Australian Journal of Crop Science
AJCS
AJCS 13(03):465-471 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.03.p1559

Biological fixation, transfer and balance of nitrogen in passion fruit (*Passiflora edulis* Sims) orchard intercropped with different green manure crops

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Abstract

Green manures can replace or supplement mineral fertilization and add organic matter to the soils, ensuring greater sustainability to fruit growing in semiarid regions. Biological fixation, transfer and balance of nitrogen were determined on an irrigated yellow passion fruit orchard (*Passiflora edulis* Sims) intercropped separately with three cover crops: sunn hemp, *Crotalaria juncea* (L.); pigeon pea, *Cajanus cajan* (L.) Mill; and jack bean, *Canavalia ensiformis* (L.) DC. In a fourth treatment, legumes were not planted, but spontaneous vegetation was left to grow freely. The legumes were croped for 90 days in three lines (0.5 m apart) inside the passion fruit plant lines (2.5 m apart). Fixation and transfers were determined by the ^{15}N natural abundance technique, using sunflower as a reference plant. The three planted legumes nodulated abundantly and fixed nitrogen in high proportions (between 50 and 90% of their N), forming symbiosis with bacteria naturally established in the soil. Jack bean produced more biomass than sunn hemp and pigeon pea, and as much as the spontaneous plants, of which 23% were legumes. The amounts of fixed N (150, 43, 30 and 29 kg ha^{-1}) were determined mainly by the biomass of legumes. More than 40% of the N of passion fruit plants came from the biological nitrogen fixation of the intercropped jack bean, which provided an amount of N higher than that exported in the fruits, generating a positive balance of more than 100 kg ha^{-1}. Therefore, it is recommended to intercrop jack bean in irrigated passion fruit orchards.

Keywords: ^{15}N natural abundance, tropical fruit, legume, natural nodulation, rhizobia.
Abbreviations: BNF_biological nitrogen fixation.

Introduction

The cultivation of cover crops, or green manures, intercropped with or preceding the main crop, is a practice used to add organic matter and nitrogen to agricultural systems (Steenwerth and Belina 2008; Ramos et al., 2010; Zotarelli et al., 2012; Ojien et al., 2014), besides providing physical protection to the soil (Snapp et al., 2005; Xavier et al., 2013; Feitosa et al., 2015). Because of the ability to fix atmospheric nitrogen through symbiosis with diazotrophic bacteria, legumes are the species most used as cover crops (Gathumbi et al., 2004; Ojien et al., 2014). Their use in cropping systems can supplement or replace the fertilization with nitrogen fertilizers (Allen et al., 2011; Zotarelli et al., 2012; Tribouillois et al., 2016), which overtax production costs and are dependent on fossil fuels for their manufacture.

The Brazilian semiarid region presents high average annual temperatures and ultraviolet sunlight, factors that lead to rapid degradation of plant debris and soil organic matter (Parton et al., 2007, King et al., 2012). In irrigated crops, increased soil water availability can further accelerate decomposition rates and nutrient losses (Lee et al., 2014), leading to rapid loss of the soil productive capacity. Therefore, green manuring is a practice that has been recommended for irrigated crops as an alternative to ensure the maintenance of the soil productive capacity in the region (Pimentel et al., 2011; Xavier et al., 2013; Feitosa et al., 2015; Mouco et al., 2015; Pereira Filho et al., 2016).

However, information on the quantities of biomass and N that can be simbiotically fixed by different legume species that can be used as green manure in the region is extremely scarce. Some species are commonly used elsewhere (Gathumbi et al., 2004; Perin et al., 2006; Xavier et al., 2013; Ojien et al., 2014), such as sunn hemp (*Crotalaria* spp.), pigeon pea (*Cajanus cajan* (L.) Mill) and jack bean (*Canavalia ensiformis* (L.) DC.). However, they are not naturally occurring in the Brazilian semiarid region (Queiroz 2009). On the other hand, the practice of inoculation with rhizobia is rare. It is not known whether the soils of the region are home to rhizobia populations capable of forming symbiotic nodules in these legumes. This information is important for the establishment of a proper crop management with green manures, as they subsidize the choice of species with potential to promote a more favorable nutrient balance to the system, in addition to defining the need for inoculation with previously selected bacteria.

Fruit growing is an activity with potential to ensure a higher income for producers of the Brazilian semiarid region than corn and bean crops, being promising for areas with water availability for irrigation. Strong sunlight and high temperatures provide better resources to tropical fruits to create high yields. Furthermore, the semiarid climate is an advantage in the phytosanitary aspect of fruits. Among the main fruit species produced in the region, passion fruit is grown on small farms, mostly in the context of family farming. It is a culture that leaves the ground little covered between the planting rows and provides little biomass to the soil, but there is little information on green manuring of passion fruit in the region.

Thus, the aim of this study was to estimate biological fixation, transfer and balance of N in an irrigated passion fruit orchard intercropped with different green manure legume species, in the semiarid region of Brazil. The ultimate objective was to find a legume species that fix enough nitrogen from the atmosphere and transfer it to the passion fruit plants to render mineral fertilization unnecessary.

Results and discussion

Biomass production and N accumulation

Jack bean produced more biomass than pigeon pea and sunn hemp, both in the evaluation at 45 days and at 90 days after sowing. However, the difference in relation to the biomass produced by spontaneous plants in the treatment without legume planting was not significant (Table 1). The yield of pigeon pea and sunn hemp was equivalent to the biomass of spontaneous legumes. These spontaneous legumes accounted for 23% of the total biomass produced by all spontaneous plants. The N contents of three planted legumes were always high (Table 1). At 45 days after sowing, jack bean accumulated more than triple the amount of N accumulated in the other two species (Table 1). At 90 days, jack bean increased its biomass production more than 2.5 times the value of the initial assessment, providing the accumulation of more than double the amount of N accumulated in the spontaneous plants. As spontaneous legumes also had high concentrations of N in their tissues, the amounts of N accumulated in weeds were similar to those accumulated by pigeon pea and sunn hemp (Table 1).

Nodulation and BNF

Symbiotic nodules were observed in the roots of the three planted legumes and the nodulation was the most abundant (higher number of nodules per plant) in jack bean (Table 2). Sunn hemp had the largest nodules, with an average biomass equivalent to twice the biomass of the nodules of the other two species (Table 2). The jack bean nodules were small but abundant assisting plants to reach a biomass over 70 kg ha^{-1}, nearly four times the biomass of pigeon pea and sunn hemp nodules. The $\delta^{15}N$ signals of all legume plants were always significantly lower than those of the reference species in the two evaluation periods and BNF were able to provide more than half of the N required for legumes at 45 days, and more than 80% at 90 days, including spontaneous legumes, with no difference between species (Table 2).

The largest amount of N added to the system via BNF came from jack bean, reaching over 65 kg ha^{-1} at 45 days and 158 kg ha^{-1} at 90 days (Table 2). The atmospheric N contribution in pigeon pea or sunn hemp cultivation was not greater than the contributions when the plants were allowed to grow naturally.

Transfer of fixed N, passion fruit yield and N balance

At 90 days after sowing, the leaves of passion fruit plants intercropped with jack bean had a significant isotopic depletion of more than 5 ‰, compared to those grown with sunflower, in which approximately 40% of the N of the passion fruit plants came from N fixed by the jack bean (Table 3). No significant differences were observed between the leaf $\delta^{15}N$ values of the passion fruit intercropped with sunn hemp or pigeon pea, or grown in plots only with spontaneous plants, and the values of the passion fruit grown with sunflower.

The average fruit yield of passion fruit during the first year of cultivation was 17.4 t ha^{-1} yr^{-1}, with no difference among species used as green manure, equivalent to an export of 49 kg ha^{-1} yr^{-1} of N. The cultivation of jack bean alone contributed to greater amount of N than that exported to the fruits, generating a positive balance of more than 16 and 109 kg ha^{-1}, when the legume was cut at 45 and 90 days, respectively. In the case of sunn hemp, the N balance was slightly negative, but with pigeon pea or spontaneous legumes, the export of N from the orchard resulted in a deficit of around 20 kg ha^{-1} N.

Spontaneous legumes growing between the passion fruit plants in this irrigated orchard accounted for 23% of the total biomass produced by all spontaneous plants, in spite of herbaceous legumes usually representing small proportions of plant biomass in native caatinga vegetation and in non-irrigated pastures established in former caatinga areas (Freitas et al., 2012a; Moreira et al., 2006). This legume biomass was similar to the biomass produced by the planted sunn hemp and pigeon pea (Table 1), indicating that growth of spontaneous plants is a better option than planting these cover species. On the other hand, jack beans produced much more than both these cover crops and spontaneous plants. It was not possible to separate and identify all species of spontaneous plants, but the isotopic signal of $\delta^{13}C$ of the

Table 1. Aboveground biomass, N concentration (%N) and content (kg ha^{-1}), C/N ratio and $\delta^{13}C$ (‰) of legume species used as cover crops in an irrgated passion fruit orchard in the Agreste region of Pernambuco state, Brazil.

Species/Evaluation date	Biomass	% N	N content	C/N	$\delta^{13}C$ (‰)
45 days after sowing					
Jack beans	1936 a	4.32 a	84.38 a	9.14 a	-26.11 a
Sunn hemp	842 b	3.87 a	27.7 b	11.18 a	-29.17 a
Pigeon pea	527 b	3.32 a	22.45 b	10.74 a	-28.78 a
90 days after sowing					
Jack beans	5152 a	3.74 a	189.26 a	11.14 b	-26.5 b
Sunn hemp	1583 bc	3.33 ab	26.95 b	11.85 b	-30.05 a
Pigeon pea	822 c	4.12 a	66.32 b	11.06 b	-30.03 a
Spontaneous plants	3175 ab	2.58 b	86.03 b	15.11 a	-23.83 c
Spontaneous legumes	744 c	4.16 a	35.54 b	11.00 b	-29.29 a

Averages followed by the same letter, in the same column and evaluation date, are not significantly different by the Tukey's at 5% probability level ($p \leq 0.05$).

Table 2. Nodulation, $\delta^{15}N$ (‰), nitrogen derived from the atmosphere (%Ndfa) and fixed N (kg ha^{-1}) of legume species used as cover crops in an irrgated passion fruit orchard in the Agreste region of Pernambuco state, Brazil.

Species/Evaluation date	Nodulation[(1)]		$\delta^{15}N$ (‰)	%Ndfa	Fixed N (kg ha^{-1})
	Nodule plant^{-1}	Biomass (mg nódulo^{-1})			
45 days after sowing					
Reference species [(2)]			12.39		
Jack bean	139 a	3.61 b	2.62 **	73 a	65 a
Sunn hemp	4 b	8.39 a	4.96 **	55 a	14 b
Pigeon pea	15 b	4.32 b	3.72 **	64 a	14 b
90 days after sowing					
Reference species [(3)]			9.25		
Jack bean	-	-	0.71 **	83 a	158 a
Sunn hemp	-	-	0.59 **	88 a	43 b
Pigeon pea	-	-	0.16 **	84 a	30 b
Spontaneous legumes	-	-	0.06 **	89 a	29 b

[(1)] Evaluation only at 45 dias
[(2)] (sun flower)
[(3)] (sun flower, *Sonchus oleraceus*, *Leonotis nepetifolia* and *Portulaca oleracea*)
** Significantly different from values of the reference plants ($p \leq 0.01$). Averages followed by the same letter, in the same column and evaluation date, are not significantly different by the Tukey at 5% probability level.

Table 3. $\delta^{15}N$ (‰) in leaves of passion fruit plants intercropped with different cover crops and N in the passion fruit plants transferred from symbiotic fixation in these cover crops and in spontaneous legumes in an irrgated passion fruit orchard in the Agreste region of Pernambuco state, Brazil.

Species	$\delta^{15}N$ (‰)	N transferred (%)
Passion fruit plants + sunflower	11.20 a	-
Passion fruit plants + jack beans	6.12 b	44.31
Passion fruit plants + pigeon pea	9.84 a	-
Passion fruit plants + sunn hemp	8.94 ab	-
Passion fruit plants + spontaneous legumes	10.08 a	-
CV(%)	13.34	

AVerages followed by the same letter are not significantly different by the Tuckey at 5% probability level.

mixture of all species, lower than all C3 legumes (Table 2), indicates a biomass contribution of grasses with C4 photosynthetic system (Ludlow et al., 1976). Jack beans discriminated less regarding ^{13}C, resulting in a $\delta^{13}C$ value greater than that of all other legumes (Table 2), which is generally attributed to an increased water use efficiency (Coletta et al., 2004; Maricle at al., 2011).

The N concentrations of the three planted and the spontaneous legumes were all similarly high (Table 1); thus, the amounts of accumulated N reflected mainly the pattern of biomass production, greater for jack beans than the other species. The high N concentrations led to low C/N ratios (Table 1), indicating that the biomass of all species may be easily decomposed when incorporated into the soil or left on the ground after cutting (Boer et al., 2007; Carneiro et al., 2008). They may also release a large part of their N to the accompanying main crop, in this case the passion fruit plants. Even the mixture of spontaneous species had a C/N ratio close to that of the planted legumes.

As already mentioned, symbiosis were observed in the roots of all three planted legumes, but jack bean showed the most abundant nodulation (higher number of nodules per plant) in (Table 2). Estimates of nodulation in the field are subject to errors due to the difficulty of collecting nodules and because sampling is limited to the top 20 cm soil layer. However, these results are useful to determine differences in behavior among species, varieties or management systems (Marinho et al., 2014; Luca et al., 2014). Sunn hemp had the largest nodules, with an average biomass equivalent to twice the biomass of the nodules of the other two species (Table 2). As inoculants were not used, the presence of nodules confirms that the populations of bacteria was naturally established in the soil houses microsymbionts compatible with the three species, which are exotic and had never been grown in the experimental area. In soils of other regions, there are reports that sunn hemp, jack beans and pigeon pea are able to obtain high proportions of their N via BNF, even grown without inoculation (Ncube et al., 2007; Ojien et al., 2007; Resende et al., 2003).

We did not escavated the plots to search for nodules of the spontaneous legumes, which were scatered in the plots and mixed with other species. However, the $\delta^{15}N$ signals of the aboveground parts were always significantly lower than those of the reference species in two evaluation periods, indicating that symbiosis with the native bacteria was effective. The signals of the planted legume were as low as those of the spontaneous legumes. Since the $\delta^{15}N$ of the reference plants were not significantly different among species and in both periods (45 and 90 days) more than 7 ‰ higher than those of the legume species, we had an appropriate situation to obtain reliable estimates of BNF (Högberg, 1997). In both sampling periods, the native symbiotic bacteria were able to provide more than half of the N of legumes at 45 days, and more than 80% at 90 days, including spontaneous legumes, with no differences among species (Table 2). This is an important result because it shows that in this particular case, inoculation with previously selected bacteria would hardly improve the fixing performance of the species. However, we should not generalize this finding to all caatinga areas, since this condition may be specific to the study area, in which native nodulating legumes have a strong presence in the spontaneous vegetation.

Since all legumes formed an efficient symbiosis, the amounts of N fixed by the different species depended mainly on their biomass, and fixation was greater for jack bean (over 65 kg ha^{-1} of N at 45 days and 158 kg ha^{-1} at 90 days; Table 2), than for sunn hemp, pigeon pea and spontaneous plants. The amounts fixed by jack bean were high compared to those reported for cropped legumes in the same semiarid region, where cowpea fixed no more than 45 kg ha^{-1} of N (Freitas et al., 2012b). The amount fixed by the spontaneous plants (29 kg ha^{-1}) was also high compared to those fixed by native herbaceous plants in fallow fields of the region (5 kg ha^{-1}; Freitas et al., 2012a). One of the causes of this higher amount was the higher proportion of legume plant biomass related to the total herb biomass (23% in the orchard and 5% in the fallow fields). Therefore, higher water availability seems to favor the competitive ability of legume species, compared to other plant families in the area. Higher water availability increased biomass production (Mokgehle et al., 2014), which largely influences N fixed amounts (Nyemba and Dakora, 2010).

There is strong isotopic evidence that the passion fruit plants absorbed N that was fixed and released by the jack bean plants, which was approximately 40% of the total N in the passion fruit plants at 90 days after sowing. This transference of fixed N in agricultural systems usually occurs by decomposition of legume tissues (nodules, roots and/or senescent leaves), by exudation of nitrogenous compounds by the roots and, to a lesser extent, by transfer from roots interconnected by mycorrhizal hyphae (Peoples et al., 2015). It is mportant to express that the sampling of the passion fruit plants was performed on the same date the cover and spontaneous legumes were cut. Hence, the aboveground biomass of the cover and spontaneous legumes had not been decomposed to release N that , could be taken up by the passion fruit plants. It is highly probable that as the decomposition of the cut biomass proceeded, the passion fruit plants could absorb even more of the previously fixed N, further increasing the contribution of the planted jack beans (Sakai et al., 2011; Peoples et al., 2015). The other legume species could also release more N, in a way that they could even have a positive contribution to the passion fruit plants. This was not detected until their aboveground biomass was cut (Vargas et al., 2017).

The average fruit yield of passion fruit during the first year of cultivation was 17.4 t ha^{-1} yr^{-1}, with no difference among the green manure species, equivalent to an export of 49 kg ha^{-1} yr^{-1} of N. The cultivation of jack bean alone contributed to an amount of N greater than the amount exported in the fruits, generating a positive balance of more than 16 kg ha^{-1}, when the legume was cut 45 days, and of more than 109 kg ha^{-1}, with cutting at 90 days. In the case of sunn hemp, the N balance was slightly negative, but with pigeon pea or spontaneous legumes, the export of N from the orchard resulted in a deficit of around 20 kg ha^{-1} N. This may mean a yield loss over time if nitrogenous fertilizers or other sources of N are not applied.

Sunn hemp, pigeon pea and jack bean nodulated and fixed nitrogen in symbiosis with bacteria naturally established in the Planosol. The cultivation of jack bean in the orchard rows secured a contribution of fixed N that was three to five times greater than sunn hemp, pigeon pea or the spontaneous legume species in plots without legume (158 vs. 29 to 43 kg ha^{-1}), as a result of the largest biomass produced, since the N contents and the proportions of N fixed in plants (83 to 89%) were similar. The cultivation of jack bean promoted a N intake greater than that exported to the fruits of passion fruit plants, generating a positive balance of more than 16 kg ha^{-1}, when the legume was cut at 45 days. This intake was more than 100 kg ha^{-1}, when the legume was cut at 90 days, by which more than 40% of the passion fruit N came from the N fixed by the legume.

Materials and methods

Characterization of the study area and experimental design

The experiment was conducted in a passion fruit orchard (*Passiflora edulis* Sims) established on the campus of IFPE (Federal Institute of Education, Science and Technology of Pernambuco) in the municipality of Belo Jardim (08° 20' 09" S and 36° 25' 26" W, altitude of 607 m). The climate was semiarid, with average annual rainfall of 890 mm and average annual temperature of 23.0 °C. The soil of the experimental area was a Planosol, with sandy loam texture, featuring the following attributes in the 0-20 cm layer, according to the methodology recommended by Embrapa (2011): 610 g kg^{-1} sand, 236 g kg^{-1} silt and 154 g kg^{-1} clay; 0.143 g kg^{-1} organic matter; pH (water 1:2.5) = 5.87; and P (Mehlich I) = 43 mg kg^{-1}. The passion fruit seedlings were planted on a trellis system, with micro-sprinkler irrigation, and spacing of 2.5 m between rows and 4 m between plants. The experiment was set up in randomized block design with four replications and four treatments, three of which assigned to the cultivation of sunn hemp (*Crotalaria juncea* (L.), pigeon pea (*Cajanus cajan* (L.) Mill) or jack bean

(*Canavalia ensiformis* (L.) DC.) between the planting rows of passion fruit. The fourth treatment (control) had no legume cultivation, but spontaneous plants were allowed to grow. The legumes were sown in four rows spaced 0.50 m, 70 days after transplanting of the passion fruit seedlings, following the sowing density of 33, 24 or 8 seeds per linear meter for sunn hemp, pigeon pea and jack bean, respectively. The plots corresponded to 8 sowing rows of legumes, with a total area of 60 m^2, and nine plants of passion fruit. At the ends of each block, extra plots were installed, in which passion fruit was grown along with sunflower (*Helianthus annuus* L.). These plots were used only for determination of the ^{15}N contents of the sunflower and of the passion fruit leaves.

Assessment of nodulation, biological fixation and transfer of N

At 45 days after sowing, the legumes were sampled to assess nodulation and biomass production. All shoots along 1 m row, randomly located in one of the two central rows, were collected, oven-dried and weighed. The roots of these plants were manually separated from the soil removed from a 0.1 m^3 volume, composed of 0.25 m from each row side, down to the depth of 0.2 m. The nodules were detached from the roots and the soil volume was sieved to recover fallen nodules. All nodules were counted, washed, oven-dried and weighed. The average size of the nodules (mg $nodule^{-1}$) was estimated in each plot by dividing the dry biomass of all nodules by the number of nodules.

At 90 days after sowing, the legume and the spontaneous plants were harvested. All the cut material was weighed and subsampled to determine the moisture content. In the plots with spontaneous plants, legume species were separated from the others and the biomass of all plants and of legumes were determined using the same procedure adopted for the plots where the legumes were planted.

The sunflower and three non-legume weeds (*Sonchus oleraceus* (L.) L., *Leonotis nepetifolia* (L.) R.Br. and *Portulaca oleracea* L.) were used as reference plants for biological nitrogen fixation (BNF) estimation, using the ^{15}N natural abundance methodology. For this, composite samples, formed by the shoots of five plants of each species, were collected in the two sampling periods (45 and 90 days), in each of the four plots with sunflower or with spontaneous plants. Samples composed of five passion fruit leaves were collected in the central plant of the plots with cover crops (legumes and weeds) and also in the plots with sunflowers, to estimate the transfer of the N fixed in the biomass of legumes to the passion fruit plants.

Of all vegetable samples, subsamples were placed in capsules and inserted into a ThermoQuest-Finnigan Delta Plus mass spectrometer (Finnigan-MAT, CA, USA), interfaced with an elemental analyzer (Carlo Erba, model 1110; Milan, Italy), at the Laboratory of Isotopic Ecology (CENA-USP, Brazil), to obtain the total concentrations (%) and the isotopic ratios of N and C. The isotopic ratios were determined according to recognized international standards. Reference materials (atropine, yeast extract and soil standard 502 - 308, LECO Corporation) were included in all analytical runs. The natural abundances of ^{15}N and ^{13}C were expressed in δ units (‰), which represents the deviation, from the standard, of the ratios of the masses of ^{15}N:^{14}N and ^{13}C:^{12}C, following the equation:

$$\delta ‰ = (Rsample \ / \ Rstandard - 1) \ x \ 1000$$

Where R_{sample} and $R_{standard}$ are the ratios ^{15}N:^{14}N and ^{13}C:^{12}C of both the sample and the standard, atmospheric N_2 being the N standard and *Pee Dee Belemnite* the C standard.

When the difference were statistically significant ($p \leq 0.05$) between the averages of the $\delta^{15}N$ signals of potentially fixing legumes and those of the references (sunflower, in the estimates made at 45 days; and sunflower and weeds *Sonchus oleraceus* (L.) L., *Leonotis nepetifolia* (L.) R.Br. and *Portulaca oleracea* L., in the estimates made at 90 days), the percentage of nitrogen derived from atmosphere (% Ndfa) was estimated by the ^{15}N natural abundance methodology (Shearer and Kohl, 1986), using the equation:

$$\%Ndfa = [(\delta 15N(reference) - \delta 15N(fixing)) \ / \ \delta 15N(reference) - B] \ x \ 100$$

Where; $\delta^{15}N_{(reference)}$ is the average value of the $\delta^{15}N$ of the reference plants of each block, $\delta^{15}N_{(fixing)}$ is the average value of the $\delta^{15}N$ of each legume for each plot and B is the $\delta^{15}N$ value for N-fixing plants grown in the absence of nitrogen. B values were as follows: -1.08 % for sunn hemp and -1.12 % for pigeon pea (Unkovich et al., 2008) and -1.00 % for jack bean (Ojiem et al., 2007). As there is no information in the literature for the spontaneous legume species, the B value was assumed to be -1.07 %, equivalent to the average value used for other species.

The amount of fixed N was calculated multiplying the proportion of N derived from the atmosphere (% Ndfa) by the N content of each species, obtained multiplying the N concentration in the sample material by the total aboveground biomass of the species in the plot.

The estimate of the transfer of the N fixed in legumes to the passion fruit was made using the equation (Peoples et al., 2015):

$$\% \ N \ transferred = [1 - (\delta 15Ntest \ plant \ / \delta 15Ncontrol \ plant)] \ x \ 100$$

Where; $\delta^{15}N_{test\ plant}$ is the $\delta^{15}N$ value obtained in the passion fruit plants grown in plots with cover crops (legumes and spontaneous plants) and $\delta^{15}N_{control\ plant}$ is the $\delta^{15}N$ value obtained in the passion fruit plants grown along with sunflower.

Fruit yield and N balance

During the period of 12 months from the orchard deployment, all produced fruits were collected and weighed. The amounts of N exported from the orchard through the fruits were estimated by the product of the fruit mass produced and their N concentrations, considering fruits with 1.88% N and 15% dry matter (Fernandes et al., 1977, Bomtempo, unpublished data). The N balance for the use of each legume was calculated by the difference between the amounts fixed by the legume of each treatment and the amounts of N exported in the fruits.

Data analysis

Data were submitted to analysis of variance and means were compared using the Tukey's test ($p \leq 0.05$), considering a randomized block design. For the assessments of nodulation, biomass production, % N, total accumulated N, C/N ratio and $\delta^{13}C$ (%), in the sampling at 45 days, the data were analyzed considering a design with three treatments and four replications. The data of number of nodules were transformed to $(x + 1)^{1/2}$. The results of $\delta^{15}N$ were analyzed considering a design with four treatments (sunn hemp, jack bean, pigeon pea and sunflower) and four replications, at 45 days, and a design with five treatments (sunn hemp, jack bean, pigeon pea and sunflower or sunflower + reference spontaneous species) and four replications, at 90 days. The results for biomass production, N contents, total accumulated and total fixed N, C/N ratio and $\delta^{13}C$ (‰), in the harvest at 90 days, were analyzed considering a randomized block design with four treatments (sunn hemp, jack bean, pigeon pea and spontaneous plants) and four replications. The data of ratios were transformed into arcsine. For both analyses, the statistical program ASSISTAT 7.6 was used.

Acknowledgments

The authors thank Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE; Processo APQ-0199-5.01/15) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Projeto Forma-Engenharia CNPq/VALE, Processo 454980/2012-4 and Proc. 403129/2013-3) for financial support and the fellowships conceeded.

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