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Nitrogen fixation by soybean in the Pampas: relationship between yield and soil nitrogen balance

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INTRODUCTION. – The Pampas is one of the most important grain producing areas of the World (SATORRE and SLAFER, 1999). Soybean (*Glycine max* (L.) Merrill) is currently the dominant cultivated crop, representing around 60% of the seeded area and grain production of the region (MINAGRI, 2010). The crop is cultivated without nitrogen fertilization and its productivity strongly depends on soil available nitrogen and the nitrogen contribution from biological fixation. It has been stated that soybean production can reach 100 Mt in the next decade by doubling the cultivated area (ALTIERI and PENGUE, 2005), but the possible impact of this crop on soil nitrogen balance and on the sustainability of the agricultural ecosystems is not known. The available local information assessing the potential of the crop to fix nitrogen from the atmosphere is scarce and it has not been integrated. Consequently, establishing reliable soil nitrogen balances is not possible in the region since there is a lack of field estimations on the input of nitrogen to the agroecosystem from the atmosphere. The use of the ^{15}N methodology is restricted by economic reasons so other simpler techniques are needed for assessing nitrogen fixation in the region.

Nitrogen fixation by soybean is highly variable representing an average of 50 to 60% of the plant requirements (PEOPLES *et al.*, 2001, SALVAGIOTTI *et al.* 2008). The apparent nitrogen balance of the crop,

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estimated from the difference between fixed nitrogen and harvested grain nitrogen, is also very variable and negative in most cases (SALVAGIOTTI *et al.*, 2008). Positive relationships between nitrogen in the shoot biomass and the fixed nitrogen has been described, that can be fitted to linear models (HERRIDGE *et al.*, 2008, SALVAGIOTTI *et al.*, 2008). Also, a good fit has been reported between soybean yield and nitrogen in aboveground organs under low fertilization scenarios (SALVAGIOTTI *et al.*, 2008). Combination of these models allows for reliable nitrogen fixation estimations based on yield data. These relationships had not yet been studied in the Pampas and it is the objective of this study to describe the relationship between soybean biomass production and yield, nitrogen fixation and soil nitrogen balance, based on a meta-analysis of locally published results of biological nitrogen fixation from field experiments performed in the region, in order to develop a local model suitable for nitrogen fixation estimation based on yield data.

MATERIALS AND METHODS. – The Pampas is a vast plain of approximately 50 Mha, located between 30º and 40º latitude South, 57º and 68º longitude East in Argentina. Rainfall varies from 200 mm in the West to 1200 mm in the East and the annual mean temperature ranges from 14ºC in the South to 21ºC in the North. Predominant soils are Mollisols developed from loessic materials of eolian origin, which present a great variation in texture, depth, organic matter content and fertility (ÁLVAREZ and LAVADO, 1998). In the humid east portion of the region fine textured soils of medium-to-high soil organic matter content predominate, meanwhile in the semiarid west, sandy soils of low organic mater level are widespread. Soybean is cultivated mainly in the humid east portion of the region on around 15 Mha yr^{-1} without fertilization.

Ten field experiments performed in the Humid Pampean Region had been published where biological nitrogen fixation in soybean crops was assessed and biomass production and yield reported (Tab. 1). Experiments were designed to compare tillage systems (6), soybean varieties (1), references crops (1), and previous crops (1) or only to determine nitrogen fixation (1). Experiments were installed on soils usually cropped with soybean. The experimental design was generally completely random blocks with 3-4 replications per treatment. Plots were seeded and harvested by hand, not harvesting plants from border rows. Seeds were inoculated with commercial *Bradyrhizobium japonicum* strains. Nitrogen fixation was assessed by isotopic methodologies applying in all experiments $^{15}(NH_4)_2SO_4$ after seedling to plots of 2.5-4 m^2. The plots received ^{15}N-fertilizer in rates varying from 10 to 20 kg N ha^{-1}, applied in solution with 5-10% a.e. ^{15}N. As non fixing control non nodulating soybean or cereal crops [corn (*Zea mays* L.), sorghum (*Sorghum bicolour* L.)] were used. Harvest was carried out at R7 growth stage when the crop has accumulated more than 90% of the biomass and nitrogen of the grain but leaf senescence and defoliation is not relevant (RITCHIE *et al.*, 1989). However, the fallen leaves were also harvested. The aboveground plant material was dried at 60ºC and weighed. Its nitrogen content was determined by the Kjeldahl method (BREMNER, 1996) and ^{15}N abundance by the Rittenberg or Dumas method (FIEDLER and PROKSH, 1975).

TABLE 1. –

Data source[1]	Site	Growing season	Rainfall (mm)	Air temperature (°C)	Soil	Organic matter[4] (%)	Texture[4]	pH[4]	Type of experiment	Design	Replications	Variety	Fertilized plot (m^2)	N rate (kg ha^{-1})	At. exc. (%)	Control crop	Harvested surface (m^2 plot^{-1})	^{15}N-methodology
Alvarez et al., (1995)	La Dulce	1982/83	358[2]	19.5[2]	Typic Argiudoll	4.01	Loam	6.0	Varieties	Randomized blocks	18	Chippewa Maple Arrow Chippewa no nod.	3	20	5	No nod. soybean No nod. soybean	2	A value
Di Ciocco et al., (2004)	Luján	2000/01	702[2]	21.6[2]	Typic Argiudoll	2.84	Silty loam	5.7	Tillage	Randomized blocks	3	D71-9289 D71-9289 no nod.	4	10	10	No nod. soybean	1	Isotopic dilution
Di Ciocco et al., (2008)	Luján	2001/02	635[2]	20.2[2]	Typic Argiudoll	4.13	Silty clay loam	5.6	Tillage	Randomized blocks	3	D71-9289 D71-9289 no nod.	4	10	10	No nod. soybean	2	Isotopic dilution
Di Ciocco et al., (2010)	Luján	2006/07	727[3]	20.3[3]	Typic Argiudoll	4.13	Silty clay loam	5.6	Tillage	Randomized blocks	3	D71-9289 D71-9289 no nod.	4	10	10	No nod. soybean	2	Isotopic dilution
	Luján	2007/08	412[3]	20.6[3]	Typic Argiudoll	4.13	Silty clay loam	5.6	Tillage	Randomized blocks	3	DM 5.2i DM 5.2i	4	10	10	Corn (*Z. mayz*) Sorghum (*S. bicolor*)	2	Isotopic dilution
Ghelfi et al., (1984)	Baradero	1980/81	542[3]	21.7[3]	Typic Argiudoll	3.03	Silty clay loam	5.5	Control crops	Randomized blocks	6	Williams	4	20	5	Sorghum (*S. bicolor*)	1.4	Isotopic dilution
López et al., (2010)	Ezeiza	2006/07	768[3]	22.0[3]	Argiacuoll	4.17	Loam	6.4	Tillage	Randomized blocks	3	Commercial variety	2	20	20	Sorghum (*S. bicolor*)	1	Isotopic dilution
	Ezeiza	2008/09	474[3]	21.7[3]	Argiacuoll	4.17	Loam	6.4	Tillage	Randomized blocks	3	Commercial variety	2	20	20	Sorghum (*S. bicolor*)	1	Isotopic dilution
	Bolivar	2008/09	251[3]	23.4[3]	Hapludoll	3.70	Sandy loam	6.3	No treatments	Randomized	4	Commercial variety	2	20	20	Wild vegetation	1	Natural abundance
Penón et al., 2010[5]	Luján	2008/09	339[3]	23.4[3]	Typic Argiudoll	4.59	Silty loam	5.6	Previous crops	Randomized blocks	4	Don Mario 4600	2.5	10	10	Sorghum (*S. bicolor*)	1	Isotopic dilution

1: cited references may be obtained from the corresponding author upon request.
2: recorded in situ during crop growing season.
3: estimated using data from meteorological observatories for the november-march period.
4: in the 0-20 cm soil layer.
5: results from different previous crops

The percentage of nitrogen derived from the atmosphere (% Ndda) was calculated in most of the experiments using the isotopic dilution method (CHALK, 1985) as:

$$\%Ndda = (1 - \frac{\%aepf}{\%aepnf})x100$$

where: *%aepf* is the percentage of a.e ^{15}N in the nitrogen fixing plant and *%aepnf* is the percentage of a.e. ^{15}N in the non nitrogen fixing plant.

In one experiment the A value concept was used for nitrogen fixing estimation (DANSO, 1986) and the natural ^{15}N abundance technique in another (BODDEY *et al*., 2000). The root nitrogen was estimated as 24% of the total content of nitrogen in the crop (SALVAGGIOTTI *et al*., 2008). This estimation agrees with local evaluations showing that 20 to 25% of the soybean biomass is allocated belowground in the 0 to 30 cm soil layer (ALVAREZ *et al*., 2008).

The apparent nitrogen balance of the agroecosystem was calculated from the difference between the nitrogen biologically fixed and the harvest of nitrogen in grain (PEOPLES *et al*., 1995). As in the five out of six experiments in which tillage systems were compared (no-till vs. plow tillage) no significant differences were reported on nitrogen fixation, yield or nitrogen balance (DI CIOCCO *et al*., 2004, 2008, LÓPEZ *et al*., 2010), results from tillage systems were averaged.

Meta-analysis of data was performed by regression and correlation analysis, testing the significance of the models by a t test. The ordinates and the slopes from the regression models were contrasted against 0 and 1 respectively using IRENE (FILA *et al*., 2003). When the intercepts from the regression models were not different from 0 they were dropped from models. The model of SALVAGIOTTI *et al*. (2008) that relate grain yield with nitrogen uptake (Fig. 1 in SALVAGIOTTI *et al.* 2008) and the model that relate fixed nitrogen with nitrogen uptake (Fig. 2 in SALVAGIOTTI *et al.* 2008) were combined in the following equation:

$$Fixed\ N\ (kg\ ha^{-1}) = -25 + 0.079\ Yield\ (kg\ DM\ ha^{-1})$$

The model allows the estimation of nitrogen fixed in the whole soybean plant assuming that root nitrogen is equivalent to 24% of total nitrogen uptake and under scenarios of low nitrogen fertilizer application. Results obtained with this model were compared to those calculated with the function fitted to pampean data.

RESULTS AND DISCUSSION. – The soybean shoot biomass production and the grain yield varied between experiments and varieties (Tab. 2). Non nodulating isolines grain yields ranged from 662 to 1910 kg DM ha^{-1}, meanwhile nodulating isolines and commercial varieties yield varied from 1360 to 4260 kg DM ha^{-1}. The mean grain yield of nodulating soybean of all the evaluated experiments was 2280 kg DM ha^{-1}, similar to mean soybean yield of 2240 kg DM ha^{-1} of the Pampean Region. The biologically fixed nitrogen varied between 19.5% (47.7 kg N ha^{-1}) and 55.2% (129 kg N ha^{-1}) with a mean value of 40 % (109 kg N ha^{-1}). Based on worldwide reviews of published reports, it has been estimated as a

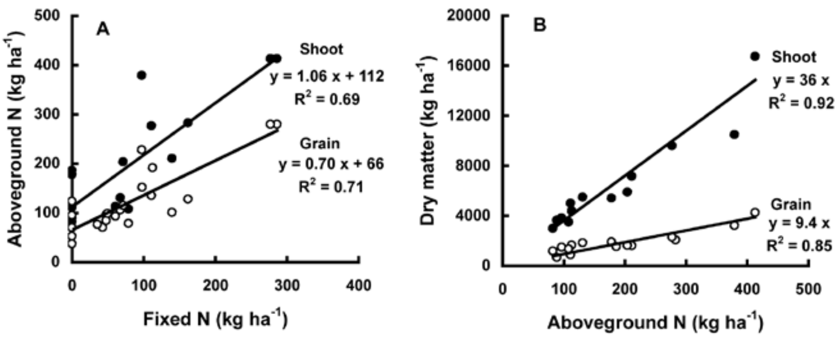

FIG. 1. – A: Relationship between fixed nitrogen in the whole plant and nitrogen in aboveground biomass. B: Relationship between nitrogen in shoot and biomass accumulation in grain or shoot.

mean that around 50 to 60% of the soybean nitrogen requirements are fixed from the atmosphere (ALVEZ *et al.*, 2003, HERRIDGE *et al.*, 2008, SALVAGIOTTI *et al.*, 2008). Thus, our observations suggest that in the agricultural systems from the Pampean Region, biological nitrogen fixation has a lower contribution to the total soybean nitrogen requirements for growth and production.

TABLE 2. – *Aboveground biomass and nitrogen in soybean, nitrogen fixed from the atmosphere and soil nitrogen balance during the growing season. DM = dry matter.*

Data source	Variety	Shoot biomass (kg DM ha^{-1})	Shoot N (kg ha^{-1})	Yield (kg DM ha^{-1})	Grain N (kg ha^{-1})	Fixed N (%)	Fixed N (kg ha^{-1})	N balance (kg ha^{-1})
Alvarez et al., (1995)	Chippewa	4400	113	1660	93.5	40.8	60.7	-32.8
	Maple Arrow	5490	131	1840	106	39.1	67.4	-38.6
	Chippewa no nod.	2990	82.2	1180	70.1	0.0	0.0	-70.1
Di Ciocco et al., (2004)	D71-9289	10500	379	3220	228	19.5	97.4	-131
	D71-9289 no nod.	5410	178	1910	124	0.0	0.0	-124
Di Ciocco et al., (2008)	D71-9289	7160	211	1620	101	50.2	139	38.3
	D71-9289 no nod.	3650	88.7	662	37.4	0.0	0.0	-37.4
Di Ciocco et al., (2010)	D71-9289	5900	204	1620	115	26.5	71.3	-43.7
	D71-9289 no nod.	5000	111	884	53	0.0	0.0	-53.0
	DM 5.2i	16800	413	4260	280	52.5	286	6.0
	DM 5.2i	16800	413	4260	280	51.1	277	-3.0
Ghelfi et al., (1984)	Williams		283	2070	128	43.5	162	33.8
López et al., (2010)	Commercial variety	3840	96.3	1490	84.7	34.5	47.7	-41.0
	Commercial variety	3500	108	1360	78.7	55.2	78.8	-0.27
	Commercial variety	9600	277	2300	135	30.3	111	-24.3
Penón et al., 2010	Don Mario 4600			1180	70.6		42.8	-27.8
	Don Mario 4600			1430	76.7		35.5	-41.2
	Don Mario 4600			1990	98.8		49.6	-49.2
	Don Mario 4600			2500	152		97.9	-54.1
	Don Mario 4600			3290	192		112.5	-79.5

The biologically fixed nitrogen strongly correlated with the aboveground nitrogen content (Fig. 1A) and this variable was also closely related with the aboveground biomass (Fig. 1B). The mean nitrogen content in non fixing soybean isolines was of 115 kg ha^{-1} while commercial varieties accumulated approximately 400 kg of N ha^{-1}. Grain yield increased markedly when nitrogen fixation rose. These results agree with previous researches performed in other parts of the World (HERRIDGE *et al.*, 2008) which showed that the aboveground dry matter production of leguminous crops is positively related with the nitrogen fixation. In average, 20 kg N are fixed by Mg aboveground biomass production, with a range of 15 to 25 kg N Mg^{-1} DM (HERRIDGE *et al.*, 2008). Pampean results fall at the lower edge of the range indicating low fixation of soybean ion the region.

Our results suggest that under the growing conditions of the Humid Pampean Region, 52 kg of N ha^{-1} are biologically fixed per Mg DM grain production (Fig. 2). The fitted regression between soybean yield and nitrogen fixation allows the local estimation of nitrogen fixation in cases where the ^{15}N methodology is not available but more experiments are needed to validate this model. Especially, it must be taken into account that results analyzed in this study were mainly generated on fine textured soils of high organic matter content. As a consequence, soil fertility was generally high and this may be depressed nitrogen fixation (HERRIDGE *et al.*, 2001). Hapludolls, Haplustolls and other sandy soils are also common in western part of the Pampas but nitrogen fixing results from these soils are not available. However, soybean overall

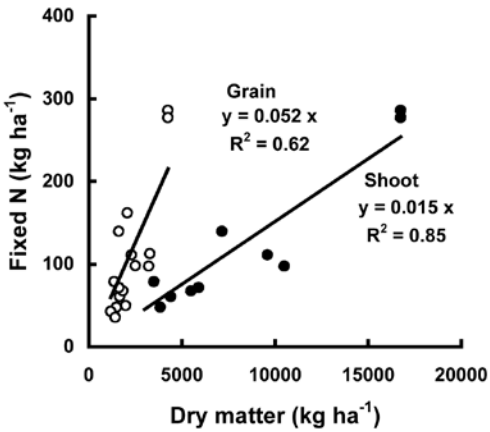

FIG. 2. – Relationship between dry matter in the aboveground biomass and the fixed nitrogen in whole plant of soybean (only nodulating varieties and isolines).

production in the region is more common on fine soils of the central and eastern Pampas.

Estimations of nitrogen fixation performed by the local pampean model are generally lower than that of the model fitted to worldwide data (Fig. 3A). Both models performed similar estimations for low yield levels but great differences existed in the medium to high range of yield. The worldwide model was calculated using data from 61 papers generated from experiments performed mainly in USA, Canada and Australia. The pampean scenario of our experiments seems not to be adequate represented by this model.

In pampean experiments the soil nitrogen balance of the soybean nodulating isolines or varieties ranged from -131 kg ha^{-1} to 46.2 kg ha^{-1}. Because the mean nitrogen content of the grains was 60 kg Mg^{-1} DM, it was estimated that approximately 8 kg of N ha^{-1} was lost from the soil with each Mg DM harvested grain and the mean lost of nitrogen in these experiments was of 21 kg N ha^{-1} yr^{-1}. This value is similar to the estimated lost of nitrogen from soybean crops in the Pampas based on the mean grain yield of the region. Our results agree with other works (PEOPLES *et al.*, 1995) which showed that the nitrogen balance in soybean crop can vary between -134 to 69 kg ha^{-1}. However, under the pampean studies the mean nitrogen balance was slightly more negative than a worldwide balance available in which an average lost of 4 kg N ha^{-1} yr^{-1} was estimated (SALVAGIOTTI *et al.*, 2008). Even, if using the worldwide model relating fixed nitrogen

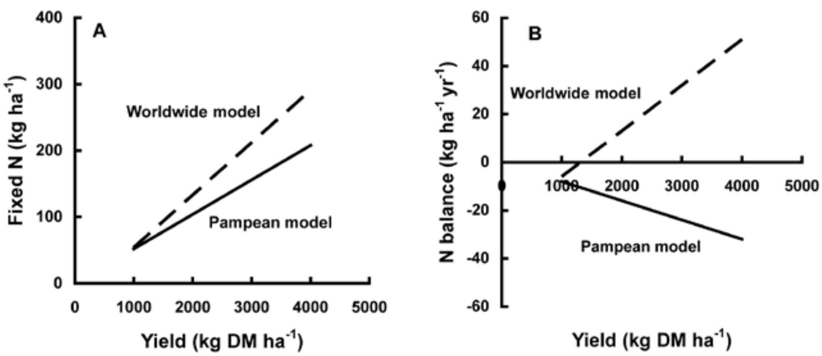

FIG. 3. – A. Estimation of biological nitrogen fixation in soybean in relation to yield by using a model fitted using worldwide data and a model fitted with local data of the Pampas. B. Apparent nitrogen balance of soybean crops in relation to yield calculated using models used in A.

and yield for nitrogen balance calculations, positive balances would be obtained instead of the negative estimations performed with the pampean model (Fig. 3B).

Future research is needed for the evaluation and development of management practices for minimizing the negative soil nitrogen balance of some common scenarios under which soybean is cropped in the Pampas. The model developed must be used with care for nitrogen fixation estimation in coarse-low fertility soils until it can be validated in these situations.

CONCLUSIONS. – In average, soybean crops fixed from the atmosphere 52 kg of N t^{-1} yield and extracted by harvest 60 kg N t^{-1} yield in the Pampas, resulting in a negative nitrogen balance of 8 kg N t^{-1} harvested dry matter grain. For the average soybean yield of the region a soil nitrogen balance of -18 kg N ha^{-1} yr^{-1} was estimated. Models developed for nitrogen fixation estimation in other parts of the World are not applicable in the region.

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SUMMARY. – Soybean (*Glycine max* (L.) Merrill) is the major grain crop in the Pampas. Its productivity strongly depends on soil nitrogen availability and biological nitrogen fixation because nitrogen fertilizers are not usually applied. Scarce local information has been generated about biological nitrogen fixation potential of soybean crops or to the soil nitrogen balance during its growing season. A meta-analysis was performed of locally published results from 10 field experiments in order to estimate average values of nitrogen fixation from the atmosphere, crop yield and soil nitrogen balance in the region and, also, to fit a regression model relating yield and fixed nitrogen. Results were compared with a nitrogen balance calculated using a model fitted to worldwide data. Nitrogen fixation in aboveground biomass was assessed by isotopic methodologies in all the experiments and an estimation of fixed nitrogen in roots was performed. Grain yield varied from 662 kg of dry matter (DM) ha^{-1} for non nodulating

isolines to 4260 kg DM ha^{-1} for commercial varieties. Nitrogen fixation accounted for 20 to 55% of the plant nitrogen. In average, 40% of the soybean nitrogen was fixed from the atmosphere, rounding 109 kg N ha^{-1}. Shoot biomass and grain yield were positively correlated to fixed nitrogen ($R^2 > 0.69$, $P = 0.05$). The slope of the regression of the amount of fixed nitrogen against yield showed that, in average, the crop fixed from the atmosphere 52 kg of N t^{-1} DM grain produced. By harvest approximately 60 kg of N t^{-1} DM were extracted from the agricultural system. Consequently, the apparent soil nitrogen balance was slightly negative and as higher the yield, the more negative the nitrogen balance. For an average soybean yield in the Pampas (2600 kg ha^{-1}, 14% water) the soil nitrogen balance can be estimated to be -18 kg N ha^{-1} yr^{-1}. Estimation of fixed nitrogen with the model adjusted to worldwide data was higher than with the local model and the calculated nitrogen balance turned to positive, so this later model seemed not to be applicable to pampean conditions. Future research is needed for the evaluation and the development of management practices and crop rotations for minimizing the negative soil nitrogen balance of the region.