

Nitrogen transfer from legume green manure in a crop rotation to an onion crop using ^{15}N natural abundance technique

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ABSTRACT

Legumes as green manure allow adding N from the air to the soil through their biological fixation. The objective was to evaluate the effect of legume crops as green manure at the beginning of the rotation on soil quality and their N input to the following onion crop (*Allium cepa* L. var. *cepa*). Five crops were sown: faba-bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), oat-vetch mixture (*Avena sativa* L. + *Vicia atropurpurea* Desf.), white lupin (*Lupinus albus* L.), and oat as control treatment, with four replicates. Prior to sowing soil was prepared with 20 Mg ha⁻¹ compost and 715 kg ha⁻¹ rock phosphate. Then, at 50% grain filling stage these crops were incorporated as green manure to the soil, followed by the onion crop. Biological N fixation (BNF) by ^{15}N natural abundance technique parameters of green manure, yield, leaf N content of the legumes and onion, and soil bio-physicochemical properties were measured and analyzed by ANOVA and Tukey's test for mean separation ($P \leq 0.05$). Faba-bean treatment had the highest BNF (388 and 369 kg N ha⁻¹ fixed in 2017 and 2018, respectively). For onion yield, just faba-bean and oat-vetch in 2018 reached the Chilean mean yield with 54.39 and 49.08 Mg ha⁻¹, respectively. Control treatment yield was not significantly different. Leaf N content in onion remained within a normal range in all treatments. Adding green manure together with compost to the soil improved soil N and K contents.

Key words: Crop rotation, green manure, legumes, ^{15}N natural abundance, nitrogen transfer, onion.

INTRODUCTION

Nitrogen is one of most important elements to sustain human life. Approximately 67.84 million tons are applied to agricultural soil as fertilizer. If synthetic N fertilizers were not used, nearly half of the world population would not exist (Chen et al., 2014). However, the overuse of synthetic N fertilizers produces a gradual degradation of soil organic matter (SOM), which deteriorates soil health by reducing the water holding capacity, increases surface and groundwater pollution, and causes multiple nutrient deficiencies (Meena et al., 2018). There is also a negative environmental impact such as greenhouse gas emissions generated by low N use efficiency (NUE); this means that nearly 50% of the applied N is taken up by the crop during the growing season and the rest is lost (Norton and Ouyang, 2019). Returning plant residues to the soil could be a good practice to enhance biological activity, improve physicochemical properties, and increase nutrient availability (Chen et al., 2014) and retention (Meena et al., 2018).

Sustainable agriculture based on agroecological criteria requires optimizing nutrient cycling and synchronizing crop nutrient demand with soil supply (De Oliveira et al., 2017). For N, cycling and using alternative N sources involve incorporating crop residues, organic amendments, green manure, cover crops, and biological N fixation (BNF), which can supply significant amounts of available soil N (De Oliveira et al., 2017). Legume species as green manure have become an important practice in sustainable agriculture due to their N fixation capacity; in many cases, their vast root

system allows major nutrient extraction and cycling (Carvalho et al., 2015). Legume green manure has a high potential for improving soil biophysicochemical properties through organic matter (OM) input (Meena et al., 2018). Crop rotation refers to adding biochemical inputs to the soil, which nourish the soil biology and crops at different times and provide a temporal biodiversity that benefits ecosystem functions such as C sequestration, pest and disease suppression and nutrient cycling, thus improving yield (Peralta et al., 2018).

Onion (*Allium cepa* L. var. *cepa*) is one of the most important commercial horticultural crops worldwide. Onion contributes to farmer income and human diet, and it is highly valued for its medicinal properties (Bua et al., 2017). It is cultivated and commercialized in south-central Chile, OM and its yield is influenced by agronomic management and soil conditions that affect bulb size and weight (González and Herrera, 2012).

Soils in south-central Chile are of volcanic origin and are characterized by amorphous clay minerals such as allophane, high P fixation capacity, and OM stabilization, which can limit the contribution of P and N to crops (Hirzel et al., 2010).

The objective of this study was to evaluate the effect of a legume crop at the beginning of a rotation on soil quality and added N to an onion crop.

MATERIALS AND METHODS

Experiment location

The experiment was set up at the Santa Rosa experimental station of the Instituto de Investigaciones Agropecuarias, INIA Quilamapu (36°32.13" S, 71°55.36" W; 190 m a.s.l.), Chillán, Ñuble Region, Chile, on a medial over loamy skeletal, amorphous, and thermic Humic Haploxerands soil (Stolpe, 2006). The biophysicochemical values were 55.1 µg g⁻¹ microbial biomass (MB), 0.81 g cm⁻³ bulk density (BD), 68.5% water-stable aggregation (WSA), 6.23 pH H₂O, 12.14% organic matter (OM), 7.14 mg kg⁻¹ N, 3.85 mg kg⁻¹ P, and 63.15 mg kg⁻¹ K.

Climatic conditions during the experiment

Atmospheric conditions at the experimental site are shown in Table 1. Mean temperatures were in the typical range of 13.1 to 13.6 °C for the study area during the 3 yr experiment. However, minimum temperatures were 1.8 and 1.2 °C lower than the 3.0 to 3.9 °C mean in July 2017 and 2018, respectively. Rainfall in 2017 reached 1076 mm, which is within the variation range for the area (1000 to 1200 mm). Rainfall in 2018 and 2019 reached 794 and 632 mm, which was a decrease of 20.6% and 36.8%, respectively.

Crop rotation establishment

An experiment was established to evaluate the effect of legume rotation on onion (*Allium cepa* L. var. *cepa*) production and soil quality. It consisted of five treatments with four replicates each, and each experimental unit had an area of 3 m × 4 m (12 m²). Treatments were faba-bean (*Vicia faba* L. 'Portuguesa', 80 kg ha⁻¹), field pea (*Pisum sativum* L. 'Enorma', 220 kg ha⁻¹), oat-vetch mixture (*Avena sativa* L. 'Urano' + *Vicia atropurpurea* Desf., 72 + 60 kg ha⁻¹), white lupin (*Lupinus albus* L. 'Alboroto', 120 kg ha⁻¹), and oat ('Urano', 120 kg ha⁻¹) as a control treatment.

Table 1. Monthly and annual mean temperatures and monthly and annual rainfall at the experimental site in 2017, 2018, and 2019.

	Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Max. temp., °C	2017	30.7	29.7	25.5	21.9	14.8	12.7	13.1	13.2	16.8	18.4	23.6	27.7	20.7
	2018	28.8	30.0	25.4	19.7	15.6	11.6	13.0	14.5	17.5	19.0	23.5	27.3	20.5
	2019	28.9	30.2	26.1	21.3	15.6	12.9	13.6	14.8	17.0	20.1	24.9	28.0	21.1
Mean temp., °C	2017	20.9	20.2	16.5	13.5	9.2	8.1	7.1	8.2	10.1	12.0	15.8	18.8	13.4
	2018	19.6	19.9	15.9	12.3	9.8	6.5	7.4	8.2	11.3	12.4	15.8	18.2	13.1
	2019	19.2	20.4	16.3	12.6	10.2	8.4	8.5	8.8	9.8	12.3	16.1	18.6	13.4
Min. temp., °C	2017	11.1	10.8	7.4	5.1	3.6	3.5	1.2	3.3	3.4	5.6	8.1	9.9	6.1
	2018	10.3	9.7	6.5	5.0	4.1	1.3	1.8	1.8	5.1	5.8	8.0	9.1	5.7
	2019	9.4	10.5	6.4	4.0	4.8	3.9	3.4	2.9	2.5	4.5	7.3	9.3	5.7
Rainfall, mm	2017	7.5	13.5	14.6	83.8	148.4	216.8	105.4	253.9	79.0	96.6	47.5	8.9	1076.0
	2018	4.9	7.1	30.9	73.8	85.7	118.0	101.3	92.8	102.2	97.2	64.8	15.3	794.0
	2019	9.1	7.8	8.2	7.4	140.6	221.6	93.9	52.1	57.3	15.1	12.6	5.8	632.0

Soil physicochemical properties were analyzed at the end of summer during the first half of March (Table 2). The soil was later prepared by plowing, harrowing, tilling with a mini tiller cultivator, and incorporated 20 Mg ha⁻¹ class A compost that contained 62% OM and 3.04% total N (155 mg kg⁻¹ NH₄ and 2520 mg kg⁻¹ NO₃) and 715 kg ha⁻¹ rock phosphate (30.5% P₂O₅, 44 % CaO, 3.6% MgO) were added to all the treatments. Crops that started the rotation as green manure were first sown on 10 May 2017 with 20 cm row spacing, while the second sowing was on 22 May 2018 with the same characteristics. The same management practices were carried out in both years.

Evaluation and determination of biological N fixation

Phytomass production was randomly collected over 0.5 m of foliage when the legumes were at the half-grain filling stage. Collected samples were dried in a forced air oven at 70 °C until constant weight to determine DM content. Afterward, a subsample (1 to 2 g) of plant material was collected and analyzed at the Laboratory of Applied Physical Chemistry of the Faculty of Bioscience Engineering, Ghent University, Belgium, to obtain total N and the ¹⁵N isotope ratio by mass spectrometry (Unkovich et al., 1993).

Biological N fixation (BNF) was estimated by the ¹⁵N natural abundance technique. The N proportion derived from biological fixation was estimated by determining three values of ¹⁵N natural abundance: δ value described by Unkovich et al. (2008), ¹⁵N-N abundance derived from the reference plants (non N₂-fixing plants, $\delta^{15}\text{N}_{\text{ref}}$), and ¹⁵N natural abundance from N₂ fixating plants ($\delta^{15}\text{N}_{\text{fix}}$). The Shearer and Kohl (1986) equation was used to calculate the BNF contribution of each legume.

The ¹⁵N content in reference plants estimated available ¹⁵N in the soil for plant growth during the whole study period. In addition, this available soil ¹⁵N pool was the same for reference plants and legumes (Boddey et al., 2000).

Establishment and evaluation of the onion crop

Onion plants ('Valinia INIA') were transplanted on the chopped and incorporated green manure residues with 20 cm row spacing. Plants were sown in July in both years and were transplanted on 24 November 2017 and 16 November 2018. There were approximately 9800 plants per hectare. Wheat straw mulch was used in every treatment for weed control along with monthly manual weed control; compost tea was applied to the leaves to prevent fungal diseases. A drip irrigation system with a flow of 2 L h⁻¹ for 3 h twice a week was used to maintain the optimal crop water status.

The first harvest was on 20 April 2018 and the second on 11 April 2019. Only onions located in the central row of each block (4 m) were evaluated; they were properly identified and placed on a mesh in the shade for curing. One sample was collected at harvest to measure leaf N content by the Kjeldahl method and yield (Mg ha⁻¹) was determined.

Determination of soil biophysicochemical characteristics

Soil fertility analyses were performed in 2017 and 2019 to determine pH, OM, available N, available Olsen P, and available K according to the methodology by Sadzawka et al. (2006). Samples were taken at a 20 cm depth for each experimental unit. Microbial biomass (MB), bulk density (BD), and water-stable aggregation (WSA) were measured according to the Jenkinson and Powlson (1976), Archad et al. (1996), and Kemper and Rosenau (1986) methodologies, respectively.

Table 2. Evolution of soil biophysicochemical properties during the experiment.

Treatment	MB	BD	WSA	pH H ₂ O	OM	N total	P Olsen	Available K
	μg g ⁻¹	g cm ⁻³	%		%	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹
March 2017								
Initial	55.10a	0.81a	68.5a	6.23a	12.14b	7.14b	3.85a	63.15b
March 2019								
Faba-bean	44.63a	0.62ab	82.0a	6.02b	13.45a	26.40a	4.69a	142.43a
Field pea	47.49a	0.56b	69.0a	6.21a	12.78ab	25.87a	3.83a	113.00a
Oat-vetch	49.82a	0.71ab	79.0a	5.98b	12.45ab	32.15a	3.87a	116.02a
White lupin	44.67a	0.64ab	74.0a	6.09ab	12.13b	23.95a	4.52a	124.52a
Oat	66.50a	0.65ab	76.0a	6.05b	13.05ab	26.86a	4.30a	143.29a

Different letters in the same column indicate significant differences according to Tukey's test ($P \leq 0.05$). MB: Microbial biomass; BD: bulk density; WSA: water-stable aggregation; OM: organic matter.

Statistical analysis

The assays were conducted using a completely randomized block design. To determine the statistical differences in the N fixation of green manure, the effect of green manure on the onion crop, and the evolution of soil properties, collected data was analyzed by ANOVA and Tukey's test for mean separation ($P \leq 0.05$). The statistical analysis was performed with the InfoStat 2020 statistical software (Grupo InfoStat, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina).

RESULTS AND DISCUSSION

Green manure yield, N content, and biological N fixation

Differences ($P \leq 0.05$) were observed between treatments in both seasons (Table 3). The faba-bean treatment obtained the highest production in both years with values of 22 and 17 Mg DM ha⁻¹ in 2017 and 2018, respectively. The white lupin and oat-vetch treatments had the lowest DM values in 2017 and field pea in 2018 due to anthracnose (*Ascochyta* spp.)

The BNF values were also significantly ($P \leq 0.05$) higher in faba-bean with 388 and 369 kg N ha⁻¹ in 2017 and 2018, respectively (Table 3).

The N fixed:DM ratio was higher in the white lupin treatment than faba-bean in both years, which indicated that white lupin had a higher N fixation efficiency with leaf N content greater than 3% in the first year. These values concur with the findings reported by Génard et al. (2016).

Field pea in 2019 had a significantly higher N content at 4.16%. As expected, the oat treatment showed the lowest N content with values of 1.18% and 1.19% in 2018 and 2019, respectively. This concurs with Adams et al. (2016), who indicate that legumes and other N-fixing plants contain higher leaf N levels than non-fixing plants.

Enrico et al. (2020) claimed that there is usually a linear relationship between the amount of N₂ fixed by grain legumes and DM production of the aerial part of the plant; the curve ranges from 15 to 25 kg N ha⁻¹ Mg⁻¹ produced DM. However, oat-vetch and white lupin treatments in 2017 surpassed these values. Nitrogen fixed by white lupin were within the ranges described by Sulas et al. (2016), and varied between 53.3 and 375 kg ha⁻¹ in a Mediterranean climate in Italy in a 2 yr study.

The N derived from atmosphere (Ndfa) values were lower in 2018 (Table 3) because of decreased DM production in all treatments, except the oat-vetch treatment. Reduced yield and Ndfa could be explained by the decrease in rainfall between May and August, which is a critical period that depends on rainfall for the vegetative growth of green manure, as well as minimum average temperature of 1.6 °C between June and August (frost presence, with temperature range of -4.1 to 10.5 °C). Low water availability directly affects the BNF potential through rhizobium viability or C supply to plant nodules; it also affects plant growth and development and its demand for N (Soper et al., 2014).

The 2018 Ndfa values for faba-bean and field pea (74.75% Ndfa and 16.57 Mg DM ha⁻¹ and 62.25% Ndfa and 2.8 Mg DM ha⁻¹, respectively), are similar to those reported by Hossain et al. (2017). Field pea values were slightly different to those obtained by Enrico et al. (2020) in plants under water stress (59% Ndfa and 3.44 Mg DM ha⁻¹). According to Enrico et al. (2020), the Ndfa value for field pea varies between 20% and 75% depending on the environmental conditions. In addition, Jensen et al. (2010) claimed that the %Ndfa from legumes is not only an attribute determined by genotype but

Table 3. Determination of N fixation using different green manure crops.

Season	Crop	DM kg ha ⁻¹	N content %	Accumulated		BNF kg ha ⁻¹	Fixed N kg N Mg ⁻¹ DM
				N kg ha ⁻¹	Ndfa %		
2017	Faba-bean	21868a	2.44c	527a	78.00a	388a	18
	Field pea	12940b	2.97b	352b	79.75a	303b	23
	Oat-vetch	5086c	2.04c	242c	85.00a	205c	40
	White lupin	5634c	3.46a	195cd	88.25a	172c	31
	Oat	11002b	1.19d	131d	-	-	-
2018	Faba-bean	16570a	2.99bc	518a	74.75a	369a	22
	Field pea	2815d	4.16a	110b	62.25bc	68c	24
	Oat-vetch	5459c	2.80c	166b	67.75b	112b	21
	White lupin	3653d	3.49b	146b	58.50c	85bc	23
	Oat	9920b	1.18d	109b	-	-	-

Different letters in the same column indicate significant differences according to Tukey's test ($P \leq 0.05$). Ndfa: N derived from atmosphere; BNF: biological N fixation.

a reflection of the interaction between soil available N and legume growth. These same authors reported Ndfa values ranging from 34% to 99%, which were lower when high fertilizer rates were applied and were close to 100% when no fertilizers were used. Also, they argue that rotating legume green manures in short periods of time will diminish nodule formation and reduce the Ndfa potential, as it was observed in the study. Some soil factors that influence N fixation are the low availability of nutrients without N, pH less than 6.7, and soil temperature less than 10 °C (Krakaliya et al., 2018).

Effect of green manure on the onion crop

The effect of green manure as a preceding crop and its incorporation in the soil caused onion yields to vary between 21.22 and 54.39 Mg ha⁻¹ and 28.68 and 46.71 Mg ha⁻¹ in 2018 and 2019, respectively (Table 4). In 2018, the lowest onion yields were obtained in the white lupin treatment and the highest occurred after faba-bean (Table 4). In 2019, onion had the highest yield after white lupin and oat green manure, respectively; however, onion yield was the lowest after field pea. This suggests that this decrease in yield was attributed to anthracnose, which affected field pea and reduced the plant population and crop development, thus decreasing BNF. The oat control treatment yield remained within the range obtained by the legume green manures in both seasons with nonsignificant differences, probably due to the use of compost in the soil preparation in both seasons. Nutrient absorption and cycling from oat roots in this soil type could be matter of future research.

Onion yields worldwide have averaged 19.7 Mg ha⁻¹ (Bua et al., 2017), while they are approximately 48 Mg ha⁻¹ in Chile (FAO, 2020). González and Herrera (2012) obtained mean yields of 69.5 t ha⁻¹ between 2001 and 2009 when using the same onion variety (Valinia INIA) in soils that share the same agroclimatic zone as the present study, which was located at the Santa Rosa experimental station of INIA Quilamapu. However, conventional management was used and the fertilizer system or rate were not mentioned.

In the present study, each treatment in 2018 was higher than the yields described by Bua et al. (2017), while the faba-bean and oat-vetch rotation exceeded the yield described by the FAO (2020). No treatment reached the yields obtained by González and Herrera (2012); this can be attributed to low soil available P, which was less than the minimum level of 10 mg P kg⁻¹ required by the onion crop according to Khokhar (2019). Suboptimal yields reflect factors such as low soil fertility, pests, and disease; therefore, several inputs are required to improve productivity, including adequate soil fertility management (Bua et al., 2017). The higher yield of onion after the white lupin green manure treatment in the second year can be attributed to the ability of white lupin to develop a cluster root system that secretes large amounts of organic acids such as malate, citrate, and acid phosphatases at the maturity stage to solubilize fixed P in the soil (Aslam et al., 2021). The first year of this green manure treatment had a low population density. Oat on the other hand, has the ability to exudate acid phosphatases that play a key role to solubilize fixed P such as phytates on P deficient soils (Zebrowska et al., 2017). Also, according to Jensen et al. (2010), long legume rotation as well as higher nitric N may delay or even inhibit nodulation, reducing N fixation. This can explain why the oat onion yield competed with legume green manures on a low P-Olsen soil.

Table 4. Effect of green manure incorporation in the crop rotation on yield and leaf N of the onion crop during the 2018 and 2019 seasons.

Season	Treatment	Yield	Leaf N
		Mg ha ⁻¹	%
2018	Faba-bean	54.39a	2.63bc
	Field pea	37.22ab	2.15c
	Oat-vetch	49.08a	2.71b
	White lupin	21.22b	3.11a
	Oat	33.08ab	2.25bc
2019	Faba-bean	35.95ab	2.11a
	Field pea	28.68b	2.31a
	Oat-vetch	33.98ab	2.23a
	White lupin	46.71a	2.14a
	Oat	42.69ab	2.18a

Different letters in the same column indicate significant differences according to Tukey's test ($P \leq 0.05$).

Abou-El-Hassan et al. (2018) mentioned yields ranging from 29.5 to 36.1 Mg ha⁻¹ using a 22.6 Mg ha⁻¹ compost rate in three different onion varieties. Onion yield values in the present study were within the ranges described by the literature or were higher. This could be due to applying 20 Mg compost ha⁻¹ when preparing the soil plus the N contribution of green manures after their incorporation, thus generating a synergistic effect. According to Caliskan et al. (2014), using combined organic amendments can improve yields *vs.* using them separately; they demonstrated the superiority of combining green manure with farmyard manure *vs.* control treatments, conventional fertilization, farmyard manure, and green manure separately by 148%, 39%, 54%, and 34.7%, respectively.

Leaf N content in onion in 2018 was significantly higher in the white lupin treatment (3.11%) and lower in field pea (2.15%). There were nonsignificant differences in 2019 and values ranged from 2.11% to 2.31%. These results are similar to those obtained by Sotomayor-Ramírez et al. (2016), which varied between 2.32% and 2.45% N in onion leaves measured 95 d after transplanting with an applied N rate of 140 to 252 kg ha⁻¹ as fertilizer. The slight difference in leaf N content could be explained by the measurement in the present study, which was carried out 147 d after transplanting *vs.* 95 d in the previously mentioned study; therefore, N could have been translocated from leaves to the bulb.

Effect of green manure on soil biophysicochemical characteristics

Values for MB, BD, and pH in all treatments, except oat in MB (Table 2), were lower compared with the initial soil analysis, which had mean inferiority values of 8%, 21%, and 3%, respectively, and WSA increased by 10%. However, there were nonsignificant differences between treatments. The OM increased between 0.31% and 1.11% in all treatments, except white lupin that decreased by 0.01%. Likewise for P, which only decreased in the field pea treatment by 0.02 mg L⁻¹. Every other treatment slightly improved soil P level from 0.02 to 0.84 mg L⁻¹, which was nonsignificant despite the use of amendments, maybe due to the high P fixation potential of the soil in study (Hirzel et al., 2010). Only soil N and K were significantly higher in 2019 with superior means of 379% and 202%, respectively. These results could indicate changes in the soil quality parameters using green manures and compost over time in contrast to the initial study values, which had sustained naturalized grassland for years. According to Meena et al. (2018), green manures decrease soil pH because they generate organic acids and CO₂ while the OM decomposition occurs. This effect can be perceived in all treatments. It also had a positive effect on the soil physical properties such as BD and WSA, which reflected the cemented effect in the soil with organic acids, amino acids, sugars, vitamins, and mucilage in the OM (Meena et al., 2018). Likewise, different studies have reported improved integral soil quality after green manures were incorporated in the short term (Carvalho et al., 2015; Ciaccia et al., 2017).

The results can be attributed to the application of compost in 2017 and 2018 as organic soil amendment as part of a management plan with agroecological criteria, that contained 3.04% total N. According to De Corato (2020), compost incorporation significantly improved soil physical properties, macro- and micronutrient dynamics, and stimulated microbial activity. Using green manures and organic amendments, such as compost, is a technique that optimizes soil fertility and quality in the long term by increasing organic C inputs (Ciaccia et al., 2017).

Given that nonsignificant differences were found between treatments for the soil N level in the autumn of 2019, it can be thought that the onion crop absorbed part of this nutrient in its growth cycle or could even have been mineralized. According to Krakaliya et al. (2018), this is a process in which there is no gain or loss in the net soil N content, but is part of the soil-plant-atmosphere system cycle.

CONCLUSIONS

Nitrogen fixation of green manure adds N to the soil. The faba-bean treatment had higher biological N fixation and DM production in the 2 yr experiment.

Management practices that incorporate green manures as well as compost can augment onion yield in sustainable agricultural systems without needing to apply mineral N fertilizers.

Green manure incorporated into the soil as well as compost added during soil preparation after 2 yr showed a positive response in total N and available K contents, with nearly 4 and 2 fold, respectively. There were nonsignificant differences between treatments. Physical properties did not change.

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