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Assessment of Contribution of Cover Crop Littering Decomposition to the N Uptake of Bearing and Non-Bearing Satsuma Mandarin Trees

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Abstract: Nitrogen (N) derived from the decomposition of litter from cover crops can contribute to the mineral nutrition of citrus trees. This study aimed to assess the prior contribution of N derived from the decomposition of forage radish (*Raphanus sativus*) and black oats (*Avena strigosa*) to Satsuma mandarin tree N demand. Forage radish and black oats were grown and enriched with ^{15}N stable isotope. Two studies were conducted on (1) non-bearing, potted satsuma mandarin seedlings for 120 days in the greenhouse (Experiment 1) and (2) bearing field-growing Satsuma mandarin trees for 270 days (Experiment 2). Tree growth and total N and ^{15}N concentrations were determined in annual and perennial organs of citrus and soil. The highest value of N derived from the decomposition of cover crop root residues was observed in the leaves and roots of non-bearing trees, while the highest amount of N derived from shoot residue decomposition was observed in the leaves of bearing trees. The results showed little contribution of the decomposition of residues of forage radish and black oats on the total N budget of annual and perennial organs of both bearing and non-bearing Satsuma mandarin trees, probably because the climatic conditions promoted a fast N mineralization and possible losses through volatilization and leaching.

Keywords: N uptake; N distribution; citrus unshiu; cover crops; stable isotopes; nutrient management

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1. Introduction

Citrus is the second most cultivated fruit tree in the world after bananas and plantains combined; its production accounts for 161.8 Mt on more than 10.2 million hectares [1]. China, Brazil, and India are the world's largest citrus producers, and Brazil is the world's largest exporter of orange juice [1]. Rio Grande do Sul (RS), located in Southern Brazil on the border with Uruguay and Argentina, is the sixth largest citrus fruit-producing state in the Country. Between 2018 and 2020, it produced an average of 346,078 t of fruit per year, equivalent to 2.1% of the total produced in Brazil [2].

In Countries with tropical and subtropical climates, such as Brazil, citrus is grown in soils with low organic matter content and, consequently, low availability of native mineral nitrogen (N) [3,4]. For this reason, N-based fertilizers are usually applied to satisfy plant needs [5–7].

Usually, soil in citrus orchards is managed with an inter-row cover crop, mainly composed of forage radish (FR) (*Raphanus sativus* L.) and black oats (BO) (*Avena strigosa*), to dissipate the kinetic energy of raindrops since heavy rainfalls are frequent [8,9]. This can reduce soil degradation and erosion [10,11] and, at the same time, protect soil structure and

increase soil organic matter content. During the season, cover crops are periodically mowed, and shoots, as well as senescent roots, remain in the soil as litter and are decomposed over time [9,12,13].

The N present in cover crop tissues can be released into the soil and absorbed by tree roots [7]; however, little is known about the contribution of shoots and roots of FR and BO to citrus nutrition. The rate of decomposition of cover crops can be affected by the chemical and biochemical characteristics of the tissue [14,15]. Once the residues are mineralized, the N released in the soil can be absorbed and transported to the growing organs, mainly leaves and fruit [13,16–18]. In addition, part of the N can also be stored in perennial organs, such as roots and stems [19–21], and then remobilized to growing organs the following season [12,22,23], reducing the demand for N [18] and the rates of N applied via fertilizers [13], thus reducing the cost of production and the potential losses of nitrate (NO_3^-) into the groundwater [24,25]. In this context, we employed the ^{15}N stable isotope technique to clarify the processes that control the dynamics of release of N and may affect the partitioning of N in citrus trees [26–31].

Therefore, our hypothesis is that the addition of cover crop residues in citrus orchards increases the N concentrations remaining in the organs of citrus trees after mowing by different amounts, with the root residues of cover crops remaining in the soil in non-bearing citrus trees and the aerial part deposited on the soil in bearing citrus trees. The aim of the present study was to evaluate the contribution of the decomposition of shoots and roots of FR and BO to the N uptake of bearing and non-bearing Satsuma mandarin (*Citrus unshiu*) trees in Southern Brazil.

2. Materials and Methods

2.1. Forage Radish and Black Oats ^{15}N Enrichment

Fifty FR and BO seeds were pre-germinated in plastic boxes (Gerbox®, Qualividros, Passos, Minas Gerais, Brazil). Briefly, seeds were placed between two sheets of paper towel (Germitest®, Germilab, Valinhos, Rio Grande do Sul, Brazil) and moistened with distilled water. The seeds were then placed in Biochemical Oxygen Demand (B.O.D.) germinators at a constant temperature of 25 °C, with an 8-h photoperiod. The pre-germination was carried out for nine days on four replicates for each species; after this period, twenty seeds were transplanted into polypropylene pots for a total of 15 pots per species. Twenty-one days after sowing, the smallest 5 plants were removed from the pots, leaving 15 plants for each species in each pot (time 0). Plants were then planted in polypropylene pots with a capacity of 5 L, filled with 4 kg of Argissolo Vermelho soil [32], corresponding to Typic Hapludalf soil [33], collected from the 0–20 cm layer. A soil sample was then air-dried, ground, passed through a 2 mm sieve, and analyzed for physical and chemical characteristics (Table 1). The plants were grown in a greenhouse with controlled temperature (25 °C) and relative humidity (60%) at the Federal University of Santa Maria (UFSM) in Santa Maria, state of Rio Grande do Sul (RS), Southern Brazil. Afterward, urea (46.6% N) ^{15}N 5.21 atom% enriched was applied to the pots; the enriched fertilizer was diluted in distilled water and applied six times (0, 5, 10, 13, 28, and 34 days after time 0) on the surface of the pots. In total, 159.1 g of urea were applied. At each application, 26.5 g of urea diluted in 2000 mL, at a rate of 65 mL per plant, was used. Pots were irrigated daily with distilled water to keep the soil at ~70% of field capacity during the experimental period, using the method of water loss as a function of time (i.e., over 24 h), as previously described [34]. At 15 and 30 days after time 0, pots were watered with 50 mL of a nutrient solution containing (in g L^{-1}) 0.46 of H_3BO_3, 1.18 of $CuSO_4$, 4.4 of $ZnSO_4$, and 88 of KH_2PO_4.

Forty-two days after time 0, the shoots of the FR and BO were cut off close to the soil surface, and a sub-sample of each cover crop was used for chemical characterization. Roots were gently separated from the soil and washed with distilled water, and a sub-sample was used for chemical characterization. The sub-samples (shoot and roots) of each species were dried in a forced-air oven at 65 °C until they reached a constant weight, ground in a Willey mill, and analyzed. Total N and C concentrations were determined using an elemental

analyzer (Thermo Scientific, Flash EA 1112, Milan, Italy) (Table 2); ^{15}N isotope enrichment was determined by mass spectrometry (Thermo Scientific, Delta V Advantage, Bremen, Germany) (Table 2). Thus, during the management of citrus orchards, the maintenance of cover crops' root residues that remain in the soil in the initial years of citrus cultivation (Experiment 1) and the deposition of the aerial part of cover crops that remain on the surface of the soils of citrus orchards in production (Experiment 2), were evaluated in this study.

Table 1. Main physical and chemical characteristics of the soil used in experiments 1 and 2, at the 0–20 cm depth.

Characteristics	Units	Experiment 1	Experiment 2
Clay (pipette method)	g kg^{-1}	110	140
Silt (pipette method)	g kg^{-1}	261	-
Sand (pipette method)	g kg^{-1}	630	-
Organic matter (Walkley and Black 1934)	g kg^{-1}	9.6	12
pH in H_2O (1:1)	-	6.5	5.8
Total N (Kjeldahl method)	%	1	0.06
Mineral N (extracted by KCl 1 mol L^{-1})	mg kg^{-1}	25	-
Exchangeable Al (extracted by KCl 1 mol L^{-1})	mg kg^{-1}	0	0
Exchangeable Mg (extracted by KCl 1 mol L^{-1})	mg kg^{-1}	135	48.6
Exchangeable Ca (extracted by KCl 1 mol $L^{-1)}$	mg kg^{-1}	542	641
Available P (extracted by Mehlich-1)	mg kg^{-1}	5.1	53.3
Available K (extracted by Mehlich-1)	mg kg^{-1}	175	44

Table 2. Chemical characterization of root and shoot residues of forage radish and black oat at the beginning of the experiment.

Variable	Forage Radish	Black Oat
Total N (g kg^{-1} dry weight)	28	18
Total organic carbon (g kg^{-1} dry weight)	430	403
C/N ratio	15	22
Root		
N (g kg^{-1})	21.7	18.3
At % ^{15}N	3.76	3.88
Shoot		
N (g kg^{-1})	20.3	19.9
At % ^{15}N	3.62	3.94

2.2. Experiment 1—Use of ^{15}N-Enriched Litter of Forage Radish and Black Oat Root as a Source of N for Non-Bearing, Potted Satsuma Mandarin Trees

In September, the experiment was set up in a greenhouse in Santa Maria, Rio Grande do Sul, in Southern Brazil, with the average air temperature inside the greenhouse at 25 °C and the average humidity at 60%. Polypropylene pots were filled with the same soil used for growing FR and BO cover crops (Table 1). The soil was air-dried, ground to pass a 2 mm mesh sieve, and fertilized with 0.25 g of P pot^{-1} and 4.0 g of K pot^{-1} as triple superphosphate and potassium chloride. According to a complete randomized experimental design, with six replicates (pot), the following treatments were compared: (1) ^{15}N-enriched FR root litter application and (2) ^{15}N-enriched BO root litter application.

The ^{15}N-enriched root litter was applied to the soil at a rate of 1.29 g DW pot^{-1}, corresponding to approximately 25 mg N pot^{-1}. Seedlings of 'Okitsu' Satsuma mandarin were transplanted into each pot, trained to a single shoot, and watered daily by gravimetric determination of water lost in the previous 24 h, to maintain soil moisture at 70% of field capacity, as described above.

Plant and Soil Analysis

One hundred and twenty days after transplanting, Satsuma mandarin were harvested. Shoots were cut off close to the soil surface and separated into leaves and stems. Roots were manually separated from the soil. Leaves, stems, and roots were dried in an oven with forced air circulation at 65 °C, ground in a ball mill, homogenized, and analyzed for total N concentration and ^{15}N. Soil from the pots was collected and homogenized, and a sub-sample was air-dried, ground, passed through a 2 mm sieve, and analyzed for total N and ^{15}N concentrations.

2.3. Experiment 2—Use of ^{15}N-Enriched Litter of Forage Radish and Black Oats Shoot as a Source of N for Bearing Satsuma Mandarin Trees

This study was carried out on 7-year-old bearing citrus trees located in the municipality of Rosário do Sul, state of Rio Grande do Sul, Southern Brazil (30°15′30″ S, 54°54′51″ W). According to Köppen–Geiger, the climate is classified as "Cfa", i.e., subtropical and humid with hot and humid summers. The average temperature during this study was 18.8 °C, and the average annual rainfall was 183.75 mm (Figure 1), being that the average annual temperature is 20 °C and the average annual rainfall is 123 mm in Rosário do Sul. The soil is the same as used in experiment 1 (Table 1). 'Okitsu' Satsuma mandarin trees were spaced 3 m between plants and 7 m between rows. According to a complete randomized experimental design with three replications, the following treatments were compared: (1) FA ^{15}N-enriched shoot litter application and (2) BO ^{15}N-enriched shoot litter application. The enriched shoots were applied at the rate of 21 g DW plant^{-1}, approximately 0.4 g of N per plant, corresponding to the rate of one cut during the growing season. Enriched residues were placed on the soil in August 2015 in an area of 0.16 m^2 (0.4 m × 0.4 m), where the center of the area was the stem of each tree. A white, 2 mm mesh net was placed above the fertilized area to prevent the litter from being scattered by the wind.

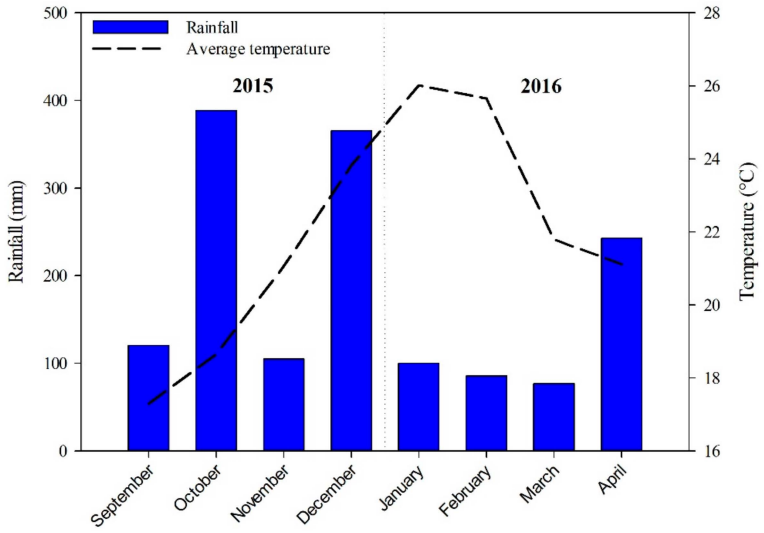

Figure 1. Monthly accumulated rainfall after deposition of litter bags in the experimental field, in Rosário do Sul, during the experiment.

Plant and Soil Analysis

In August 2015, at the same time of treatment application, leaves were collected from the middle third of the shoot of the year, from the four quadrants of each tree, dried in a forced-air oven at 65 °C until they reached a constant weight, ground in Willey-type mills (2 mm mesh), and submitted to chemical analysis. In the study area, all cover crops were removed to avoid competition with trees. Later, from September (corresponding to March in the Northern Hemisphere) 2015, tree leaves were collected monthly until February

(corresponding to August in the Northern Hemisphere), as described above, dried at 65 °C, ground, and analyzed for total N and ^{15}N concentration.

In April 2016, all the fruits from the treated trees were collected and counted, and the total yield was recorded. Ten fruits per plant were used for analysis of the total N and ^{15}N concentrations.

After harvest, a trench, in the same position of 0.16 m^2 where ^{15}N-enriched litter was placed, was opened, and soil samples were collected at 0–5, 6–10, 11–15, and 16–20 cm of depth. The soil was air-dried, ground, and passed through a 2 mm mesh sieve. The soil was analyzed for total N and ^{15}N concentration.

In April 2016 (270 days after treatment application), trees were destructively harvested, and the canopy was divided into stems, branches (age > 2 years), current-year shoots (1-year-old), and leaves. All the roots were removed, separated from the soil, and washed with tap water and then with distilled water. All organs were weighed, and then a subsample was taken and dried at 65 °C, ground, and analyzed for total N and ^{15}N.

2.4. Calculations and Statistical ANALYSIS

The excess ^{15}N atoms in the tissue and soil samples was calculated using equations proposed by [35] using Equation (1):

$$\text{Excess } ^{15}\text{N sample (\%)} = \%^{15}\text{N atom.sample-0.3663\%} \quad (1)$$

The percentage of N derived from the shoot residues (NDFS) or roots residues (NDFR) was calculated using Equation (2):

$$\text{NDFRES (\%)} = [(\text{\% excess } ^{15}\text{N sample})/(\text{\% excess } ^{15}\text{N residue})] \times 100 \quad (2)$$

where NDFRES is N derived from residues.

The results obtained in experiment 1 were tested for normality using the Shapiro–Wilk test and homogeneity of variance using the Levene test, and when the assumptions were met, the *t*-test was used to compare the two cover plant species. When no difference was observed in the variables between the cover plant species, analysis of variance (ANOVA) was carried out with the mean value of the treatments to compare the citrus organs (leaves, stem, and roots) and their influence on plant growth, total N, and atoms of excess ^{15}N, NDFS, and NDFR. ANOVA was carried out to check for significance using the F test, and when the effect was significant, the data were subjected to Tukey's mean comparison test ($p < 0.05$). The N concentration derived from the shoot of the residues, as well as the total N and ^{15}N of the citrus leaves collected at different times, were checked for normality using the Lilliefors test ($p < 0.01$). When normality was not met, the analysis of variance was carried out using the Kruskal–Wallis test ($p < 0.05$), and the means were separated using the Tukey test ($p < 0.05$).

The results of the variables analyzed at the end of experiment 2 were tested for normality using the Shapiro–Wilk test and homogeneity of variance using the Levene test. When the assumptions were met, the *t*-test was used to compare the cover plant species. When no difference was observed between the cover plant species, ANOVA was carried out to compare the citrus organs (leaves, pulp, branches of the year, twigs of the year, stems, and roots) and their influence on plant growth, total N, and atoms of excess ^{15}N, NDFS, and NDFR. A repeated time design was used to compare the effect of the cover crop over time on total N, ^{15}N atom excess, and NDFS in the leaves of bearing trees. When ANOVA was significant at $p < 0.05$, mean comparisons were made using the Skott Knott test ($p < 0.05$). The soil layers were subjected to ANOVA using the F-test, and when significant, the means of the treatments were separated using the Tukey test at $p < 0.05$ probability.

3. Results

3.1. Experiment 1—Use of ^{15}N-Enriched Litter Derived from the Decomposition of Forage Radish and Black Oat Root Residues as a Source of N for Non-Bearing Young Potted Satsuma Mandarin Trees

The application of root residues of FR and BO did not affect tree growth, total N concentration, ^{15}N atom excess, and NDFR (Table 3). Roots of citrus accounted for the highest dry weight, with 28.8% and 52.8% more dry weight than stem and leaves, respectively (Table 4). Total N was 148% and 169% higher in roots and leaves, respectively, than in stems, while ^{15}N atom excess was higher in leaves than in stems, while roots showed intermediate values (Table 4). The NDFR and total N were higher in roots and leaves than in stems, 206% and 347% more NDFR in roots and leaves, respectively (Table 4).

Table 3. Effect forage radish and black oat root residues application on non-bearing potted satsuma mandarin dry matter, total N, ^{15}N atom excess, N derived from forage radish and black oat root residues in young potted citrus plants grown in greenhouse.

Cover Crop [(1)]	Citrus Tree ORGANS		
	Leaves	**Stems**	**Roots**
	Dry Matter (g tree^{-1})		
Forage Radish	32.4 ± 2.11	36.5 ± 1.63	51.0 ± 0.87
Black Oats	31.9 ± 0.48	39.9 ± 0.31	47.3 ± 2.73
Significance	ns	ns	ns
	Total N (mg tree^{-1})		
Forage Radish	586 ± 58	216 ± 15	645 ± 82
Black Oats	814 ± 52	305 ± 19	648 ± 74
Significance	ns	ns	ns
	^{15}N atom % excess		
Forage Radish	0.120 ± 0.012	0.088 ± 0.013	0.108 ± 0.011
Black Oats	0.154 ± 0.028	0.082 ± 0.012	0.100 ± 0.003
Significance	ns	ns	ns
	N derived from roots litter (NDFR) (mg tree^{-1})		
Forage Radish	18.9 ± 3.17	5.08 ± 0.90	18.9 ± 3.91
Black Oats	32.8 ± 7.74	6.47 ± 1.11	16.6 ± 1.66
Significance	ns	ns	ns

[(1)] Control treatment = Natural abundance of ^{15}N (0.3663 atoms%). Means ± SE (n = 3). ns: not significant.

Table 4. Distribution of dry matter (DM), total N and ^{15}N atom% excess, N derived from root litter (NDFR) in non-bearing citrus trees.

Organ	DM (g Tree^{-1})	Total N (mg Tree^{-1})	^{15}N (atom% Excess)	NDFR (%)	NDFR (mg Tree^{-1})
Leaves	32.2 ± 0.97 c [(1)]	700 ± 62 a	0.137 ± 1.18 a	3.57 ± 0.39 a	25.8 ± 4.87 a
Stem	38.2 ± 1.06 b	260 ± 23 b	0.085 ± 0.04 b	2.23 ± 0.22 b	5.78 ± 0.71 b
Roots	49.2 ± 1.53 a	646 ± 49 a	0.104 ± 0.08 ab	2.71 ± 0.14 ab	17.7 ± 1.97 a
Significance	*	*	*	*	*
CV (%)	7.02	18.17			40.81

[(1)] Means ± SE (n = 3) values followed by the same letter are not statistically different by the Tukey test ($p > 0.05$). * Effect significant at $p < 0.05$. CV: Coefficient of variation in cover crop residue error 1.

One hundred and twenty days after root residue deposition, a higher total N, ^{15}N atom excess (%), and NDFR in the soil with FR compared to BO root deposition was observed (Table 5).

Table 5. Effect of cover crop root residues on the concentrations of total N, ^{15}N atom excess, and N derived from roots (NDFR) in soil of experiment 1 after 120 days from the deposition of root residues.

Cover Crop [(1)]	Total N (%)	^{15}N at.% Excess	NDFR (%)
Forage radish	0.069 ± 0.0057	0.014 ± 0.0003	0.358 ± 0.0087
Black oat	0.054 ± 0.0011	0.010 ± 0.0006	0.264 ± 0.0171
Significance	**	ns	ns

[(1)] Control treatment = Natural abundance of ^{15}N (0.3663 atoms%). **, ns: effect significant at $p \leq 0.01$ or not significant, respectively. (Means ± SE n = 3).

3.2. Experiment 2—Use of ^{15}N-Enriched Litter Derived from the Decomposition of Forage Radish and Black Oats Shoot as a Source of N for Bearing, Field-Grown Satsuma Mandarin Citrus Trees

Interaction between sampling time and cover crop was not significant for total N, ^{15}N atom excess, and NDFS (%) in citrus leaves; in addition, no effect of soil application of FR and BO residues was observed on the three fractions of N (Figure 2). Total N was higher in September after the residues were positioned, and after a sharp decrease was observed in October, the values remained almost stable (Figure 2). The opposite was observed for ^{15}N atom excess and NDFS, which showed the lowest values in September and then increased in October and remained almost stable (Figure 2).

Organ dry weight, total N concentration, ^{15}N atom excess (%), NDFS, and amount of N were not statistically influenced by the type of residues applied to the soil, and therefore, the data of the two cover crops were pooled together (Table 6). Citrus stems and roots accounted for the highest biomass, followed by branches, leaves, twigs of the year, and pulp (Table 7). The highest concentration of total N (g kg^{-1}) was observed in citrus leaves, representing 35.3% of the total N of the organs. The highest amount of N (mg plant^{-1}) was found in leaves and roots, which together represented 57.1% of N per plant. The highest percentages of ^{15}N atom excess and NDFS (g kg^{-1}) were observed in the pulp and leaves of citrus plants (Table 7). The highest amount of NDFS (mg plant^{-1}) was found in the leaves of bearing citrus plants, which represented 52.5% of NDFS of the organs.

Soil application of plant residues did not affect the concentration of total N, ^{15}N atom excess, and the N derived from shoot residues in the different soil depths investigated (Table 8).

Table 6. Effect forage radish and black oat shoot residue application on bearing Satsuma mandarin tree dry matter (DM), total N concentration, ^{15}N atom excess, and N derived from shoot litter (NDFS).

Citrus Tree Organ	Cover Crop	
	Forage Radish	Black Oats
	Dry Matter (kg tree^{-1})	
Pulp	0.53 ± 0.05	0.55 ± 0.10
Leaves	1.66 ± 0.27	1.97 ± 0.32
Twigs of the year	0.62 ± 0.10	0.55 ± 0.13
Branches	2.57 ± 0.47	2.78 ± 0.28
Stems	4.16 ± 0.58	3.86 ± 0.61
Roots	4.30 ± 0.82	4.95 ± 1.20
Significance	ns	ns
	Total N (g organ^{-1})	
Pulp	5.13 ± 0.98	6.05 ± 1.22
Leaves	34.38 ± 5.54	41.11 ± 6.17
Twigs of the year	6.61 ± 1.35	5.27 ± 1.18
Branches	17.17 ± 4.73	18.01 ± 1.05
Stems	20.81 ± 4.08	20.74 ± 0.83
Roots	27.34 ± 9.77	26.53 ± 5.69
Significance	ns	ns

Table 6. *Cont.*

Citrus Tree Organ	Cover Crop	
	Forage Radish	**Black Oats**
	^{15}N (atom% excess)	
Pulp	0.0069 ± 0.0017	0.0068 ± 0.0004
Leaves	0.0060 ± 0.0006	0.0048 ± 0.0003
Twigs of the year	0.0039 ± 0.0008	0.0034 ± 0.0007
Branches	0.0025 ± 0.0007	0.0026 ± 0.0005
Stems	0.0017 ± 0.0001	0.0019 ± 0.0000
Roots	0.0009 ± 0.0000	0.0011 ± 0.0000
Significance	ns	ns
	NDFS (mg organ^{-1})	
Pulp	9.10 ± 4.02	10.41 ± 2.43
Leaves	46.80 ± 4.03	50.18 ± 9.38
Twigs of the year	6.02 ± 1.57	4.85 ± 1.86
Branches	8.98 ± 0.96	12.17 ± 3.00
Stems	7.95 ± 0.92	9.63 ± 0.15
Roots	6.15 ± 2.67	7.56 ± 1.61
Significance	ns	ns

Means ± SE (n = 3). ns: effect not significant.

Table 7. Distribution of dry matter (DM), total N concentration, ^{15}N atom excess, and N derived from shoot litter (NDFS) of bearing Satsuma mandarin tree.

Organ	DM	N		^{15}N	NDFS	
	(kg Tree^{-1})	(g kg^{-1})	(g Organ^{-1})	(Atom% Excess)	(% of Total N in the Organ)	(mg Organ^{-1})
Pulp	0.544 ± 0.05 d	10.2 ± 0.61 b	5.596 ± 0.73 c	0.0068 ± 0.0008 a	0.16 ± 0.02 a	9.75 ± 2.12 b
Leaves	1.820 ± 0.20 c	20.8 ± 0.23 a	37.746 ± 4.00 a	0.0054 ± 0.0004 a	0.13 ± 0.01 a	48.5 ± 4.63 a
Twigs of the year	0.626 ± 0.08 d	10.2 ± 0.40 b	6.409 ± 0.84 c	0.0039 ± 0.0005 b	0.09 ± 0.01 b	6.24 ± 1.11 b
Branches	2.757 ± 0.25 b	6.53 ± 0.32 c	18.154 ± 2.17 b	0.0027 ± 0.0004 c	0.07 ± 0.01 c	11.5 ± 1.66 b
Stems	4.011 ± 0.38 a	5.29 ± 0.43 c	20.774 ± 1.86 b	0.0018 ± 0.0000 c	0.04 ± 0.00 c	8.79 ± 0.56 b
Roots	5.045 ± 0.69 a	5.91 ± 0.49 c	30.093 ± 5.06 a	0.0011 ± 0.0000 d	0.02 ± 0.00 d	7.69 ± 1.43 b
Significance	*	*	*	*	*	*
CV (%)	16.44	6.20	18.1			19.6

* Effect significant for $p < 0.05$ by the F-test. Means ± SE (n = 3) followed by the same letter are not statistically different ($p > 0.05$). CV: Coefficient of variation in cover crop shoot residue error 1.

Table 8. Effect of soil application of black oat and forage radish shoot residues on the concentration of total N, atoms of excess ^{15}N, and N derived from shoot residues at different depths of soil cultivated with bearing citrus tree.

Cover Crop	Total N (%)	^{15}N (at.% Excess)	N Derived from Shoot (%)
forage radish	0.0315 ± 0.0037	0.0025 ± 0.0004	0.0597 ± 0.0099
black oat	0.0575 ± 0.0191	0.0050 ± 0.0022	0.1242 ± 0.0553
Siginificance	ns	ns	ns
Depth (cm)	Total N (%)	^{15}N (at.% excess)	N derived from shoot (%)
0–5	0.0411 ± 0.0046	0.0028 ± 0.0005	0.07 ± 0.01
6–10	0.0360 ± 0.0026	0.0022 ± 0.0003	0.05 ± 0.01
11–15	0.0726 ± 0.0391	0.0022 ± 0.0002	0.05 ± 0.01
16–20	0.0281 ± 0.0036	0.0078 ± 0.0043	0.19 ± 0.11
Siginificance	ns	ns	ns
CV%	4.05	0.66	12.14

ns: not significant for the F-test. CV: Coefficient of variation related to the cover plant residue type factor of error 1. Means ± SE (n = 3).

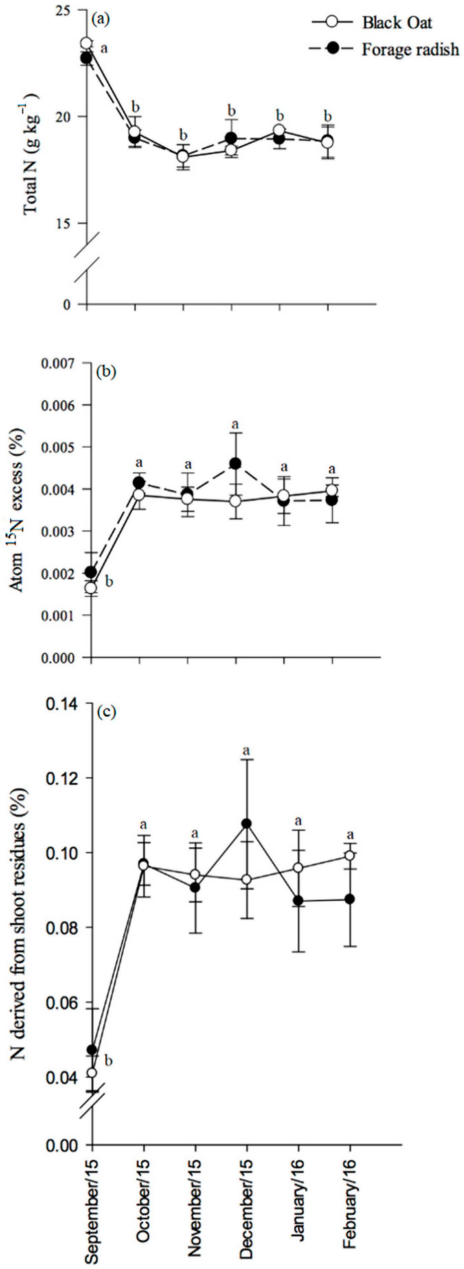

Figure 2. Effect of soil application of black oat and forage radish litter on the concentration of total N (**a**), atom ^{15}N excess (**b**), and N derived from shoots—NDFS (**c**) in bearing satsuma mandarin leaves during the vegetative season. Vertical bars indicate standard error (n = 3). Means followed by the same letter are not statistically different by Tukey test ($p < 0.05$).

4. Discussion

4.1. Experiment 1

Application of two different root residues to young non-bearing potted citrus seedlings showed a similar effect in terms of N uptake; however, the soil fertilized with forage radish showed higher total N, ^{15}N atom excess, and total N than that fertilized with black oat. The different responses can be explained by considering different C and N compositions of the two cover crop residues that promoted a different release of N into the soil. Probably, the low C/N ratio (<20) of cruciferous species, such as forage radish, resulted in a higher mineralization rate, increasing the availability of N [36,37] than black oats.

Leaves and roots showed higher concentrations of total N and N derived from root litter than the other tree organs (stem, one-year-old shoot) due to the higher sink strength of

these organs. Part of the N existing in roots can be accumulated in the form of amino acids at the end of the season [38] and can be degraded in spring, leading to N redistribution to other growing annual organs, especially leaves [18,39,40]. Growing leaves have intense cell division and, therefore, are a sink of N [41]. However, less than 4% of the N existing in all the organs of young citrus was derived from root decomposition, while more than 96% was derived from the soil N and the mineralization of native organic matter since N fertilizers were not applied. However, even in orchards subjected to fertilizer application, studies reported that young citrus plants often take advantage of small amounts of N supplied by cover crop residues [42–45]. Considering the short-term nature of the investigation, the results obtained in this study confirm the importance of correct soil management strategies, such as the use of cover crops as a source of N, such as organic matter, which can be mineralized, increasing the forms of mineral N available for plant uptake [11,45].

4.2. Experiment 2

The C/N ratio of forage radish and black oat (15 and 22, respectively) that we obtained in our study may have contributed to the availability of mineral N derived from the decomposition of the residues of the two cover crops increased in the soil shortly after deposition; however, less than 0.14% of the N present in citrus leaves was derived from the decomposing shoot residues of the black oat and forage radish, meaning that this fraction was irrelevant in comparison to mineral N forms derived from the mineralization of organic matter and N fertilization carried out in previous cycles that accounted for more than 99% of the N present in leaves. The low percentage of N derived from the decomposition of shoot residues of forage radish and black oats in citrus leaves could be caused by the loss of N by volatilization [46], especially in high-temperature regions such as those of Southern Brazil, leaching [47,48], or by surface runoff [49,50] or even moved far from the citrus root system, decreasing the root interception and uptake.

The decrease in total N concentration in citrus leaves over the months was probably due to dilution, caused by the increase in leaf area and, consequently, dry matter [51]; in addition, part of the N present in leaves may have been redistributed to other growing annual organs, especially the fruit [44,52,53].

The higher percentages of ^{15}N atoms excess and NDFS in leaves and pulp at harvest time were due to intense cell division and clogging, which increases the dry matter of these two annual organs, which are normally a sink of nutrients such as N [54,55]. However, more than 96% of the N present in roots and stems was derived from the mineralization of organic matter [22,56,57] since no nitrogen fertilizer was applied throughout the experiment. Thus, the results indicate that the N derived from the decomposition of the shoot of cover crops such as forage radish and black oats contributes little to citrus nutrition. However, the residues can contribute to dissipating raindrop energy and reduce erosion [58–60].

In light of the above, orchard management can be very complex and compromise the long-term viability of exploration. This is because, usually, the productivity of an orchard can last 15 to 20 years, during which time it is affected by the management practices imposed by the farmer. In this way, short-term studies contribute to supporting the establishment of management strategies for the viability of orchards, as well as increasing sustainability and the cost/benefit ratio for the producer [61–63].

5. Conclusions

In our experimental conditions, the contribution of cover crops to citrus nutritional status was little. This result could be related to the short duration of the experiment; however, we believe that losses of N through leaching, volatilization, and possible runoff played an important role in the small amount of N from residues found in citrus plants. Pot experiment showed that the mineralization of residues is quite fast; consequently, it is important to use this source of N when root uptake is intense to reduce losses in the environment; otherwise, cover crops can be used to produce high C:N manure with a low mineralization rate to apply as an amendment.

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