenses inherent to the production process with the base year 2020 for green corn and cherry tomato (AGRIANUAL, 2021): operation (soil preparation, weed control, fertilization, sow, transplanting, irrigation, transporting, sprouts, harvest), inputs (fertilizers and seeds), administration (light, water, travel, tax, accounting, land lease). The yield of green corn and cherry tomato was expressed kg ha^{-1} and 2021 as the base year for marketing prices of these products. The years 2011/ 2012 and 2012/2013 of yield was called year A and B.

Considering there are no systematic official data to estimate marketing prices for organic products in Brazil, we used the conventional products' base of prices in "Companhia de Entrepostos e Armazens Gerais de São Paulo" (CEAGESP, 2021). We added 83% for cherry tomato and 25% for green corn in the marketing price. This increase was based on the research of "Departamento de Economia Rural" (PARANÁ, 2021) and Watanabe, Abreu e Luiz (2020) that showed the difference in marketing price between organic and conventional products in Brazil.

The net profit and benefits to costs ratio were calculated as shown in Equation:

$$\text{Net profit} = \text{Revenue} - \text{Cost}. \tag{6}$$

$$\frac{Benefits}{cost} ratio = \left(\frac{Revenue}{cost}\right) - 1 \tag{7}$$

This calculation was used separately to cherry tomato and green corn for each year of yield, then we calculated it for the systems. The total cost included 1.8 % of tax rates under the revenue.

6.3. Results

Cherry tomato

The production cost of cherry tomato was divided into different managements of this crop: cherry tomato without straw (CWS), cherry tomato with straw (CS), cherry tomato in an intercropping system with jack bean (JB), sun hemp (SH), dwarf velvet bean (DVB), mung bean (MB), white lupine (WL) or cowpea bean (CB) (Table 30). The cost of mechanized operation, manual operation, and administration were the same for all managements of cherry tomato. The inputs were different because of the legume cost (seed and sowing). Year A and B had similar costs; only year B did not have the eucalyptus posts and wire that the producers can use for 10 and 3 more cycles of cherry tomato, respectively.

The lowest total cost was the WL in year A, followed by SH, MB, CS, CB, DVB, CWS, and JB (Table 31). The difference in the operation cost between the managements was determined by legumes cost (inputs) and the yield/revenue because the higher the productivity, the higher the tax (1.8%) paid. In the same year, the highest yield, revenue, and net profit were CWS, followed by DVB, CS, CB, JB, MB, SH, and WL. The managements of cherry tomato with jack bean, the cost of jack bean seed significantly increased the cost value (Tables 30 and 31). The management of CWS (90%), CS (89%), DVB (89%), and CB (89%) had the highest benefits/costs ratio, followed by JB (88%), MB (87%), SH (87%) and WL (85%).

Regarding year B, the lowest operation cost was the CB, followed by CWS, SH, MB, JB, DVB, WL, and CS (Table 31). The highest yield, revenue, and net profit in the same year were DVB, followed by WL, JB, CS, MB, CWS, SH, and CB. The benefits/cost ratio was above 80% for all managements with cherry tomatoes. However, the yield in year B was 53% lower than year A, on average, which reduced the tax of operation cost and 53% in the revenue, and 50% in the net profit (Table 31).

The cost and revenue accumulated in the two years of organic cherry tomato showed the highest net profit was CS, followed by CB, DVB, CWS, JB, MB, SH, and WL (Table 31). The benefits/cost ratio was above 83% of organic cherry tomatoes. Furthermore, 77.27% of the operation cost of cherry tomato crop was inputs, 18.17% manual operation, 4.51% administrations (included tax), 0.05% mechanized operation (Figure 13).

Table 30. Estimate of production operating cost of organic cherry tomato in an intercropping system with different legumes per hectare. independently of the year

Description	Specification	R$ unit-1*	Unit	CWS	CS	JB	SH	DVB	MB	WL	CB
				Total (R$)							
Mechanized operation											
Irrigation	Equipment	60.00	1	60.00	60.00	60.00	60.00	60.00	60.00	60.00	60.00
Subtotal				**60.00**	**60.00**	**60.00**	**60.00**	**60.00**	**60.00**	**60.00**	**60.00**
Manual operation											
Transplantation	Worker-day	71.30	10	713.00	713.00	713.00	713.00	713.00	713.00	713.00	713.00
Tomato stakes	Worker-day	71.30	15	1.069.50	1.069.50	1.069.50	1.069.50	1.069.50	1.069.50	1.069.50	1.069.50
Tomato tying	Worker-day	71.30	36	2.566.80	2.566.80	2.566.80	2.566.80	2.566.80	2.566.80	2.566.80	2.566.80
Fertilization	Worker-day	71.30	10	713.00	713.00	713.00	713.00	713.00	713.00	713.00	713.00
Removal of sprout	Worker-day	71.30	36	2.566.80	2.566.80	2.566.80	2.566.80	2.566.80	2.566.80	2.566.80	2.566.80
Weeding	Worker-day	71.30	30	2.139.00	2.139.00	2.139.00	2.139.00	2.139.00	2.139.00	2.139.00	2.139.00
Harvest and classification	Worker-day	71.30	130	9.269.00	9.269.00	9.269.00	9.269.00	9.269.00	9.269.00	9.269.00	9.269.00
Irrigation	Worker-day	71.30	41	2.923.30	2.923.30	2.923.30	2.923.30	2.923.30	2.923.30	2.923.30	2.923.30
Subtotal				**21.960.40**	**21.960.40**	**21.960.40**	**21.960.40**	**21.960.40**	**21.960.40**	**21.960.40**	**21.960.40**
Inputs											
Thermophosphate	R$ tonne-1	1.264.50	1.1	1.422.56	1.422.56	1.422.56	1.422.56	1.422.56	1.422.56	1.422.56	1.422.56
Potassium sulfate	R$ tonne-1	1.891.00	0.1	236.38	236.38	236.38	236.38	236.38	236.38	236.38	236.38
Tomato seeds	R$ seed envelope-1	896.30	25	22.407.50	22.407.50	22.407.50	22.407.50	22.407.50	22.407.50	22.407.50	22.407.50
Seedling making	R$ outsourced unit-1	0.50	50000	25.000.00	25.000.00	25.000.00	25.000.00	25.000.00	25.000.00	25.000.00	25.000.00
Bamboo stakes	R$ dozen-1	7.00	1625	11.375.00	11.375.00	11.375.00	11.375.00	11.375.00	11.375.00	11.375.00	11.375.00
Eucalyptus posts	R$ unit-1	7.00	2404	16.826.92	16.826.92	16.826.92	16.826.92	16.826.92	16.826.92	16.826.92	16.826.92
Wire nº 16	R$ kg-1	16.60	12	199.52	199.52	199.52	199.52	199.52	199.52	199.52	199.52
Wire nº 20	R$ kg-1	24.20	6	145.43	145.43	145.43	145.43	145.43	145.43	145.43	145.43
Jack bean seeds	R$ bag (25 kg)-1	589.00	6.3			3.681.25					
Sun hemp seeds	R$ bag (25 kg)-1	450.00	0.6				247.50				
Dwarf velvet bean seeds	R$ bag (25 kg)-1	243.20	2.6					627.46			
Mung bean seeds	R$ bag (40 kg)-1	334.40	0.7						246.62		

White lupine seeds	R$ bag (25kg)$^{-1}$	350.00	2.2							759.50	
Cowpea bean seeds	R$ bag (25kg)$^{-1}$	233.00	3.7								862.10
sowing	Man-day	71.30	1.0			71.30	71.30	71.30	71.30	71.30	71.30
Subtotal				**77.613.31**	**77.613.31**	**81.365.86**	**77.932.11**	**78.312.07**	**77.931.23**	**78.444.11**	**78.546.71**
Administration											
Lease	R$ ha^{-1}	1.600.00	1	1.600.00	1.600.00	1.600.00	1.600.00	1.600.00	1.600.00	1.600.00	1.600.00
Administrative labor	R$ ha^{-1}	2.094.40	1	2.094.40	2.094.40	2.094.40	2.094.40	2.094.40	2.094.40	2.094.40	2.094.40
Accounting/office	R$ ha^{-1}	290.90	1	290.90	290.90	290.90	290.90	290.90	290.90	290.90	290.90
light/ phone	R$ ha^{-1}	1.163.60	1	1.163.60	1.163.60	1.163.60	1.163.60	1.163.60	1.163.60	1.163.60	1.163.60
Travels	R$ ha^{-1}	305.80	1	305.80	305.80	305.80	305.80	305.80	305.80	305.80	305.80
Subtotal				**5454.7**	**5454.7**	**5454.7**	**5454.7**	**5454.7**	**5454.7**	**5454.7**	**5454.7**

Note: CWS – cherry tomato without straw. CS – cherry tomato with straw. JB – cherry tomato with jack bean. SH – cherry tomato with sun hemp, DVB – cherry tomato with dwarf velvet bean. MB – cherry tomato with mung bean. WL - cherry tomato with white lupine. CB – cherry tomato with cowpea bean in an intercropping system. *The unit value was taken from the Agrianual 2020.

Table 31. Estimate of operation cost and revenue based in the yield of organic cherry tomato in an intercropping system with different legumes per hectare

	Unit	Year	CWS	CS	JB	SH	DVB	MB	WL	CB
Operation cost + tax (1.8%)	R$ ha^{-1}	A	127.494.81	126.155.61	128.410.56	122.111.21	126.868.77	122.830.33	120.405.61	126.858.61
Yield	Kg ha^{-1}		69.155.56	65.022.22	60.400.00	51.555.56	65.066.67	53.777.78	44.711.11	64.311.11
Average price received	R$ kg^{-1}		18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Revenue	R$ ha^{-1}		1.244.800.00	1.170.400.00	1.087.200.00	928.000.00	1.171.200.00	968.000.00	804.800.00	1.157.600.00
Net profit	R$ ha^{-}		1.117.305.19	1.044.244.39	958.789.44	805.888.79	1.044.331.23	845.169.67	684.394.39	1.030.741.39
Benefits/cost ratio	%		90	89	88	87	89	87	85	89
Operationl cost + tax (1.8%)	R$ ha^{-1}	B	97.377.34	99.868.54	102.469.09	96.572.94	99.559.29	97.954.46	97.833.74	100.240.34
Yield	Kg ha^{-1}		29.200.00	36.888.89	33.333.33	25.733.33	33.777.78	30.000.00	28.044.44	35.155.56
Average price received	R$ kg^{-1}		18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Revenue	R$ ha^{-1}		525.600.00	664.000.00	600.000.00	463.200.00	608.000.00	540.000.00	504.800.00	632.800.00
Net profit	R$ ha^{-}		428.222.66	564.131.46	497.530.91	366.627.06	508.440.71	442.045.54	406.966.26	532.559.66
Benefits/cost ratio	%		81	85	83	79	84	82	81	84
Operation cost + tax (1.8%)	R$ ha^{-1}	A+B	224.872.15	226.024.15	230.879.65	218.684.15	226.428.06	220.784.79	218.239.35	227.098.95
Yield	Kg ha^{-1}		98.355.56	101.911.11	93.733.33	77.288.89	98.844.44	83.777.78	72.755.56	99.466.67
Average price received	R$ kg^{-1}		18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Revenue	R$ ha^{-1}		1.770.400.00	1.834.400.00	1.687.200.00	1.391.200.00	1.779.200.00	1.508.000.00	1.309.600.00	1.790.400.00
Net profit	R$ ha^{-}		1.545.527.85	1.608.375.85	1.456.320.35	1.172.515.85	1.552.771.94	1.287.215.21	1.091.360.65	1.563.301.05
Benefits/cost ratio	%		87	88	86	84	87	85	83	87

Note: CWS – cherry tomato without straw. CS – cherry tomato with straw. JB – cherry tomato with jack bean. SH – cherry tomato with sun hemp, DVB – cherry tomato with dwarf velvet bean. MB – cherry tomato with mung bean. WL - cherry tomato with white lupine. CB – cherry tomato with cowpea bean in an intercropping system.

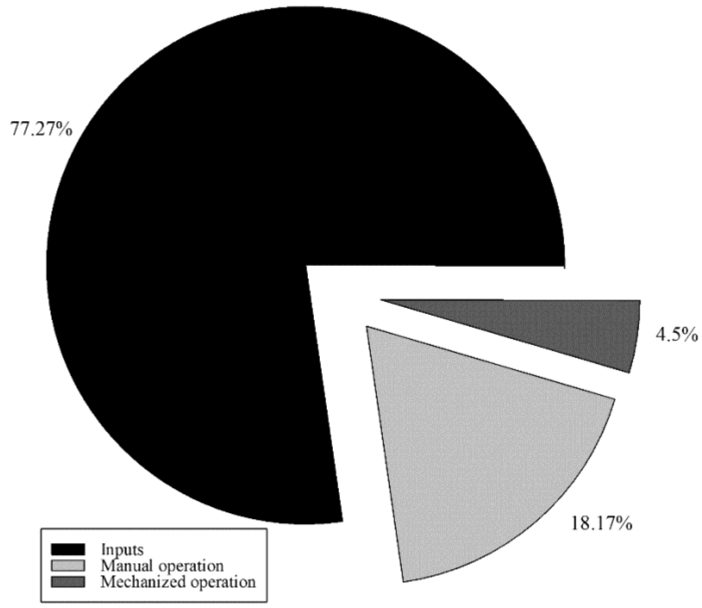

Figure 13. Division of the operation cost of cherry tomato in the two years of production, on average

Green corn

The operation cost of organic green corn was the same for all green con in succession to cherry tomato and legumes in an intercropping system (Table 32). However, the economic analysis was divided into different managements of cherry tomatoes in succession due to the difference in green corn yield (Table 33).

The lowest operation cost was the green corn in succession to CS in year A, followed by MB, DVB, SH, WL, JB, CB, and CWS (Table 33). The highest yield, revenue, and net profit in the same year was the green corn in succession to CWS, followed by CB, JB, WL, SH, DVB, MB, and CS. The benefits/cost ratio was higher in the green corn in succession to CWS and CB (Table 33). Likewise, the lowest operation cost was the green corn in succession to CS in year B, followed by MB, CWS, DVB, WL, CB, SH, and JB. The highest yield, revenue, and net profit was the green corn in succession to JB, followed by SH, CB, WL, DVB, CWS, MB, and CS.

The cost and revenue accumulated in the two years of organic green corn showed the highest net profit was the green corn in succession to JB, followed by CB, SH, and WL, in which the benefits/cost ratio was above 50% (Table 33). Moreover, approximately 66% of the total cost of the organic green corn crop was the operations, 24% inputs, 7% administrations (included tax), 4% post-harvest (Figure 14).

Table 32. Estimate of operation cost of organic green corn in succession to cherry tomato and legumes in an intercropping system per hectare

Description	Specification	R$ unit^{-1*}	Unit	Green corn
			Total (R$)	
Operations				
Planting	seeder	181.03	0.40	72.41
Manual labor for planting	Worker-day	8.91	8.00	71.30
Internal transport for planting	truck	143.73	0.50	71.87
Weeding control	Worker-day	8.91	64.00	570.40
Internal transport for weeding	truck	143.73	0.10	14.37
Harvest - Manual labor	Worker-day	8.91	40.00	356.50
Internal transport for harvest	truck	143.73	4.00	574.92
Irrigation		2.15	180.00	387.00
Subtotal				**2,118.77**
Inputs				
Seeds	R$ 60.0000 seeds^{-1}	780	1.00	780.00
Subtotal				**780.00**
Administration				
Administrative labor	R$ ha^{-1}	39.19	1.00	39.19
light/ phone	R$ ha^{-1}	13.06	1.00	13.06
Processing/conservation	R$ ha^{-1}	6.92	1.00	6.92
Travels	R$ ha^{-1}	38.66	1.00	38.66
Subtotal				**97.83**
Post-harvest				
Transport	R$ tonne^{-1}	19.55	0.93	37.73
Receiving and cleaning	R$ tonne^{-1}	37.50	1.93	72.38
Administrative tax	R$ tonne^{-1}	5.25	1.93	10.13
Subtotal				**120.24**

Note: *The unit value was taken from the Agrianual 2020.

Table 33. Estimate of operation cost and revenue based in the yield of organic green corn in succession to cherry tomato in an intercropping system with legumes per hectare

	Unit	Year	CWS	CS	JB	SH	DVB	MB	WL	CB
Total cost + tax (1.8%)	R$ ha^{-1}	A	3,253.63	3,215.00	3,242.08	3,233.36	3,229.46	3,228.40	3,237.61	3,253.34
Yield	Kg ha^{-1}		1,930.00	1,385.00	1,767.00	1,644.00	1,589.00	1,574.00	1,704.00	1,926.00
Average price received	R$ kg^{-1}		3.94	3.94	3.94	3.94	3.94	3.94	3.94	3.94
Revenue	R$ ha^{-1}		7,599.38	5,453.44	6,957.56	6,473.25	6,256.69	6,197.63	6,709.50	7,583.63
Net profit	R$ ha^{-}		4,345.75	2,238.44	3,715.49	3,239.89	3,027.23	2,969.23	3,471.89	4,330.28
Benefits/cost ratio	%		57	41	53	50	48	48	52	57
Total cost + tax (1.8%)	R$ ha^{-1}	B	3,217.27	3,209.33	3,295.66	3,253.70	3,222.37	3,215.28	3,245.90	3,246.47
Yield	Kg ha^{-1}		1,417.00	1,305.00	2,523.00	1,931.00	1,489.00	1,389.00	1,821.00	1,829.00
Average price received	R$ kg^{-1}		3,94	3,94	3,94	3,94	3,94	3,94	3,94	3,94
Revenue	R$ ha^{-1}		5,579.44	5,138.44	9,934.31	7,603.31	5,862.94	5,469.19	7,170.19	7,201.69
Net profit	R$ ha^{-}		2,362.17	1,929.11	6,638.66	4,349.61	2,640.57	2,253.90	3,924.29	3,955.22
Benefits/cost ratio	%		42	38	67	57	45	41	55	55
Total cost + tax (1.8%)	R$ ha^{-1}	A+B	6,470.90	6,424.33	6,537.73	6,487.06	6,451.83	6,443.68	6,483.51	6,499.81
Yield	Kg ha^{-1}		3,347.00	2,690.00	4,290.00	3,575.00	3,078.00	2,963.00	3,525.00	3,755.00
Average price received	R$ kg^{-1}		3.94	3.94	3.94	3.94	3.94	3.94	3.94	3.94
Revenue	R$ ha^{-1}		13,178.81	10,591.88	16,891.88	14,076.56	12,119.63	11,666.81	13,879.69	14,785.31
Net profit	R$ ha^{-}		6,707.92	4,167.54	10,354.14	7,589.51	5,667.79	5,223.13	7,396.18	8,285.50
Benefits/cost ratio	%		51	39	61	54	47	45	53	56

Note: green corn in succession to CWS – cherry tomato without straw, CS – cherry tomato with straw. JB – cherry tomato with jack bean. SH – cherry tomato with sun hemp, DVB – cherry tomato with dwarf velvet bean. MB – cherry tomato with mung bean. WL - cherry tomato with white lupine or CB – cherry tomato with cowpea bean in an intercropping system.

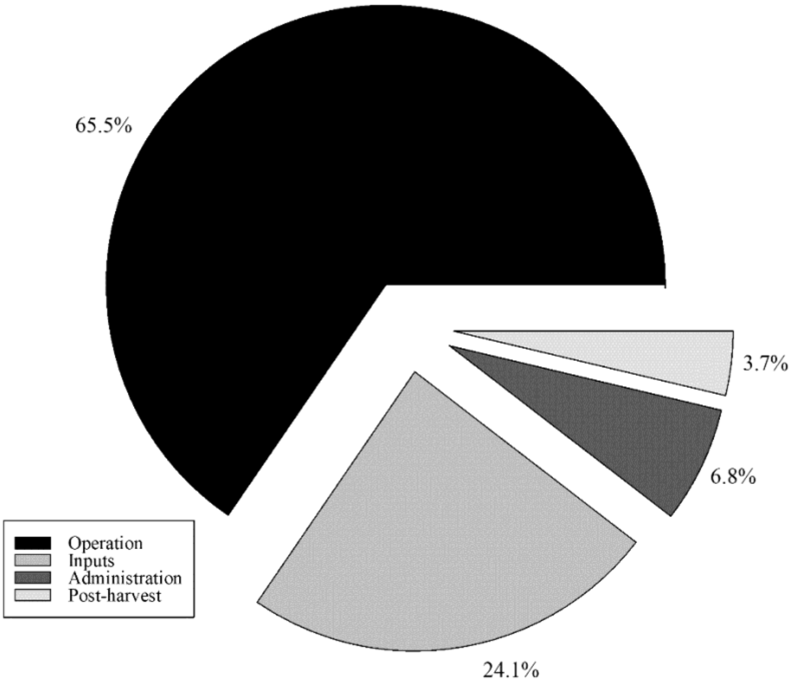

Figure 14. Division of the total operating cost of green corn in the two years of production, on average

The organic system with cherry tomato and green corn

The inputs were 76% of the system's total cost in two years, and 18% of this cost was the manual operation (Table 34 and Figure 15). Furthermore, the operation cost of the cherry tomato was 97% of the total operation cost (cherry tomato + green corn) (Table 34). However, 99% of the system's total revenue came from the cherry tomatoes crop (Table 34). The net profit in two years was R$ 1,399,017.53, with 68% coming from year A and 32% - year B (Table 34).

Table 34. Estimate of total operation cost and revenue of the organic system per year of yield each crop

	Unit	A	B	A+B
Subtotal Mechanized operation	R$	2,178.77	2,178.77	4,357.54
Subtotal Manual operation		21,960.40	21,960.40	43,920.80
Subtotal Inputs		98,661.05	89,675.45	188,336.50
Subtotal Administration		5,672.30	5,673.94	11,346.24
Subtotal Post-harvest		110.11	110.11	220.21
Operation cost – Cherry tomato	R$ ha^{-1}	125,356.15	116,370.55	241,726.70
Operation cost – Green corn		3,226.48	3,228.12	6,454.59
Operation cost - Total		128,582.63	119,598.67	248,181.29
Revenue - Tomato		1,066,500.00	567,300.00	1,633,800.00
Revenue – Green corn		6,653.88	6,744.94	13,398.82
Revenue -Total		1,073,153.88	574,044.94	1,647,198.82
Net profit		944,571.26	454,446.27	1,399,017.53
Benefits/cost ratio	%	88	79	85

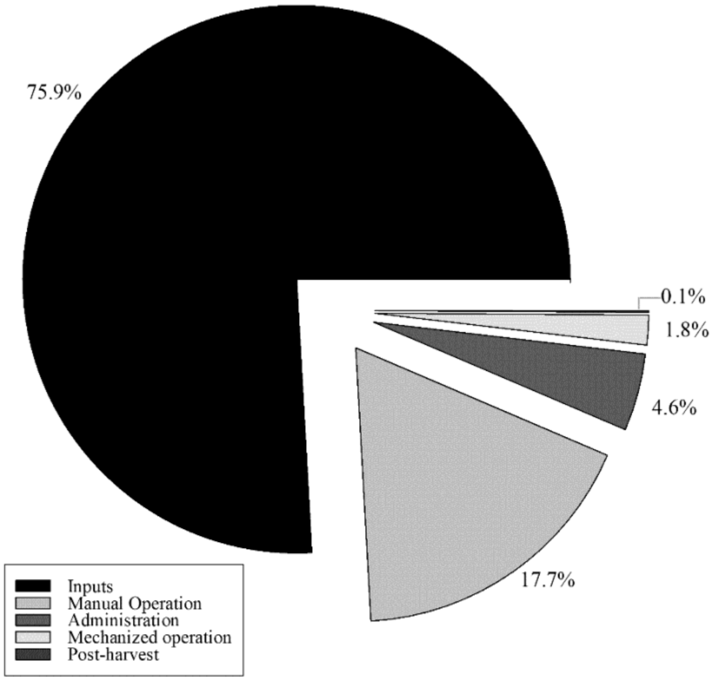

Figure 15. Division of the total operation cost of organic system

6.4. Discussion

The tomatoes crop has been one of the main vegetables in Brazil (IBGE, 2021). This crop production has a high cost and risk of investments due to dependence on hired labor and price fluctuation related between supply and demand (HF, 2021). In the current study, the operational cost of the organic cherry tomato was R$ 112,277.41 year^{-1}, on average, and the inputs were responsible for more than half of this cost. Likewise, the operation cost in conventional tomato crop was R$ 102,805.59 in 2020/2021, and 54% of this cost was the inputs and 31% the hired labor (HF, 2021; CONAB, 2021). The economic analysis for convectional cherry tomato in 2014, the operational cost was R$137,244.60, and the inputs and hired labor represented 60% and 40% of this cost (NEGRISOLE et al., 2015). However, the fertilizers and defensive accounted for 72% of inputs costs in the conventional system, and the seedling production accounted for 60% of the inputs system in the present organic study (Table 30) (HF, 2021; CONAB, 2021).

In the present study, the increased line spacing, consequently reducing the plant's number per hectare, and increasing the number of the hast per plant from 2 to 3, would reduce the cost with seeds and seedling making. According to Azevedo (2006), the production of organic cherry tomato cv. "Super-Sweet" (16,666 plants ha^{-1}) with three hast per plant (25 t ha^{-1}) had more yield than cherry tomato with two hast per plant (22 t ha^{-1}).

The benefits/cost ratio in the current organic cherry tomato was 88%; thus, 88% of the revenue represented the net profit. The conventional cherry tomato cv. Sweet, this cost/benefits ratio was 36% for large producers in 2014, considering the operation cost only (NEGRISOLE et al., 2015). In 2021, this ratio was 21% for conventional tomato crops in São Paulo State, Brazil (HF BRASIL, 2021). This ratio difference between organic and conventional has been in the premium price paid to the organic products. Crowder e Reganold (2015) studied the financial performance of organic and conventional agriculture using 44 research from Asia, North America, South America, Europe, Oceania showed the premium prices determined the organic system was more profitable than a conventional system. According to the same authors, the median breakeven for the organic system needed to be 7% more than the conventional system for its net present value (NLV) to match between them. However, this study showed this difference was 29%; thus, organic products with premium values significantly higher NPV than the conventional system. Moreover, the premium prices compensated for the lower yield of organic than conventional system (SEUFERT; RAMANKUTTY; FOLEY, 2012).

The research developed in Paraná State, Brazil, with familiar agriculture in the organic and conventional system showed that organic tomato and green corn were sold 83% and 25% higher than conventional in 2020 (PARANÁ, 2021). Another study developed in Campinas city, São Paulo State, Brazil, in 2017 showed the prices of organic vary among the vegetable and local in which were sold, and the organic vegetable in the can be 73%, 123%, and 95% higher than conventional for carrot, lettuce and tomato in the supermarket (WATANABE; DE ABREU; LUIZ, 2020).

The organic green corn has had a premium price, but it was lower than cherry tomatoes, beyond the cost of operation and the revenue was also lower than a cherry tomato. The operation and inputs (seeds) accounted for 65.5% and 24.1% of the operation cost, respectively (Figure 14). Furthermore, the hired labor was 31% of the operation cost. However, the study with organic green corn at Maranhão state, Brazil, in 2016 showed inputs, operation, and certification represented 60%, 15%, and 23% of the operation cost, respectively (PONTES, 2018). According to the same authors, the specific cost with certification and manure accounted for 24% of the operation cost, was the highest annual cost.

In the current study, the net profit was lower due to the low yield despite of the low-cost operation (Table 33). The green corn had a lower yield than the São Paulo state yield of 5.8 Mg ha^{-1} in 20120/2019 and of variety yield average of 5.7 Mg ha^{-1} (SÃO PAULO, 2021).

Nevertheless, this crop had a net profit and a benefit/cost ratio of over 39% (Table 33). The green corn in Brazil has been sold as horticultural due to its perishability. Brazilian corn industry association (AbiMilho) showed that consumption in nature of maize was 1.7 million tonnes in 2020, which increased 68% compared to 2013; thus, the consumption of green corn has been grown in this country. Possibly, the investment in fertilizer in the organic green corn would increase productivity, consequently, the revenue despite increasing the cost of operation in the present study. Pontes (2018) showed that the producers of green corn in the organic system in 2016 received a net profit of R$ 10,166.4 per year, on average, even with the high cost of fertilizer and certification.

In two years of production, the organic green corn had higher benefits/cost ratio when in succession with cherry tomato and legumes in an intercropping system (mainly in JB and CB) due to its superior yield, consequently, the superior revenue (Table 33).

6.5. Conclusion

The organic system with cherry tomato and legumes in an intercropping system and the green corn in succession was high profitability under the conditions in which this study was conducted.

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7 Spatial distributions of enzymes in the rhizosphere of tomato-jack bean intercropping

Abstract

The work aimed to evaluate the activity and spatial distribution of enzymes (Phosphatase, Chitinase, and β-Glucosidase) of single and intercropping with tomato and jack bean. The plants have grown for 14 days to direct soil zymography evaluation. Zymography is applied as in situ technique to study the spatial distribution of exogenous enzyme activity around the roots. The enzyme activity, hotspots, and rhizosphere were extracted through zymography images. The roots of tomato and tomato + jack bean have grown and filled all rhizotron. The treatment of jack bean, tomato, and tomato + jack bean increased the enzyme activity compared with soil treatment independently of the enzymes. The enzymatic spatial distribution is presented along and around the roots, and the roots' presence in the soil increases enzymes activity in rhizotron. The jack bean, tomato, and jack bean + tomato treatments showed different enzymatic spatial distribution. The growth of tomato and jack bean on the same rhizotron induced the combination of the enzymatic distribution patterns of the two plants' roots. The tomato + jack bean treatment was not increased the enzyme activity compared to tomato and jack bean single crop treatment, although numerally, the average of β-glucosidase activity has been a gradual increase in the tomato + jack bean compared to tomato and jack bean single crop. According to the type of enzyme evaluated, the hotspots and rhizosphere showed different results. The reduction of hotspots in the tomato + jack bean treatment compared with jack bean treatment can be showing competition between the roots and the soil microbial. Corroborating to the data of the rhizosphere that was bigger in the single tomato and jack bean for the enzyme chitinase and phosphatase.

Keywords: zymography, rhizosphere extent, phosphatase, chitinase, β-Glucosidase

Distribuição especial das enzimas na rizosfera do tomate e feijão-de-porco em consórcio

Resumo

O objetivo do trabalho foi avaliar a atividade e distribuição espacial de enzimas (fosfatase, quitinase e β-glucosidase) em monocultura ou consórcio de tomate e feijão-de-porco. As plantas cresceram durante 14 dias para a avaliação zimográfica direta do solo. A zimografia é uma técnica aplica *in situ* para estudar a distribuição espacial da atividade enzimática exógena arredor das raízes. A atividade enzimática, *hotspots* e a rizosfera fora extraída das imagens zimográficas. As raízes do tomate e tomate + feijão-de-porco cresceram e ocuparam todo o rizotron. O tratamento de feijão-de-porco, tomate e tomate + feijão-de-porco aumentou a atividade enzimática compara com o tratamento de solo independente da enzima testada. A distribuição espacial enzimática foi apresentada ao longo e ao redor das raízes, assim como, a presença das raízes no solo aumento a atividade no rizotron. Os tratamentos apresentaram diferentes distribuição espacial das enzimas. O crescimento do tomate e do feijão-de-porco no mesmo rizotron acarretou uma combinação no padrão da distribuição espacial enzimático das duas raízes. O tratamento tomate + feijão-de-porco não aumentou a atividade enzimática comparado ao tomate em monocultura, apesar que numericamente a média da atividade da β-glucosidase apresentou uma tendência de aumento no tomate + feijão-de-porco comparado com tomate e feijão em monocultura. De acordo com o tipo de enzima avaliado, o *hotspots* e a rizosfera apresentaram diferentes resultados. A redução do *hotspots* no tratamento tomate + feijão-de-porco comparado com o feijão-de-porco pode ser um indicativo de competição entre as raízes e os microrganismos do solo. Corroborando com os dados da rizosfera que aumentou no consórcio se compara ao tomate e feijão-de-porco para as enzimas fosfatase e chitinase.

Palavras-chave: zimografia, extensão da rizosfera, fosfatase, chitinase, β-Glucosidase

7.1. Introduction

Legumes are used in agriculture in intercropping and crop rotation systems to increase soil fertility and the availability of nitrogen (N) in the system, thereby reducing the use of nitrogen fertilizers that are costly to farmers and can be harmful to the environment (AMBROSANO et al., 2011; OBERSON et al., 2013; WICHERN et al., 2007b). For legumes in the intercropping system, the knowledge base on belowground interaction (roots) is fundamental for understanding the interaction between plants under intercrop and potential N supply to companion and subsequent crops (WICHERN et al., 2007a; 2008; ZANG et al., 2015).

The intercropping benefits the development of different types of roots and changes their distribution and architecture beyond affecting the rhizodeposition. The rhizodeposition releases organic and inorganic compounds in the rhizosphere such as enzymes, exudate, ions and represents an essential flow of carbon (C) into the soil (MARSCHNER, 2012; PAUSCH et al., 2013; DUCHENE; VIAN; CELETTE, 2017). Taschen et al. (2017) demonstrated that intercropping between legume and non-legume plants can modify the bacterial community in the rhizosphere compared to the same species in monoculture, suggesting a synergic interaction in the mixed rhizosphere plant.

The rhizosphere of plants not only interacts with soil microorganisms but also with other rhizospheres/roots of other plants that may or may not be of the same species. Root exudates play an important role in the interaction between neighbouring plants, and there may be changes in root allocation at physiological and biochemical levels in response to specific neighbouring plants (BADRI; VIVANCO, 2009; HERZ et al., 2018; DAM; HARRO J., 2016). According to Badri and Vivanco (2009), neighbouring plants of different species (polyculture) could alter the balance between defense and growth and other physiological characteristics. The study by Schmid et al. (2013) on the root-root perception of *Arabidopsis thalia* and *Hieracium pilosella* species by transcriptome analysis found that detecting neighbouring plants belowground can occur independently of resource depletion and that the presence of neighbouring roots impacted the distribution of species roots and gene transcription.

Furthermore, rhizodeposits are considered one of the potential transfer mechanisms of N or even other nutrients beyond it is essential in the rhizosphere process like the production of enzymes (FUSTEC et al., 2010; PEOPLES et al., 2015; THILAKARATHNA et al., 2016). There are numerous enzymes involved in the C and N cycle, and they are important for the decomposition of soil organic matter and nutrient cycles (KOCH; TSCHERKO; KANDELER, 2007). The soil enzymes are necessary for the decomposition of organic matter and nutrient cycling; thus, they influence soil fertility and agronomic yield (RAO et al., 2017). The enzymatic activity of soil is influenced by different biotic and abiotic factors and interactions between plants and microorganisms (GEA et al., 2017). During plants growth and development, the roots release root exudates and other rhizodeposits that stimulate and increase the activity of microorganisms and the synthesis of extracellular enzymes (RAZAVI et al., 2016; KUZYAKOV; BLAGODATSKAYA, 2015).

Thus, this work aimed to evaluate the activity and spatial distribution of enzymes (Phosphatase, Chitinase, and β-Glucosidase) of single and intercropping tomato and jack bean using the zymography technique. We hypothesized that the intercropping system would increase chitinase, β-glucosidase, phosphatase activities, and hotspots areas.

7.2. Materials and Methods

Soil

The soil was samples from the top 20 cm of the Rhodic Kandiudox (USDA soil taxonomy). The area is agroecological experimental station in Piracicaba, Sao Paulo, Brazil (altitude of 540 m, 22°43'S, 47°38'W). The soil was sieved (< 2 mm) and had the following physical and chemical properties: 28% of clay; 12% of silt; 16% of fine sand, 14% of coarse sand; density: 1.2 g cm^{-3}; pH ($CaCl_2$): 5.2; organic matter (OM): 46 g dm^{-3}; P: 33 mg dm^{-3}; K: 10.2 mmolc dm^{-3}; Ca: 55.0 mmolc dm^{-3}; Mg: 24 mmolc dm^{-3}; H+Al: 28 mmolc dm^{-3}; Al: 1 mmolc dm^{-3}, S: 8.0 mg dm^{-3}; B (hot water): 0.18 mg dm^{-3}; Cu: 3.1 mg dm^{-3}; Fe: 38 mg dm^{-3}; Mn: 26.8 mg dm^{-3}; Zn: 11.2 mg dm^{-3}; base saturation (BS): 89 $mmol_c$ dm^{-3}; cation exchange capacity (CEC): 117 $mmol_c$ dm^{-3}; percentage of base saturation (V): 76%; aluminium saturation (m): 1%.

Initial plant growth

The jack bean (*Canavalia ensiformis* DC) seeds were from Brazil. The seeds were soaked in aerated distilled water overnight and transferred to a tray containing moist filter paper at 25ºC (Figure 1) (GEA et al., 2017). The seeds received distilled water every day until the paper was wet. The jack bean was transferred to the rhizotron seven days after germination. The tomato seedling ('Red Brandywine' variety) was obtained from a United Kingdom supplier and was transplanted to the rhizotron with 12,6 g of dry weight (aerial part and roots) and 15 cm of height on average.

Experiment management

The experimental design was a complete randomized design with four treatments and five replications. The treatments were soil (without plants), tomato single, jack bean single and tomato plus jack bean grew in the rhizotron. The plants grow in separated rhizotrons with an inner dimension of 23 x 23 x 1.8 cm contained one kilogram of soil. In the treatment with tomato and jack bean, the plants were grown with 4 cm of distance between them. The rhizotrons were closed and kept inclined at an angle of 45°; thus, roots grow along the lower wall (lateral of the openable rhizotron). The rhizotrons were kept in a climate-controlled grown chamber at 23°C of temperature and a daily light period of 16 hours with 500 µmol m^{-2} s^{-1} of photosynthetically active radiation intensity (SCRASE et al., 2018). The soil was maintained in 60% of water holding capacity by irrigation with distilled water.

Direct soil zymography

The plants have grown for 14 days to direct soil zymography evaluation. The zymography is applied as *in situ* technique to study the spatial distribution of exogenous enzyme activity around the roots (GEA et al., 2017; RAZAVI et al., 2016). This study followed the protocol optimized by Razavi et al., 2016, 2019. The visualization of enzyme activity involves using membrane saturated with 4-Methylumbelliferyl (MUF)-substrate that becomes fluorescent when reacting with a substrate-specific enzyme (RAZAVI et al., 2019). The enzymes evaluated were β-glucosidase, *N*-acetylglucosamine (chitinase), and phosphatase detected by 4-Methylumbelliferyl-β-D-glucopyranoside, 4-Methylumbelliferyl-*N*-acetyl-β-D-glucosaminide, and 4-Methylumbelliferyl-phosphate, respectively. Separately, each substrate was dissolved in MES buffer to a concentration of 10 mM. The MES buffer was made with distilled water and 0.1 M concentration of MES hemisodium ($C_6H_{13}NO_4 \cdot 0.5Na$, Sigma-Aldrich, Germany).

Polyamide membrane filters (Tao Yuacan, China) with a dimension of 20 x 20 cm and pore size of 0.45 µm were saturated with the substrate for each enzyme. The rhizotrons were open, rooted size, and the saturated membranes were applied directly to the soil surface (GEA et al., 2017). After the membrane was incubated on the soil surface for one hour, it was carefully lifted from the soil surface, and any soil particles were gently removed using a brush. This membrane was put on the background, and the photos was taken on the UV-light with an excitation wavelength of 355 nm and emission wavelength of 460 nm in the dark room

(Figure 16). Fluorescent on the membrane with the substrate is visible under light UV that shows the area where the substrate was enzymatically hydrolysed (GEA et al., 2017; RAZAVI et al., 2019). The intensity of fluoresce is proportional with the enzyme activity (RAZAVI et al., 2016). The photographic camera CANON D100 was positioned on the tripod at 68.4 cm of high over the black ground, and four UV-light were positioned in the four corners of membrane at 6 cm over the black ground (Figure 16).

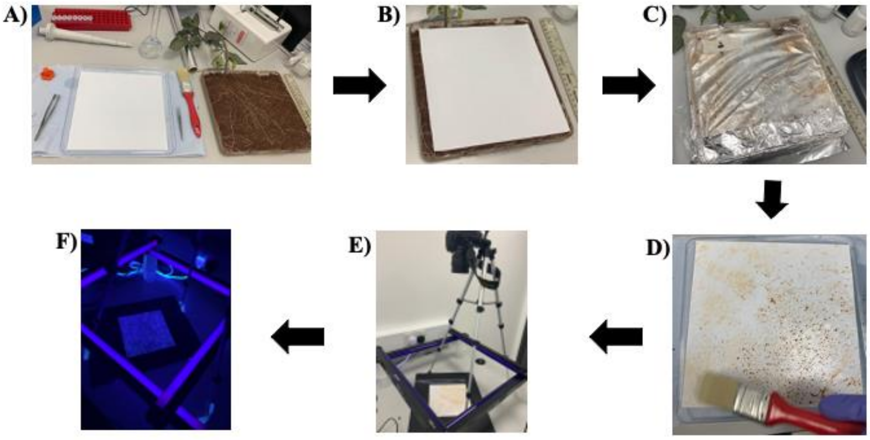

Figure 16. Steps of direct soil zymography: A) saturation of membranes with the substrate, B) application of saturated membranes directly to the soil surface; C) incubation of membrane for one hour, D) remotion of any soil particles, E) putting the membrane on the background, E) take photos on the UV-light

Image processing

The zymography images were processed in toolbox Matlab (MATLAB, the MathWorks) and Image J, according to Ravazi et al. (2019) and Gea et al. (2017). The image processing included five steps: 1. Image transformed into 16-bit gray-scale; 2. Background correct; 3. Root segmentation; 4. Roots skeletonization and 5. Conversion of grayscale values to enzyme activity. Briefly, the zymograms were transformed to 16-bit gray-scale images as matrices and corrected for light variation and camera noise (MENON et al., 2007). We used the gray-value obtained from blank sides of the sample as the referencing point. After that, we calculated an average background gray-value through the zymograms of calibration lines at zero concentration point on the calibration line and subtracted this value from all the zymograms. The standard calibration for colouration the activity of enzymes and the gray-value of zymograms fluorescence was prepared to quantify the zymograms image. The calibration function was obtained by zymography of 4 cm^2 membranes soaked in a solution of MUF with the concentration of 0.01, 0.2, 0.5, 1, 2, 4, 6 and 10 mM. One of the membranes was put

1 ml of each concentration, separately. The membrane of the calibration was imaged under UV light and analysed in the same way that the samples were. The pixel-wise gray-value in the zymograms were converted to enzyme activity using the calibration function. The gray-value of calibration function was correlated with their substrate concentration and enzyme activity by fitting with the linear correlation.

The resulting images were then used for calculating the enzyme activity on the surface of the roots, hotpots and rhizosphere. The roots were segmented as threshold method in Matlab that was used to detect the boundaries of the roots distinguishing the difference between roots and soil surface (CHAUDHURI et al., 1989). The segment roots, their length and radius were calculated using Euclidean distance map function in Matlab to calculate overall enzyme activity on the surface of the roots. Hotpots were distinguished from the surrounding area by the intensity of their colour contrast in the digital images basing on the image references and calibration lines (RAVAZI et al., 2019). The boundaries were confirmed to one-way analysis of variance (ANOVA) that was applied to assess the significant differences between independent variables (e.g. mean values of a specific number of adjacent pixels, for example, equal to 0.1 mm).

Dry matter

After taking the photos, the plants have sampled and dried at 65 ° C until a constant mass to determine the dry weight. The statistical analysis was performed using the R software (Version 1.3.1093). After descriptive and exploratory analysis of the data, analysis of variance (ANOVA) was applied, and the comparison was performed using the Tukey test with 5% significance level.

7.3. Results

The roots of tomato and tomato + jack bean have grown and filled all rhizotron (Figure 17). In contrast, the jack bean roots has grown slower than tomato and its roots have not filled all rhizotron. Moreover, the dry weight of the tomato (5.8 g rhizotron^{-1}) was higher than jack bean (4.8 g rhizotron^{-1}) ($p < 0.05$), independently they were cultivated single or combined. The treatment of jack bean, tomato, and tomato + jack bean increased the enzyme activity compared with soil treatment independently of the enzymes (Figures 18, 19, 20 and 21B).

The enzymatic spatial distribution is presented along and around the roots (Figures 18, 19, and 20), and the roots' presence in the soil increases enzymes activity in rhizotron (Figure 21B). The jack bean, tomato, and jack bean + tomato treatments showed different enzymatic spatial distribution. The areas with high enzyme activity in the jack bean treatments have been slightly increased closer to the stem base (Figures 18b, 19b, 20b). On the other hand, the areas with high enzyme activity in the tomato roots have been a slight increase in the lower part of the rhizotron, farther from the base of the stem (Figures 18c, 19c, 20c). Therefore, the growth of tomato and jack bean on the same rhizotron induced the combination of the enzymatic distribution patterns of the two plants' roots. Furthermore, the tomato + jack bean treatment was not increased the enzyme activity compared to tomato and jack bean single crop treatment ($p < 0.05$), although numerally, the average of β-glucosidase activity has been a gradual increase in the tomato + jack bean compared to tomato and jack bean single crop (Figure 21B).

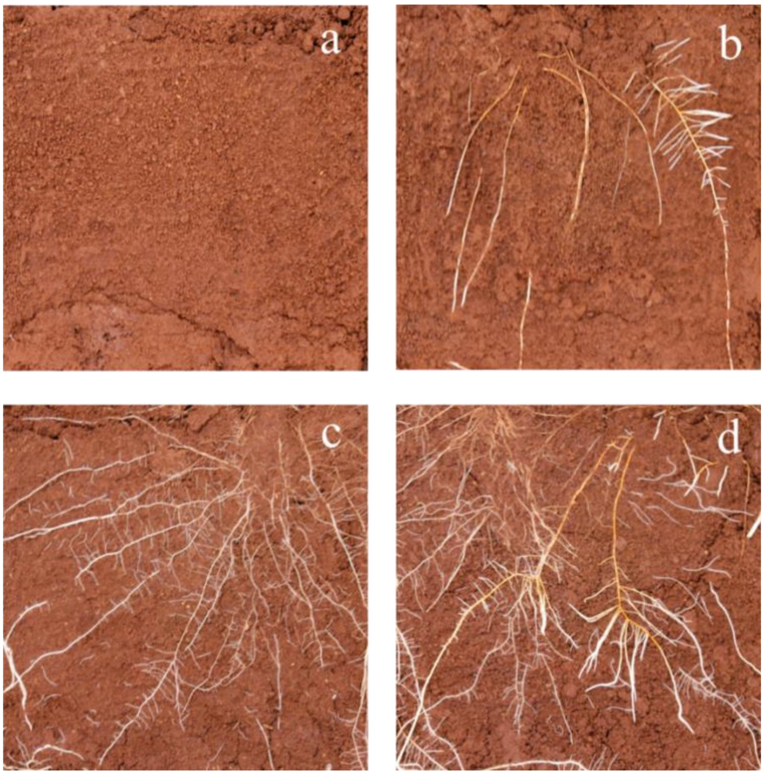

Figure 17. Soil (a) and roots grown of jack bean (b), tomato (c) and tomato + jack bean(d) in the rhizotron after 14 days of growth

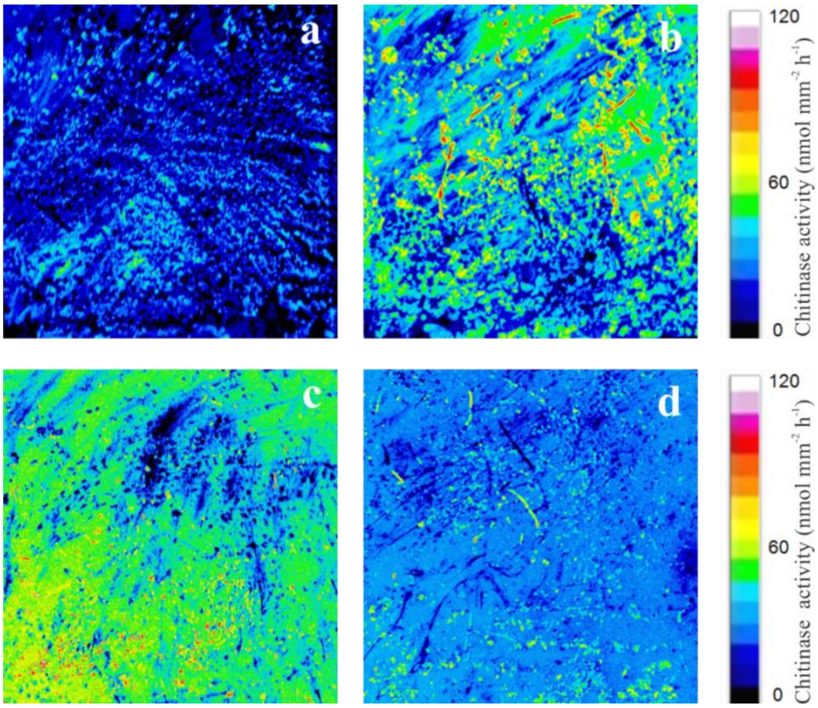

Figure 18. Spatial distribution of chitinase activity on the soil (a), jack bean (b), tomato (c) and tomato + jack bean after 14 day of growth

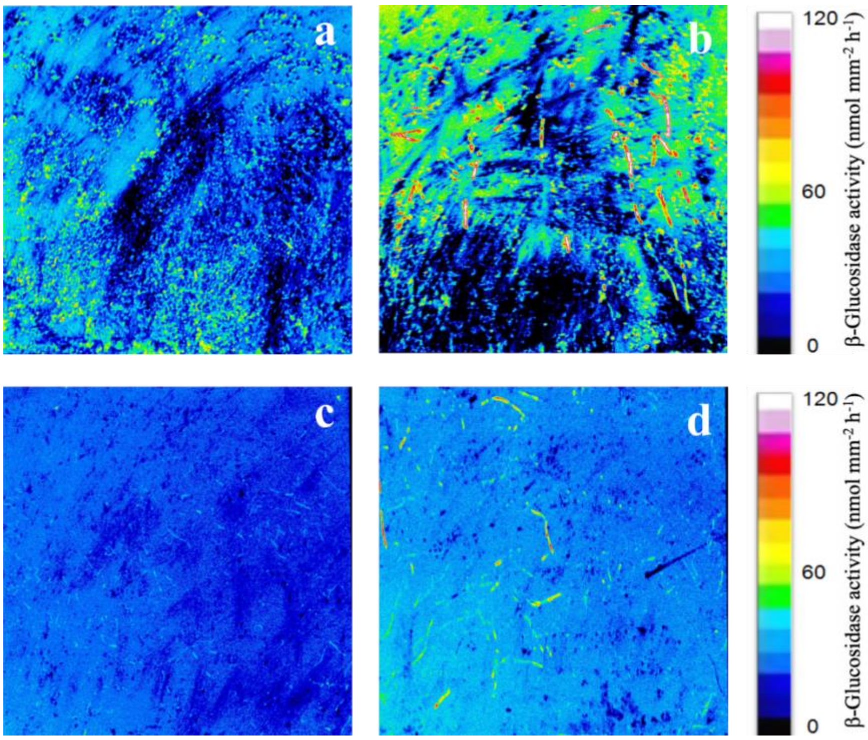

Figure 19. Spatial distribution of β-Glucosidase activity on the soil (a), jack bean (b), tomato (c) and tomato + jack bean after 14 days of growth

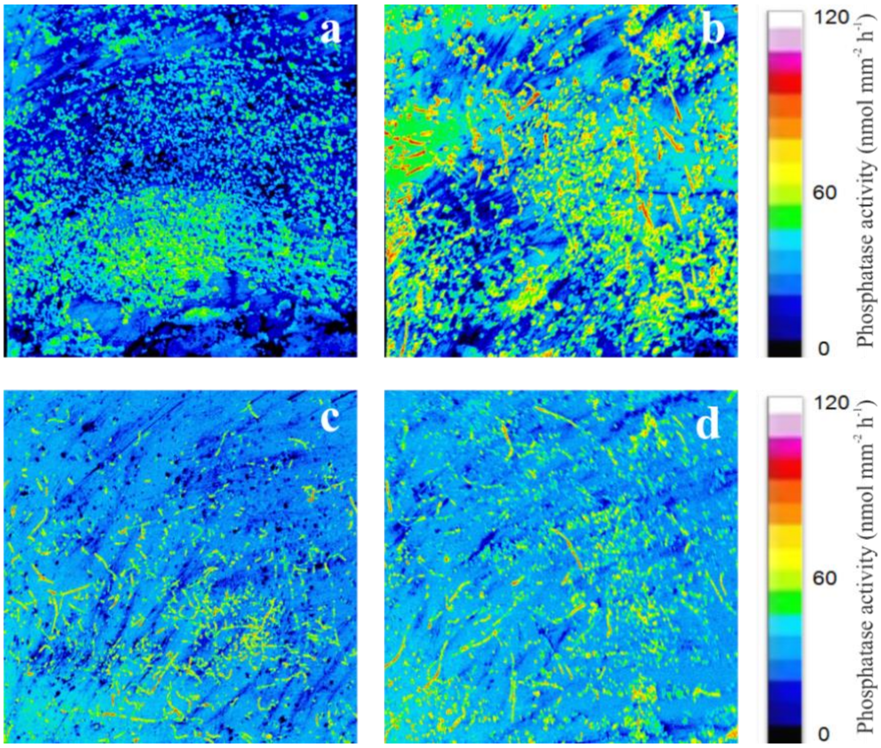

Figure 20. Spatial distribution of Phosphatase activity on the soil (a), jack bean (b), tomato (c) and tomato + jack bean after 14 days of growth

According to the type of enzyme evaluated, the hotpots and rhizosphere showed different results (Figure 20A and C). The chitinase hotpots in the tomato and jack bean single crop were higher than tomato + jack bean and soil treatment (Figure 20C). On the other hand, the β-glucosidase and phosphatase hotpots were higher in the jack bean single crop than soil and tomato single crop, and they did not show a difference between soil and tomato single crop treatment. However, the β-glucosidase and phosphatase hotpots in the tomato + jack bean treatment did not differ among jack bean and tomato single crop ($p > 0.05$); thus, its hotpots were in the middle of the hotpot's percentage of treatment jack bean and tomato.

The rhizosphere in the tomato + jack bean treatment was higher than jack bean and tomato single for chitinase enzyme ($p < 0.05$) (Figure 21A). For phosphatase, the rhizosphere was higher in the tomato + jack bean than single tomato crop. Although the rhizosphere for β-glucosidase did not differ among the treatments, it is observed that numerically the rhizosphere average was higher in the tomato + jack bean than single jack bean and tomato crop.

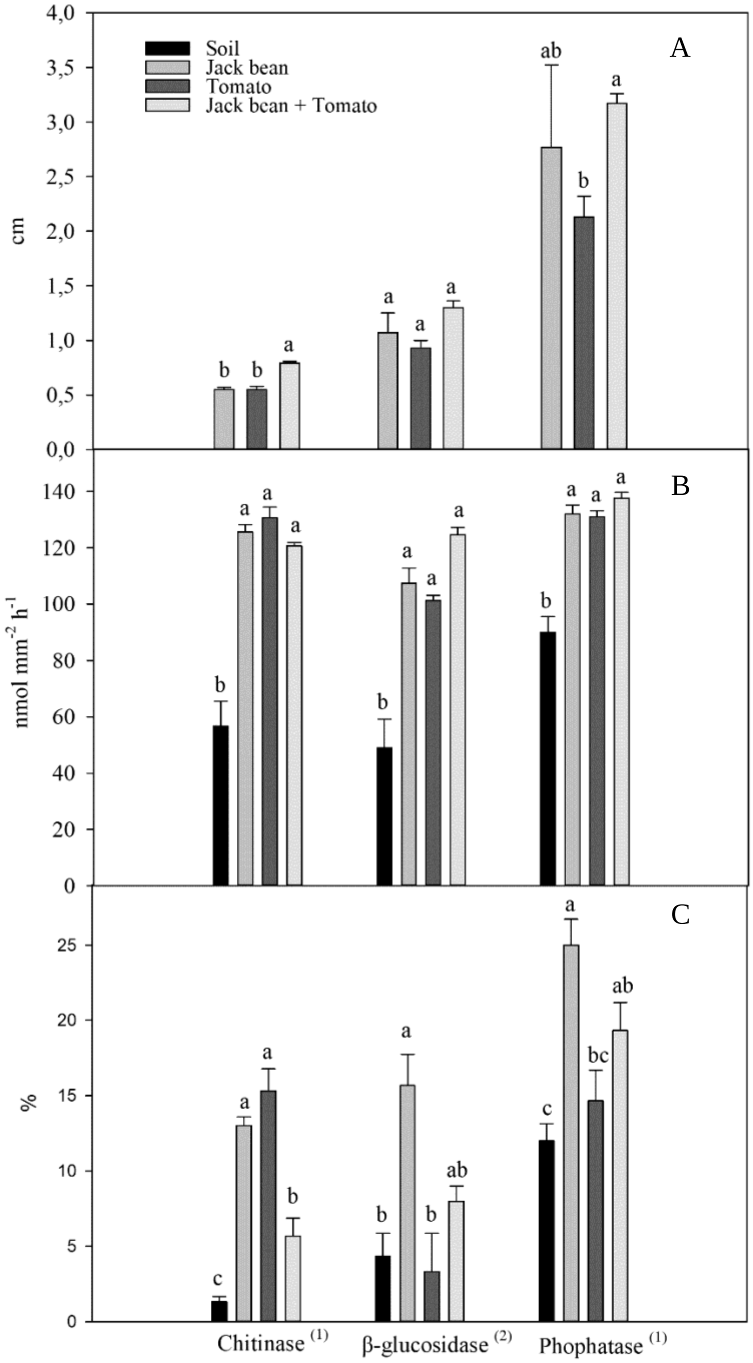

Figure 21. Rhizosphere (A), enzyme activity (B) and hotpots (A) of chitinase, β-glucosidase and phosphatase enzymes. The means followed by the same lowercase letter do not differ by the Tukey ($p < 0.05$). For hotpots data, (1) Statistic for transformed data for log(x) and (2) $\sqrt{x}$

7.4. Discussion

The intercropping system is the growth of two or more crops in the same area at the same time increasing the use efficiency of land and environmental resources such as water, light, and nutrients (BEDOUSSAC et al., 2015; LITHOURGIDIS et al., 2011). In this system can occur three types of plant-plant interaction in the intercropping system: (a) competition, when one resource became limited, (b) complementary, when the plants did not compete for the same resource in the time and space, and (b) facilitation, when the modification of the environment is beneficial for one species at least (BEUDOSSAC et al., 2015). There is a balance between them that are influenced soil nutrients concentration, environmental condition, species companion, and the yield is one ways to assess whether competitiveness between plants is taking place (BEDOUSSAC et al., 2015; DUCHENE; VIAN; CELETTE, 2017).

The tomato + jack bean treatment has not indicated interspecific competition among the plants in the rhizotron because they did not differ the dry mass of plants aerial part in monoculture. Similar results were observed by Salgado et al. (2020) in which the intercropping of cherry tomato and jack bean in the field also did not indicate competition among these species. However, the difference in the root's development of tomato and jack bean is due to planting in the rhizotron. The jack bean was transferred to rhizotron seven days after the germination, and the tomato was transferred as a seedling. We choose this way because the jack bean grows and develops faster than tomato, beyond simulating the management carried out in the field (section 2.2).

The soil enzymes are necessary for the decomposition of organic matter and nutrient cycling; thus, they influence soil fertility and agronomic yield (RAO et al., 2017). The enzymatic activity of soil is influenced by different biotic and abiotic factors and interactions between plants and microorganisms (GEA et al., 2017). The roots, during their growth and development, release exudates and other rhizodeposits that stimulate and increase the activity of microorganisms and the synthesis of extracellular enzymes (RAZAVI et al., 2016; KUZYAKOV; BLAGODATSKAYA, 2015), a factor in which the rhizotron with plants had more enzyme activity than bulk soil (Figure 21B).

The factors that can affect the enzyme's activity were agronomic management, plants species, soil organic matter, for example. Although the treatments with plants did not show the difference in enzyme activity (Figure 21B), Taschen et al. (2017) demonstrated that intercropping between legume and the non-legume plants can modify the bacterial community in the rhizosphere compared to the same species in monoculture, suggesting a synergic interaction in the mix rhizosphere plant. Thus, this synergic integration in the tomato + jack bean altered the spatial pattern distribution of the enzyme activity compared to the single tomato and jack bean crop (Figures 18, 19, 20). Moreover, the enzyme activity distribution can be modified with the growth of the plants and with the morphology of the roots (MA et al., 2018). Razavi et al. (2016) showed the spatial distribution of enzymes activity (phosphatase, β-glucosidase, leucine-aminopeptidase, and cellobiohydrolase) in the lentil was uniform and homogeneous along with the roots, on the other hand, the maize has had higher the apical and proximal parts of the roots.

The hotpots are small soil volume that has a high microbial process rate compare with the average soil condition, thereby they are controlled by factors that limiting the microbial activity mainly the availability of carbon and other nutrients (GEA et al., 2017; KUZYAKOV; BLAGODATSKAYA, 2015). Therefore, the reduction of hotpots in the tomato + jack bean treatment compared with jack bean treatment could be showing competition between the roots and the soil microbial. Corroborating to the rhizosphere data (Figure 21A). The rhizosphere was bigger in the treatment of tomato + jack bean than single tomato crop for the enzyme chitinase and phosphatase that shows that the roots need extent the area of enzyme activity in the rhizosphere to acquire the nutrient like P and N (KUZYAKOV; RAZAVI, 2019; GEA et al., 2017).

According to Kuzyakov and Razavi (2019) the maximum extent rhizosphere is shown by the enzyme of the most limited nutrient cycle for plant nutrition that normally is P following by N and C. For the currently study the rhizosphere, size in this study was higher in the phosphatase (1.9-3.3 mm) followed by β-glucosidase (0.8-1.4 mm) and chitinase (0.5-0.8 mm).

7.5. Conclusion

The jack bean, tomato, and jack bean + tomato treatments showed different enzymatic spatial distribution. Moreover, the growth of tomato and jack bean on the same rhizotron induced the combination of the enzymatic distribution patterns of the two plants' roots.

The tomato + jack bean treatment was not increased the enzyme activity compared to tomato and jack bean single crop treatment.

The reduction of hotspots in the tomato + jack bean treatment compared with jack bean treatment can be showing competition between the roots and the soil microbial. Corroborating to the data of the rhizosphere that was bigger in the single tomato and jack bean for the enzyme chitinase and phosphatase.

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8 CONCLUSION

In the first year (2011), the weights of total and marketable tomato fruit (weight of total yield and of individual fruit) were lower in the treatments where cherry tomatoes were intercropped with white lupine or sun hemp, compared with the controls, indicating interspecific competition between the cherry tomatoes and these legumes. Although there were no indications of competition between the crops in 2012, it was recommended that white lupine and the sun hemp should be used as intercrop green manures with some caution, due to the high dry biomass yields produced in both years by these green manures.

The jack beans were highlighted as a promising green manure for this cropping system due to the high yield and nutrients accumulated by this legume and as there were no indications of competition between the jack beans and the cherry tomatoes.

No treatment affected the yield of green corn grown in the rotation, but the cropping system by itself was not thought to have supplied sufficient nutrients to meet the demand of the successive green corn crops.

The BNF was responsible for more than half of the N accumulated in the legumes.

The N of legumes was transferred to cherry tomato in similar quantities, and the leaves and fruits of cherry tomato received more N transfer than shoots. It was shown that N transfer increases with the growth/development of cherry tomato.

The intercropping system with legumes did not affect the ^{15}N natural abundance of leaves and the aboveground biomass of green corn cultivated in succession. The legume in an intercropping system with cherry tomato cultivated in the succession of green corn does not provide sufficient nitrogen to supply the green corn demand.

The residual green corn straw under cherry tomato with different legumes (source of N) in an intercropping system did not increase the straw decomposition rate and the release of the C and N from the straw decomposition.

The C: N ratio declined gradually over the days of the decomposition, considering the maintenance of the C concentration and the increase of the N concentration during the decomposition time, independently of treatment.

The $\delta^{13}C$ decreased in the green corn straw and the $\delta^{15}N$ tended to increase during the decomposition time.

The organic system of cherry tomato intercropping with green manures in succession to green corn reduced the soil fertility because it reduced the SOM and consequently the S and P concentration and the CEC, beyond the reduced concentration of same other nutrients.

Therefore, this system demands nutrients from the soil faster than the crop residues can release through decomposition. However, the N and C total stock in the soil increased over the years showing the cropping system capacity to sequestration C and N that will compose the SOM.

The nitrogen was a limiting factor for the increase in yield in this cropping system, mainly due to the crop repetition. The crops demand nutrients faster than the soil microorganisms are able to cycle the nutrients contained in the plant residue, which leads to a reduction in soil organic matter, capacity exchange cations and O and S content.

The organic system with cherry tomato and legumes in an intercropping system and the green corn in succession was high profitability under the conditions in which this study was conducted.

The jack bean, tomato, and jack bean + tomato treatments showed different enzymatic spatial distribution. Moreover, the growth of tomato and jack bean on the same rhizotron induced the combination of the enzymatic distribution patterns of the two plants' roots.

The tomato + jack bean treatment was not increased the enzyme activity compared to tomato and jack bean single crop treatment.

The reduction of hotspots in the tomato + jack bean treatment compared with jack bean treatment can be showing competition between the roots and the soil microbial. Corroborating to the data of the rhizosphere that was bigger in the single tomato and jack bean for the enzyme chitinase and phosphatase.