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N transfer from green manures to lettuce in an intercropping cultivation system

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ABSTRACT. The aim of this work was to evaluate the transfer of N via biological nitrogen fixation from green manures to intercropped lettuce using the ^{15}N abundance method. The treatments consisted of lettuce with black oats, lettuce with cowpea, lettuce with white lupin and monocropped lettuce (control). Green manures were sown at two different times including the following: 60 days before transplanting of lettuce seedlings and simultaneous to transplanting of lettuce seedlings. N transfer was estimated using the natural ^{15}N abundance method. It was observed that lettuce intercropped with green manures reached the same fresh mass of monocropped lettuce when the green manures were sown at the same time as the vegetables were planted. However, when the green manures were sown previously, the fresh mass of the monocropped lettuce was higher, and independent of the sowing time of green manures, the monocropped lettuce reached a higher dry mass. White lupin and cowpea transferred higher amounts of N regardless of sowing time, and the C:N ratio of white lupin to cowpea was lower compared to black oats. Intercropping with white lupin, cowpea and black oat transferred 18, 17 and 7% of N, respectively, to lettuce.

Keywords: *Avena strigosa* Schreb, *Lupinus albus* L., *Lactuca sativa* L., *Vigna unguiculata* (L.) Walp., natural ^{15}N abundance.

RESUMO. Transferência de nitrogênio de adubos verdes para a alface cultivados em sistema de consórcio. O objetivo deste trabalho foi estudar a transferência do nitrogênio de adubos verdes para alface em cultivo consorciado, através da técnica da abundância natural de ^{15}N. Os tratamentos foram: alface em monocultivo (testemunha), alface consorciado com aveia-preta, com feijão-caupi e com tremoço-branco, em duas épocas de semeadura dos adubos verdes: 60 dias antes e no transplante das mudas de alface. Observou-se que a alface consorciada com adubos verdes adquiriu a mesma massa fresca das alfaces solteiras quando a semeadura do adubo verde foi simultânea com o plantio da mesma; no entanto quando os adubos verdes foram semeados com 60 dias de antecedência as alfaces solteiras obtiveram maior massa fresca ; independentemente da época de semeadura as alfaces solteiras alcançaram maior massa seca; o tremoço-branco e o caupi transferiram maior quantidade de N para a alface, independentemente de sua época de semeadura; a relação C:N do feijão-caupi e tremoço-branco foram menores que a da aveia-preta; o cultivo consorciado de tremoço-branco, caupi e a aveia-preta transferiram 18, 17 e 7% de N para a alface; e que em cultivo consorciado há transferência de N proveniente da fixação biológica do nitrogênio (FBN) dos adubos verdes tremoço-branco e caupi para a alface.

Palavras-chave: *Avena strigosa* Schreb, *Lupinus albus* L., *Lactuca sativa* L., *Vigna unguiculata* (L.) Walp., abundância natural de ^{15}N.

Introduction

In Brazil, leaf vegetable production is mainly performed by small farmers (COSTA; SALA, 2005). For such farmers, especially those who practice alternative agriculture, the increase and maintenance of soil fertility at low input and low energetic and economic cost is a difficult task. On the other hand, in the tropics soils it is constantly necessary to make use of different fertilizing techniques to minimize soil exhaustion and maintain an adequate level of nutrients.

Green manuring consists of using plants in rotation, using plants in succession or in intercropping with cash crops. The green manures can be incorporated or used as mulch, which aims to protect soil as well as improve its physical, chemical and biological characteristics (COSTA, 1992). Green manuring is an important tool to increase soil fertility with an emphasis on N fixation and supply, and improves the sustainability of production systems at a relatively low cost (COBO et al., 2002a and b; FAGERIA; BALIGAR, 2005).

Any amount N fixed by a legume can become a nutrient source for the next crop. According to Giller and Wilson (2001), there are two ways through which N from one plant can become available to other plants including the following: (1) from a plants below ground; through the senescence of roots and nodules, root exudates and direct transfer between roots by mycorrhizal connexions, and (2) from plants above ground; through the decomposition of leaves and other plant residues.

Castro et al. (2004) emphasized that fixed N becomes available as soon as the legume is cut in an intercropping system through excretion of root N compounds and decomposition of root nodules but mainly by the decomposition and release of nutrients above ground after it is cut. However, the availability of N depends on the mineralization rate, and its synchronization with nutrient demand and uptake rate of the cash crop are key for the maximization of green manuring benefits. Ambrosano et al. (2009), using stable isotope-labeled techniques, observed the utilization of up to 35% of N from legumes by corn crops, and Giller and Wilson (2001) observed that 40% or more of N is available after two weeks of incorporation of new branches of legumes into the soil, which could result in N waste if the main crop does not reach its higher nutrient demand within two weeks time. Synchronization of the rate of N release by green manure with cash crop demand is an important management strategy (WIVSTAD, 1999); thus, the use of green manures as mulch makes the mineralization process slower, and the chance of synchronization with the demand of the cash crop is higher.

Several studies have examined the use of green manuring in succession or rotation (MOGOR; ARAUJO CAMARA, 2009; NOSSE et al., 2008); however, these techniques imply the cultivation of a non-cash crop probably without immediate economic revenue, which constitutes a problem for small farmers who need to cultivate their land intensively. Intercropping between vegetables and green manures could be an alternative to succeeding or rotation cropping. Although intercropping between two or more cash crops is common and has been well studied, intercropping between cash crops and green manures is still under investigation.

The natural ^{15}N abundance technique ($\delta^{15}N$) measures the amount of N_2 fixed by plants, and this method has been used successfully in studies of N transfer (SIERRA et al., 2007). The method relies on the comparison between low soil mineral N and atmospheric N_2 resulting from the isotope fractionation between ^{14}N and ^{15}N that occurs during physical, chemical and biological processes that involve N from soil and organic matter (SHEARER; KOHL, 1986). A non-N-fixing plant would have a ^{15}N composition similar to the amount of N available in the soil, while an N-fixing plant would have lower levels of ^{15}N (negative values or nearly zero), according to Shearer and Kohl (1986). This observation may occur because of an isotope abundance dilution effect by the N absorbed from the soil from the N fixed in the atmosphere; also, fractioning in the N_2 fixation can result in negative values of $\delta^{15}N$. Thus, using a non-N-fixing plant as a marker of ^{15}N from soil mineral N, the amount of fixed N can be calculated from the proportion that the ^{15}N was diluted.

White lupins (*Lupinus albus* L.) and black oats (*Avena strigosa* Schreb.) are frequently cultivated in southern Brazil as green manures, cover crops and forage (COSTA, 1992), whereas Cowpea [*Vigna unguiculata* (L.) Walp.] is often cultivated in the northeastern area of Brazil and in several tropical and subtropical regions (FERY, 2002; STEELE et al., 1985). Lettuce (*Lactuca sativa* L.) is a vegetable which demands high amounts of N in a short period of time (KATAYAMA, 1993). Despite several existing studies concerning the potential of biological N fixation (BNF) of legumes (BECKER et al., 1995; CHOI et al., 2008; FAGERIA, 2007; PERIN et al., 2004), there is a lack of research about the contribution of green manures on N transfer in intercropping systems (SILVA et al., 2008). Thus, the goal of this study was to evaluate the transfer of N through biological nitrogen fixation from green manures to intercropped lettuce using the ^{15}N abundance method.

Material and methods

The experiment was carried out at the Polo Regional Centro Sul from the Agencia Paulista de Tecnología dos Agronegócios (APTA) in Piracicaba, São Paulo State, Brazil (22°42'S, 47°38'W; altitude 560 m above sea level) between July and November 2005.

The soil on the site is classified as a Paleudalf and was chemically characterized after lettuce plants were harvested at depth (0-20 cm): pH ($CaCl_2$) 5.35; M.O. 34.75 g dm^{-3}; P 25.50 mg dm^{-3}; S-SO_4 3.75 mg dm^{-3}; K 5.5 $mmol_c$ dm^{-3}; Ca 55.25 $mmol_c$ dm^{-3}; Mg 25 $mmol_c$ dm^{-3}; H+Al 32 $mmol_c$ dm^{-3}; SB 85.8 $mmol_c$ dm^{-3}; CTC 117.7 $mmol_c$ dm^{-3}; V% 72.7; B, 0.42 mg dm^{-3}; Cu 4.5 mg dm^{-3}; Fe 51 mg dm^{-3}; Mn 45 mg dm^{-3}; Zn 3.1 mg dm^{-3}. Total rainfall during the experimental period was 436 mm, and the temperature ranged from a minimum 11.4°C to a maximum 30.4°C with a mean of 21.9°C.

The experimental plot was arranged in a randomized block design using split plots and six replications of each treatment. The main plots were the green manure's sowing dates for the lettuce transplant (DAT) (with treatment 0 consisting of the simultaneous planting of green manures and lettuce, and treatment 60 consisting of the sowing of green manures 60 days prior to lettuce transplant), and the sub-plots consisted of the cultivated crops (monocropping and intercropping lettuce with the green manures black oat [*Avena strigosa* Schreb] cv. comum, cowpea [*Vigna unguiculata* (L.) Walp.] cv. IPA-206 and white lupin [*Lupinus albus* L.] cv. comum). The sub-plots measured 1.80 x 1.20 m.

Field plots were established on July 22, 2005 with the sowing of the first green manures corresponding to treatment 60 days before transplant (DBT) of lettuce. The lettuce cultivar adopted was Piraroxa, and the seedlings were sown in polyethylene trays of 288 cells with commercial substrate. The seedlings were grown in a greenhouse with automatic irrigation. Twenty-eight days after sowing, the lettuce seedlings were transplanted to the field plots. No fertilizing, liming or inoculation was performed during the experimental period.

The intercropping was planted in an additive design; therefore, with the same number of plants, each crop would have a respective single cultivation. The green manures were sown in three rows spaced 0.3 m from each other, and the sowing densities were 68 seeds m^{-1} for black oat (1.5 cm apart), 15 seeds m^{-1} for cowpea (6.6 cm apart) and 15 seeds m^{-1} for white lupin (6.6 cm apart). The lettuce seedlings were transplanted at 0.3 x 0.3 m spacing with a total of 24 plants per plot. When in intercropping, the vegetables were transplanted between the green manure rows.

Automatic drip irrigation was used twice a day before lettuce transplant (for irrigating the 60 DBT treatments) and three times a day after transplant for the rest of the growth cycle. The experimental area was periodically weeded by hand.

According to Portes (1984), light is the main biotic factor for the success of an intercropping system. Thus, to avoid shading of lettuce plants, and consequently sunlight competition, the green manures were periodically mowed at 0.20 m from the soil. The 60 DBT green manure plots were mowed once a day before lettuce transplanting because their biomass could negatively influence the vegetables' development in the shade. During the experimental period, a total of four mowings were performed in the 60 DBT treatments and two for the 0 DBT (simultaneous planting). Fresh weight of aboveground mass was determined, and 10% of this material was separated for dry weight determination. The remaining fresh mass was laid on the soil surface as mulch.

Lettuce plants were harvested 49 days after transplanting on 11/9/2005. Four, central lettuce plants were harvested per plot. The measured parameters were fresh and dry weight of green manures, C:N ratio of green manures, % of N fixed by the green manures, fresh and dry mass of the lettuce, content of N in lettuce plants, amount of N transferred from green manures to lettuce and $\delta^{15}N$. The measurement of the green manures' fresh and dry weight was made after each mowing.

The measurements of fresh and dry mass of the sampled plants were made at the laboratory of the Polo Regional Centro Sul from the Agencia Paulista de Tecnologia dos Agronegócios (APTA). The samples were dried in a forced-air oven at 65°C until constant mass and grinded in a Willey grinder with sieve mesh 4. At the Stables Isotope Laboratory from CENA/USP (Centro de Energia Nuclear na Agricultura da Universidade de São Paulo), between 5 and 10 g of sample from each treatment was weighed in a precision balance. The samples were placed in tin capsules for $\delta^{15}N$ analysis in the mass spectrometer SERCON Co., U.K., model 20-20 with automatic analyzer ANCA GSL at the Laboratory of Soil Fertility CENA/USP.

The natural abundance of ^{15}N technique (SHEARER; KOHL, 1986) was used for determination of NBF contribution of legumes, and the determination of N accumulated was made with the mass spectrometer from the Stables Isotope Laboratory of CENA/USP. For quantification of biological nitrogen fixation (BNF), the following equation was used: $\%BNF = 100\ [\delta^{15}N_{control} - \delta^{15}N_{legume})/(\delta^{15}N_{legume} - B)]$, in which: % BNF: amount of N in % originating from BNF, $\delta^{15}N_{control}$ amount of ^{15}N, in ‰ originating from control plant (non-N-fixing), $\delta^{15}N_{legume}$: amount of ^{15}N, in ‰, originating from the N-fixing plant; and B: isotopic discrimination value of ^{15}N from the N-fixing plant without the presence of N in the soil. The value of

B for white lupin was -1.16 and for cowpea it was -1.48 according to data from Boddey et al. (2000) and Nguluu et al. (2002).

The following equation was used for measuring the N transferred from green manures to lettuce: $\%N_{Tgml} = 100\ [\delta^{15}N_{single\ lettuce} - \delta^{15}N_{lettuce\ +\ green\ manure}/\delta^{15}N_{single\ lettuce}]$, in which: $\%N_{Tgml}$: amount of N of lettuces provided by the green manures; $\delta^{15}N_{single\ lettuce}$, amount of ^{15}N, in ‰, from lettuce in monocropping; $\delta^{15}N_{lettuce\ +green\ manure}$, amount of ^{15}N in ‰ from lettuce intercropped with green manures (in this case the monocropped lettuces were considered a control, according to Boddey et al. (2000)). Thus, the biological N fixation was estimated comparing the natural ^{15}N abundance in the legumes and in the black-oats, which is a non-N-fixing plant.

The F test and Tukey at 5% of probability were used for Comparison between means of sowing times and cultivation systems. To satisfy the assumptions of the analysis of variance, the data referring to fresh mass of green manures (FMGM) and dry mass of green manures (DMGM) were transformed by ln (*x*). All data were analyzed through analysis of variance using the program SANEST (ZONTA; MACHADO, 1984).

Results and discussion

The fresh and dry biomass yields varied according to the time of establishment of the intercropping as shown in Table 1. Cowpea had a significantly higher yield compared to black oat and white lupin given that these did not differ in fresh mass. In terms of dry mass, cowpea yielded 445 and 5,645 kg ha^{-1}, respectively for the treatments 0 and 60 DBT, white lupin yielded 158 and 2,621 kg ha^{-1} and black oats 136 and 2,043 kg ha^{-1}. These results are inferior to those reported by Giacomini et al. (2003), Borkert et al. (2003) and Queiroga et al. (2002).

The C:N ratio of the green manures was not affected by sowing time (Table 1). As expected, black oat showed higher values of C:N compared to the two legumes. These results are in accordance with those of Yoneiama and Ohtani (1983), Fowler et al. (2004) and Pypers et al. (2005) who compared the C:N ratio of legumes and Poaceae grasses. However, Giacomini et al. (2003) reported C:N values three times higher for black oats regarding common vetch (*Vicia sativa*). The probable reason for the low C:N ratio of black oats in this study, compared to other literature reports, is that during the experiment, the temperature in spring and summer was high and is considered harmful to black oat development, resulting in a lower C accumulation (BARRADAS et al., 2001). The legumes cowpea and white lupin presented a similar C:N ratio to that reported by Clement et al. (1998) who evaluated this parameter for several green manures.

Table 1. Fresh mass, dry mass, C:N relation of the above ground part of the green manures, total N content and N fixed by the legumes.

	Treatments			
Sowing time	Black oats	Cowpea	White lupin	SEM**
DBT*	--Fresh mass (kg ha^{-1}) --			
0	478	3850	1100	1800 ± 862 b
60	11073	49477	20143	26897 ± 862 a
Mean	5775 C	26570 A	10622 B	
SEM**	1051	1051	1051	
CV% treatments: 4.2; CV% sowing time: 5.2				
DBT*	--Dry mass (kg ha^{-1})--			
0	135.74	447.31	158.19	247.07 ± 214.67 b
60	2042.50	5644.58	2620.69	3435.93 ± 214.67 a
Mean	1089.12 B	3045.97 A	1389.44 B	
SEM**	371.85	371.85	371.85	
CV% treatments: 4.8; CV% sowing time: 6.7				
DBT*	-- C:N relation --			
0	-	10.6 (6)	10.9 (4)	10.7 ± 1.7 (10) a
60	17.3 (5)	11.7 (2)	10.5 (4)	13.8 ± 3.7 (11) a
Mean	17.3 (5) B	10.9 (8) A	10.7 (8) A	
SEM**	2.1	0.8	2.0	
CV% treatments: 8.11; CV% sowing time: 23.76				
DBT*	-- Total N content (%) --			
0	-	4.0 (6)	4.1 (4)	4.1 ± 0.7 (10) a
60	2.5 (5)	3.8 (2)	4.2 (4)	3.3 ± 0.9 (11) a
Mean	2.5 (5) B	4.0 (8) A	4.2 (8) A	
SEM**	0.3	0.3	0.8	
CV% treatments: 13.8; CV% sowing time: 16.27				
DBT*	-- N fixed by legumes (kg ha^{-1}) --			
0	-	6.2	3.7	4.7 ± 2.1 b
60	-	80.9	69.2	71.3 ± 3.2 a
Mean		41.1 A	34.9 B	
SEM**		2.2	2.4	
CV% 29.52				

Means followed by different letters, lower case in columns and upper case in rows, indicate significant differences, by F and Tukey tests ($p < 0.05$), respectively. The values between brackets refer to number of observations. *Number of days of green manure sowing before lettuce transplant. ** Standard error of the mean.

Cowpea produced a higher amount of fresh and dry mass compared to white lupin. While cowpea reached values higher than 80 kg ha^{-1}, white lupin had an average mass of 69 kg ha^{-1} (Table 1). Castro et al. (2004) found around 60 kg ha^{-1} of N in cowpea intercropped with eggplant, and Ofori et al. (1987) reported from 32 to 87 kg ha^{-1} in monocropped cowpea and from 18 to 58 kg ha^{-1} in intercropped cowpea given that the percentage of N fixed varied from 72 to 42%. The amount of N fixed by white lupin in this study was significantly inferior to the results published by Fragstein (1995) that reported 541 kg ha^{-1} of N fixed by this legume.

No difference was seen between the amounts of total N regarding the sowing times of green manures; however, the legumes reached higher values of total N fixation compared to black oats (Table 1). The warm and rainy climate during the experiment favored the rapid decomposition of the residues.

Barradas et al. (2001) found similar results to those in this study regarding the total N in white lupin and black oat. The higher accumulation of total N in the legume plants occurs because they have the ability to fix N. The lower amounts of N accumulated at 0 DBT are due to the relatively low development of the green manures, which had been sown 49 DBT, compared to the 60 DBT, and thus accumulated more biomass.

Intercropping legume and lettuce is a promising biological strategy to achieve and to keep N in the production system under tropical conditions.

The green manure sowing time regarding lettuce planting did not influence the accumulation of fresh mass in the vegetables. However, the monocropped lettuce accumulated a higher amount of fresh mass compared to the intercropped lettuce for the treatment 60 DBT (Table 2). For the simultaneous planting (0 DBT) plot, the amount of fresh mass produced by lettuce was similar in all treatments because it reached classification 15 according to the CEASA (2011) standard. Thus, the simultaneous planting does not affect the commercial production of lettuce intercropped with green manures. These results agree with data from Costa et al. (2003, 2007) and Rezende et al. (2006), which involved studying the behavior of lettuce intercropped with other vegetables. In the 60 DBT treatments, there was competition between crops because the green manures had a considerable amount of biomass when the lettuce seedlings were transplanted. In this treatment the green manures dominated the lettuces, which affected production and resulted in a lower yield. Paula et al. (2005) observed the same effect of yield variation and transplanting time in an intercropping between onion and lettuce.

The dry mass of the intercropped lettuce was considerably lower than the monocropped lettuce; intercroppings with black oat, cowpea and with lupin yielded 74, 67, and 74%, respectively, of the monocropped control. No influence was observed on sowing time in green manures by the accumulation of dry mass of lettuce (Table 2).

The content of N in lettuce plants was not affected under simultaneous planting (0 DBT); however, in the 60 DBT, lettuce intercropped with cowpea had higher amounts of N compared to the monocropped lettuce or to intercropped with white lupin (Table 3). This effect is probably due to the lower C:N ratio and higher biomass of cowpea (Table 1) compared to the other green manures. Cowpea mulch was laid in the plots and, thus, released higher amounts of N to the intercropped lettuce plants. These results are in accordance with those of Sorensen and Thorup-Kristensen (2003) who studied succession of lettuce after green manures and found that the control plots (without green manures) had a lower amount of N compared to those with previous green manure cultivation.

Regarding N accumulated per lettuce plant, there was no influence of green manure on sowing time; however, the monocropped lettuce (control) accumulated a higher amount of N compared to the intercropped (Table 3). These results show that the monocropped lettuce accumulated more biomass compared to the intercropped once it grew with no competition from another crop (Table 2). The competition in an intercropping system is not only for light but also for nutrients and water. Sometimes there is more than one factor of competition simultaneously occurring, and one source of competition can lead to another. However, intercropping light is usually the most problematic abiotic factor (PORTES, 1984).

Monocropped lettuce and those intercropped with black oat had a higher $\delta^{15}N$ at 0 DBT than the lettuce intercropped with white lupin, although $\delta^{15}N$ was similar to those intercropped with cowpea (Table 3). When the green manures were sown 60 days before lettuce transplanting, both monocropped plants and those intercropped with black oats had a higher value of $\delta^{15}N$ compared to the plants intercropped with legumes.

Table 2. Fresh and dry mass of lettuce head.

	Treatments				
Sowing time	Monocropped lettuce	Lettuce with black oats	Lettuce with cowpea	Lettuce with white lupin	SEM**
DBT*	-- fresh mass (g plant^{-1}) --				
0	195Aa	200 Aa	160 Aa	180 Aa	183 ± 14
60	223 Ba	164 Ba	148 Aa	150 Ba	171 ± 14
Mean	209	182	153	165	
SEM**	12	12	12	12	
CV% treatments:7.7; CV% sowing time: 25.0					
DBT*	-- dry mass (g planta^{-1}) --				
0	12.5	10.5	8.7	10.0	10.5 ± 0.6 a
60	12.9	8.3	8.4	8.7	9.6 ± 0.6 a
Mean	12.7 A	9.4 B	8.5 B	9.4 B	
SEM**	0.6	0.6	0.6	0.6	
CV% treatments: 9.5;CV% sowing time: 22.3					

Means followed by different letters, lower case in columns and upper case in rows, indicate significant differences, by F and Tukey tests ($p < 0.05$), respectively. *Number of days of green manure sowing before lettuce transplant. **Standard error of the mean.

Table 3. Nitrogen accumulated in lettuce shoots, amount of nitrogen in shoots, values of δ15N, amounts of N transfered from green manures to lettuce.**

Sowing time	Monocropped lettuce	Lettuce with black oats	Lettuce with cowpea	Lettuce with white lupin	SEM**
			Nitrogen accumulated in lettuce shoots		
DBT*			-- g kg^{-1} --		
0	33.6 Aa	31.6 Ab	30.7 Ab	32.2 Aa	32.0 ± 0.5
60	32.5 Ba	35.2 ABa	39.2 Aa	32.5 Ba	35.1 ± 0.5
Mean	33.0	33.92	34.9	32.4	
SEM***	0.7	0.7	0.7	0.7	
CV% treatments: 5.5; CV% sowing time: 7.2					
			Amounts of nitrogen in lettuce shoots		
			-- g plant^{-1} --		
0	0.4	0.3	0.3	0.3	0.3 ± 0.017 a
60	0.4	0.3	0.3	0.3	0.3 ± 0.017 a
Mean	0.4 A	0.3 B	0.3 B	0.3 B	
SEM***	0.02	0.02	0.02	0.02	
CV% treatments: 11.7; CV% sowing time: 22.5					
			Values of $\delta^{15}N$ in lettuce shoots		
			-- ‰ --		
0	+6.3 Ab	+6.2 Aa	+5.6 ABa	+5.3 Ba	+5.8 ± 0.2
60	+7.4 Aa	+6.3 ABa	+5.4 Ba	+5.4 Ba	+6.1 ± 0.2
Mean	+6.8	+6.22	+5.5	+5.3	
SEM***	0.2	0.2	0.2	0.2	
CV% treatments: 8.84; CV% sowing time: 12.2					
			N transferred from green manures to lettuce		
			-- % --		
0	-	6	15	20	14 ± 3 a
60	-	8	19	15	14 ± 4 a
Mean	-	7 B	17 A	18 A	
SEM***	-	3	3	3	
CV% treatments: 34,5; CV% sowing time: 58,5					
			Amount of N transferred from the green manure for lettuce		
			-- kg ha^{-1} --		
0	-	2.6	4.1	6.4	4.4 ± 1 a
60	-	2.6	6.3	6.1	5.0 ± 2 a
Mean	-	2.6 B	5.2 A	6.25 A	
SEM***	-	1.4	1.3	1.4	
CV% treatments: 31.0; CV% sowing time: 76.0					

Means followed by different letters, lower case in columns and upper case in rows, indicate significant differences, by F and Tukey tests ($p < 0.05$), respectively.* Number of days of green manure sowing before lettuce transplant. **Population of 100,000 lettuce plants per hectare. ***Standard error of the mean.

Thus, comparing the values of $\delta^{15}N$ for the monocropping of lettuce treatments with lettuce intercropped with green manures, it is clear that the legumes had the tendency to provide N to lettuce plants. The legumes contributed more to the transfer of N than black oats, as shown on Table 3, for the amount of N transferred (kg ha^{-1}) and percentage of N transferred from green manures to lettuce. The sowing time of green manures did not affect N transfer.

Despite the high nitrogen content of soils, most N is in not available, and available and non-available forms are not necessarily in equilibrium. In addition, because of N's high mobility in soil in available forms of N, usually there are no direct residual effects of N fertilizers (RAIJ, 1981). Mineralization of organic N of plant material added to the soil is initially fast because of the breakdown of the more easily decomposable components, but it slows down subsequently until stabilization of the organic residue.

Legumes have a higher amount of N and a lower C:N ratio (Table 1) that might have favoured the mineralization. However, even for the Poaceae grass, there was some N transfer due to the mineralization of N from its aboveground portion, which was mowed and laid as mulch in the plots. Silva et al. (2006) observed that sunnhemp (*Crotalaria spectabilis*) had a higher residual effect of N in its mulch than millet (*Pennisetum glaucum*) and, thus, transferred a higher amount to the following corn crop.

As observed in the present study, the C:N ratio of sunnhemp, which is also a legume, is lower than that of millet, therefore the nutrient release through the biomass decomposition is more efficient. Dias et al. (2007) reported that in an intercropping between legume trees and Survenola digitgrass, the transfer of N from the trees to the grass ranged from 0 to 37.7% depending on the distance between crops. N transfer may have occurred mainly via root exudation.

Conclusion

The results of this study indicate that in spite of the competition associated between crops that have a negative effect on lettuce yield, intercropping with white lupin and cowpea contributed significantly to

N transfer (originating from BNF) to lettuce, nutritionally independent of the time of sowing of green manures.

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