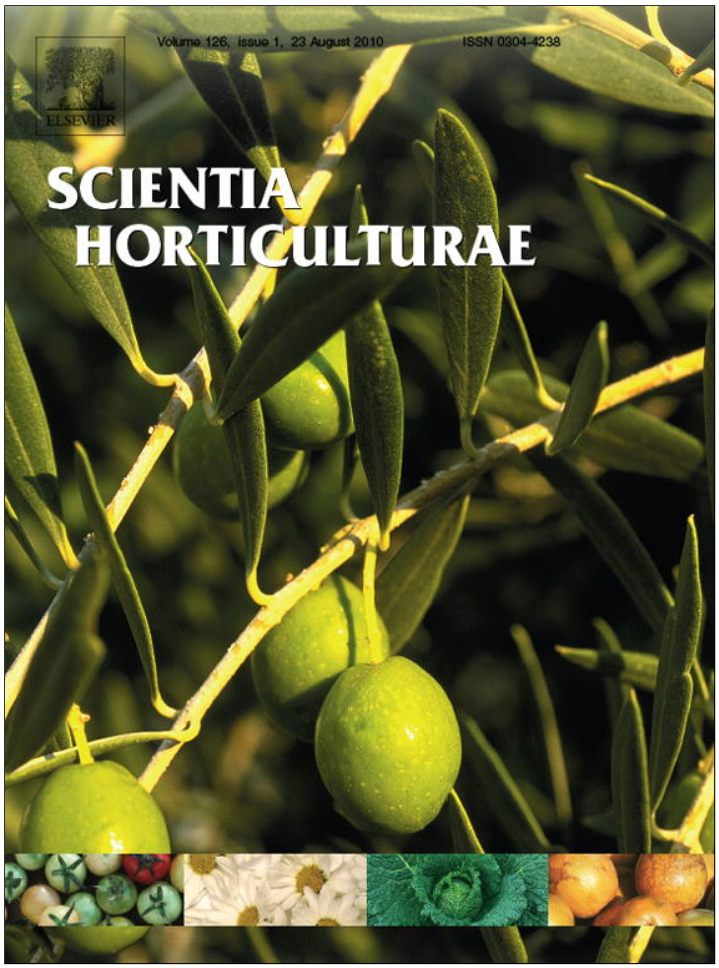

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Isotopically-labelled nitrogen uptake and partitioning in sweet cherry as influenced by timing of fertilizer application

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ABSTRACT

Sweet cherry (*Prunus avium* L.) is a fruit of increasing economic importance though it is less significant than other stone fruit species such as peach. Cherry has received little attention concerning nitrogen (N) uptake and dynamics in mature trees. The aim of this work was to determine N uptake and partitioning as influenced by the timing of fertilizer application in 7-year-old sweet cherry trees cultivated in a cold region (Los Antiguos, Santa Cruz, Argentina; 71°38′ W, 46°32′ S). Nitrogen (95 kg ha^{-1}) was applied as ammonium nitrate to a soil with 'Bing' sweet cherry trees grafted onto *Prunus mahaleb* rootstocks. Fertilization was split into two equal applications per treatment, involving either the commercial fertilizer ammonium nitrate or the same fertilizer labelled with ^{15}N isotope (10% atom.). Treatments consisted of one early spring (full bloom, October 2005) or one summer (late January 2006, 15 days after harvest) application of ^{15}N ammonium nitrate to three replicate trees. Fruit were harvested in early January and leaves were collected at both full canopy and leaf fall. All trees were excavated in winter (August, 2006). Trees were partitioned into their components: trunk, branches (current-season shoots, 1-year-old and over-1-year-old branches), buds of the same age, small roots (less than 1 mm thick), large roots, leaves (sampled in February and April), and fruit (collected at harvest). Those components were dried and analysed for total N and ^{15}N content. Total N per tree and N content derived from the fertilizer did not differ between treatments. Summer postharvest ^{15}N application partitioned not only to structural components (trunk and roots) but also to buds and leaves. Uptake efficiency was significantly ($p = 0.0113$) higher in the spring than in the summer application (65.7% vs. 37.44%). Nevertheless, 52.5% of N applied in spring was lost due to harvest and summer pruning. This emphasizes the importance of the postharvest N fertilization which increases N accumulation in both reserve organs and buds though, according to our data, it is less efficiently used. The extent of nitrogen uptake, efficiency of use and partitioning in the following growing seasons are still open questions that deserve further research.

1. Introduction

Nitrogen is a highly mobile nutrient that plays a major role in different plant metabolic processes supporting tree growth, flower induction and quality, ovule fertilization, and fruit set and development (Sánchez et al., 1995; Sanzol and Herrero, 2001; Tagliavini and Millard, 2005). In sweet cherry (*Prunus avium* L.) a deciduous tree usually grown in temperate regions with moderately cold winter temperatures, the flowering period starts in the absence of leaves at the beginning of spring, thus depending upon the reserves stored in the tree during the previous season (Flore and Layne, 1999; Lichou et al., 1990; Longstroth and Perry, 1996). Fruit development, spring flush of vegetative growth and bud differentiation occur simultaneously resulting in competition between organs for the available resources (Hanson and Proebsting, 1996). In addition, sweet cherry is the earliest deciduous tree fruit to mature each summer. The whole fruit ontogeny may only extend 60–80 days from flowering to fruit maturity, with a rapid period of cell division that can last just 2 weeks (Kappel, 1991; San-Martino et al., 2008).

Many previous studies have analysed the effects of N fertilizer application in different fruit tree crops, but there is little published information about the effects of N, either in young (Dencker and Hansen, 1994; Neilsen et al., 2004) or adult sweet cherry trees (Azarenko et al., 2008). Seasonal patterns in deciduous fruit trees (Jordan et al., 1998) suggest that N uptake is low during spring. Thus, fruit and leaf demand for N is mainly fulfilled by redistribution of the N accumulated during the previous season and situated in structural tree components, such as roots, trunk and branches (Grassi et al., 2002; Quartieri et al., 2002; Tagliavini et al., 1996). Subsequently, N uptake peaks during maximum tree growth, slows

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during fall and reaches a minimum at leaf fall (Jordan et al., 1998; Peterson and Stevens, 1994). During this last period, N is stored as proteins in different above-ground organs and as amino acids in the roots (Tagliavini and Millard, 2005).

Knowledge of tree internal N dynamics is important to determine the timing and amount of N supply in spring and summer. For example, in sweet cherry trees, N applied early in the spring–before bud burst– will not be available for fruit cell division, which influences final fruit size. Three weeks later, N supply distributes to aerial organs (Grassi et al., 2002). Nitrogen applied after harvest is stored mainly in roots, as previously described for sweet cherry (Azarenko et al., 2008), apple (Aguirre et al., 2001; Khemira et al., 1998), pear (Quartieri et al., 2002) and peach trees (Tagliavini et al., 1999).

Utilization of ^{15}N tracers is the main method used to estimate N storage pools and redistribution in trees (Kraimer et al., 2001). Using labelled N in forest trees, Millard (1993) determined that N uptake in fall may contribute to N storage and that N translocation from leaves may be very low. In 2-year-old sweet cherry trees, Grassi et al. (2002) determined that N remobilization began soon after budbreak, that is, prior to N acquisition and lasted up to 60 days if trees had sufficient N stored. According to this, the best management practice for sweet cherry orchards consists of increasing the amount of storage N and its availability at the beginning of the season. This could be achieved via N application during late spring or early summer for the subsequent year and would not affect N levels in fruit. The amount, timing and kinetics of N taken up by sweet cherry trees over a season are valuable data for guiding N fertilization in orchards. However, little information is available on N uptake and partitioning in mature sweet cherry trees, as influenced by timing of fertilizer application.

On account of the economic importance of cherries, there is a scientific interest in identifying the magnitude of N uptake and seasonal patterns of N partitioning at the whole-canopy level. The aim of this work was to determine N uptake and partitioning in mature sweet cherry trees grown in a cold region with a short growing season, as influenced by timing of fertilizer application. The hypothesis was that, due to the early harvest of the fruit, the application of N fertilizer immediately after harvest would optimize N storage for this species.

2. Materials and methods

2.1. Experiment site and crop characteristics

Research was performed in the Los Antiguos valley (71° 38′, W 46° 32′ S), province of Santa Cruz, Argentina. Sweet cherry orchards have been established and managed in the valley since the early 1970s. The climate is cold and semiarid with high global irradiation and low relative humidity. On account of the relatively high latitude, this zone has a longer daytime duration in summer; this advantage partly offsets its low mean daily light values. Mean monthly temperatures vary between 2.5 °C (June) and 14.9 °C (January), with an absolute minimum of −11.6 °C in July. Rainfall is not strictly seasonal, but almost 60% of total rainfall takes place in winter, with a mean of 192 mm per year (San-Martino and Manavella, 2004). Since effective rainfall reaches only 47.6 mm from October (full bloom) to May (leaf fall), sweet cherry orchards were irrigated daily between October and March, at an average rate of 50 m^{-3} ha^{-1} day^{-1} to fulfil tree water requirements. Soils are mostly associations between Entic Haploxerols of different texture, Torrifluvents and Torriortents (Irisarri et al., 1990).

The orchard soil characteristics were determined as previously described (Sparks et al., 1996) for 0–30 cm and 30–60 cm depth: pH (soil:water, 1:2.5): 6.1 and 7.0, respectively; organic matter content (Walkley–Black method): 3.02% and 1.10%; N (Kjeldhal method): 0.162% and 0.074%; available P (Bray method): 251 mg kg^{-1} and 25 mg kg^{-1}; K (ammonium acetate method): 3 meq kg^{-1} at both depths. The soil texture (Bouyoucos method; Klute, 1986) has been classified as loam at both depths. No N fertilizer was applied in the previous year to attain a stronger N uptake but K, Zn, Ca and Mg were applied as previously described (San Martino et al., 2006). In brief, each tree received 100 g K_2O (as K_2SO_4), and the orchard was foliar fertilized 3 times during spring with 82 g Zn in 100 L water (as $ZnSO_4$) and 150 g Mn in 100 L water (as $MnSO_4$). Also 175 g Ca in 100 L^{-1} water (as $CaCl_2$) were applied twice during fruit development to overcome site specific deficiencies. Leaves were sampled for foliar analysis in January, prior to labelled nitrogen application. According to Temminghoff (2000), leaves showed normal values of N (2.22%), P (0.21%), K (1.48%), Mg (0.62%), and B (24 ppm), and low concentrations of Ca (1.17%), Mn (23 ppm) and Zn (9 ppm). Cherry is considered one of the most susceptible crops to Zn deficiency. In our experiment, plants did not develop severe or visible symptoms of stress (rosettes and small yellow leaves), but sub-clinical or marginal deficiencies could have affected tree growth and fruit yield. Nevertheless, previous attempts to introduce higher Zn supplementations did not improve Zn levels in leaves.

2.2. Field experiment and analysis

Six 7-year-old sweet cherry (*P. avium* L. 'Bing') trees grafted onto *Prunus mahaleb* rootstocks were selected among others with similar growth and trunk cross-sectional area (TCSA) in winter, prior to fertilizer treatments and randomly assigned to two treatments (3 trees per treatment). The training system was a Spanish bush system (vase-shaped tree, non-supported, with 4 main branches) with a density of 1000 trees ha^{-1} (tree spacing: 2 m × 5 m). Fertilizer was applied as a split application during early spring (full bloom, October) and summer (after harvest, January) in both treatments. Each tree received a total amount of 95 g N, which is the recommended mean rate in the valley. The total amount was split into two equal doses applied in spring and in summer. Treatments were: (1) ^{15}N labelled ammonium nitrate applied in spring (designated SP) followed by commercial ammonium nitrate applied in summer and (2) commercial ammonium nitrate applied in spring followed by ^{15}N labelled ammonium nitrate applied in summer (designated SU). Ammonium nitrate was isotopically-labelled both in the ammonium and in the nitrate ion (10% atom). It was diluted in water and hand-applied around each tree, to 8 holes 5–30 cm depth located 20–50 cm apart from the trunk. Holes were made with a stainless steel cylindrical auger of 8 cm diameter. After application, holes were covered with soil to avoid gaseous loss. As the orchard was furrow irrigated and in order to avoid lateral displacement of the fertilizer, plastic barriers were placed around each tree, 2 m apart from the trunk and 1 m deep. Barriers protruded 30 cm above the soil surface. Trees were watered during the experiment as previously described.

In order to confirm tree uniformity, leaf area (LA) and leaf area index (LAI), fruit number (F), and F:LA ratio were determined at harvest (January). LA and LAI were assessed by sampling 1% of the leaves at random over each whole tree. Leaves were scanned and data were processed using the UTHSCSA Image Tool 3.0 programme (http://ddsdx.uthscsa.edu/dig/itdesc.html) as previously described (Cittadini and Peri, 2006).

Two hundred ripe cherries per tree (600 fruit per treatment) were picked at random at commercial harvest and used for the assessment of different fruit quality indices: average fresh fruit weight, fruit diameter (Centre Interprofessionnel des Fruits et Légumes, 1995); firmness (Durofel, Agrotechnologie, France; Hilaire et al., 2000); soluble solids content with a hand-held temperature-compensated refractometer (Atago Co., Tokyo, Japan;

Calvo and Sozzi, 2009); titratable acidity by potentiometric titration of a 10 ml juice sample with 0.1 N NaOH to an endpoint of pH 8.2 (expressed as mmol H^+ per litre juice; Calvo and Sozzi, 2009); and colour (L, C, *h*, with a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan); Calvo and Sozzi, 2009). 600 replicates per treatment were measured for fruit diameter, fresh weight, firmness and colour. Fifteen 40-fruit independent replicates were evaluated for soluble solids content and titratable acidity. Trunk growth was estimated by measuring TCSA at the beginning and at the end of the growing season.

2.3. Tissue samples and sampling time

All fruit were harvested at full commercial maturity in January, 10 days prior to summer N application, then counted and weighed. Leaves were sampled in February (full canopy) and April (senescence), counted and weighed. The February sample (1% of the leaves of each tree) was also used to determine the LA, as previously described. In order to avoid leaf loss, branches were surrounded by nets, so that senescent leaves were captured at fall.

Trees were sampled at the end of winter, while still dormant (August). The trees were cut at the graft point and in trunk, branches (current-season shoots, 1-year-old and over-1-year-old branches called older branches), buds of the same age. Then the soil was thoroughly removed up to 1 m but very few roots were found to develop below 0.8 m. Roots were collected, washed and classified as small roots (less than 1 mm thick) and large roots. Samples were weighed in the field for total fresh weight and subsamples were taken, dried to constant weight, ground and analysed for total N concentration (Semi-micro Kjeldahl; Temminghoff, 2000) and ^{15}N (Dumas, Jasco emission spectrometry; International Atomic Energy Agency, 2001). Fresh:dry weight ratio and dry matter (DM) partition, expressed as a percentage of each organ on the whole tree DM, were also determined. Pruned wood was estimated to calculate N removal from the field, by weighing pruned branches of the same age removed from six adjacent trees.

^{15}N enrichment (a') was calculated by subtracting ^{15}N natural abundance (a) from the total ^{15}N of each organ. Natural abundance was determined by mass spectrometry (International Atomic Energy Agency, 2001) in tissue samples of branches, leaves and fruit of nearby trees. The in situ value (0.3735%) was slightly higher than the standard (0.366%) (International Atomic Energy Agency, 2001). Total N and ^{15}N were determined at CATNAS (Centro de Aplicaciones de Tecnología Nuclear en Agricultura Sostenible-Facultad de Agronomía, Montevideo, Uruguay). Standard calculations were made as follows (International Atomic Energy Agency, 2001):

$$\text{N derived from fertilizer (NdFF) (\%)} = \frac{^{15}\text{N atom.exc. } (a') \text{ in the plant or organ}}{\%\ ^{15}\text{N atom. exc.}(a') \text{ in the fertilizer}} \times 100$$

$$\text{N yield (g)} = \text{dry matter yield (g)} \times (\%\ \text{N}/100)$$

$$\text{N fertilizer yield (NFY) (g)} = \text{N yield (g)} \times (\%\ \text{Ndff}/100)$$

$$\text{N uptake efficiency (NUE) (\%)} = (\text{NFY}/\text{rate of N application}) \times 100$$

To determine N removal from the field, fruit, leaves and pruned wood were considered. Pruned wood was estimated in 6 adjacent trees with similar TCSA. Total N removal (kg ha^{-1}) was calculated considering N percentage and dry weight of each tree component and multiplying these values by orchard density (1000 trees ha^{-1}).

2.4. Statistical analysis

A *t*-test for independent samples was used to compare the means of two fertilization treatments for all the evaluated variables.

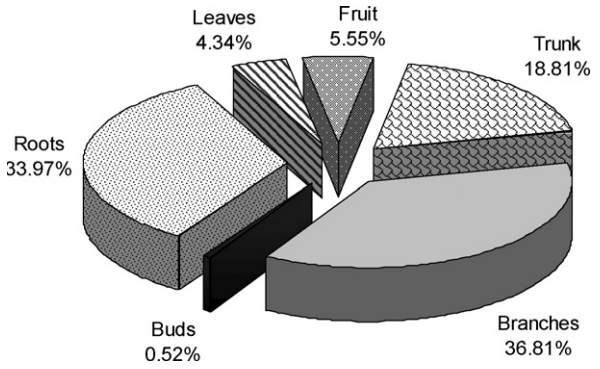

Fig. 1. Average biomass partitioning (% dry weight) for the six 'Bing' sweet cherry trees.

The same test was used to verify the homogeneity of the trees using leaf area determinations, F:LA relation and quality indices. Data were analysed using the PC-SAS software package (SAS Institute Inc., 1989). The mean and standard deviation (SD) for dry weight, percentage of N (% N) and percentage of Ndff (% Ndff) of each component are presented herein. For TCSA, a repeated measures analysis of variance was used (Littell et al., 1996; Vittinghoff et al., 2005).

3. Results

No differences between treatments were detected in fruit yield, LAI and fruit quality indices (Table 1). Since total N applied was the same for both treatments, results indicate uniformity of tree and fruit characteristics between treatments. Indicators of tree uniformity were the following (mean ± SD): leaf area per tree = 50.49 ± 2.54 m^2; LAI = 5.05 ± 0.25. Fruit number per tree was relatively low for all trees (645.67 ± 49.71 fruit), considering the tree age. F:LA ratio was similar in both treatments ($p = 0.83$) (12.81 ± 1.17 fruit m^{-2}). Mean yield was 5510.84 ± 595.37 kg (Table 1) and did not differ between treatments, implying no short term effects of timing of ^{15}N application on tree variables. Neither year*treatment interaction ($p = 0.0968$) nor treatment differences ($p = 0.8654$) were found when analyzing TCSA, measured at the start and the end of each year. On the other hand, differences in TCSA ($p = 0.0001$) were found between the beginning and the end of the experiment (61.50 ± 8.10 cm^2 and 105.85 ± 12.80 cm^2, respectively).

Dry weight mean comparison (Table 2) showed no differences between times of application for the various organs, except for current-season buds, which were higher for spring fertilized trees. Roots and branches were the main components of biomass partition (collectively, 70% of total DM), followed by the trunk, fruit, leaves and buds (Fig. 1). The aerial/subterranean dry weight ratio was 1.94, and roots were distributed in the soil from 70 cm depth to 90 cm width from the tree trunk. Large roots were the main component of subterranean biomass (99.44% for large roots vs. 0.56% for small roots). Branches over 1-year-old comprised 87.8% of total branch mass while current-season and 1-year-old branches were 3.28% and 8.82%, respectively. Only 11% DM (2.94 kg) was partitioned to current-season growth (branches, buds, leaves and fruit). Among these components, 50% of current-season growth was partitioned to fruit production, 39% to leaf growth and only 11% to branch and bud growth. Total fresh weight per tree was 32.84 ± 5.5 kg and total dry weight per tree was 27.43 ± 6.3 kg DM (data not shown). DM content for each tree component was the following: fruit: 26.7%; leaves: 56.70%; branches: 61.16%; roots: 62.11%; and trunk: 80.74% (data not shown).

Table 1
Leaf area index, yield and fruit quality indices, expressed as mean and standard deviation (SD) according to the application time of the ^{15}N fertilizer: spring (SP) or summer (SU). No significant differences were detected between treatments according to p values ($p > 0.05$).

Yield and fruit quality indices	Application time				p-Value
	SP		SU		
	Mean	SD	Mean	SD	
Leaf area index (LAI):	5.03	0.28	5.07	0.28	0.8700
Yield (kg ha^{-1}):	5518.40	919.13	5503.29	202.99	0.9792
Fruit weight (g)	8.27	0.14	8.78	0.44	0.1269
Fruit size (mm)	24.42	0.63	25.20	0.46	0.0727
Firmness (ID)	0.66	0.02	0.63	0.03	0.2588
Lightness (L^*)	29.65	2.54	28.69	1.24	0.5146
Chroma (C^*)	20.29	8.08	17.21	4.12	0.5885
Hue angle (h°)	11.91	4.44	10.63	2.12	0.6767
Titratable acidity (mmol H$^+$ L^{-1})	89.55	4.48	91.04	5.97	0.7362
SSC (%)	19.67	3.13	19.08	1.38	0.5451

Total N per tree was 158.73 and 134.22 ($p = 0.240$ g) for spring and summer treatments, respectively. The % N in 1-year-old branches differed with time of N application (Table 3), with higher values for trees fertilized with ^{15}N in SP. In both treatments, more N accumulated in large roots (75.64 g N and 71.80 g N), relative to 1-year-old branches (30.57 g N and 20.51 g N) and leaves (18.16 g N and 16.18 g N) for SP and SU application, respectively (data not shown).

Pruned biomass of the different components was: 0.68 ± 0.24 kg for current-season branches; 0.83 ± 0.18 kg for 1-year-old branches and 1.92 ± 0.32 kg for older branches. Therefore, total pruned DM was estimated to be 3.44 ± 0.38 kg per tree (data not shown). Total N removal from the field (Table 4) considered also the DM of fruit and senescent leaves (since leaves are burned annually) (Fig. 1) and % N in each organ (Table 3). Using the estimated fruit yield (5500 kg ha^{-1}) and planting density (1000 trees ha^{-1} in this study) total N removal from the field was 33.35 kg N ha^{-1}. As the burning of leaves is not recommended, and considering N present only in pruned wood and fruit, N removal from the field was 26.11 kg N ha^{-1}.

No differences ($p = 0.1285$) were found between treatments for total Ndff (57.62 ± 9.89 g for SP and 39.65 ± 5.05 g Ndff tree^{-1} for SU application), though fruit were not considered in the SU treatment because ^{15}N was applied after harvest. The time of ^{15}N application affected % Ndff for some organs (Table 3). When ^{15}N was applied after harvest (SU treatment), % Ndff accumulated in roots (large and small), trunk, and 1-year-old and older buds. The highest percentage of Ndff in the SU treatment was accumulated in roots (over 25%), while % Ndff in roots for the SP treatment was under 13%. Instead, % Ndff was significantly higher in current-season branches and in both green and senescent leaves after applying ^{15}N in SP. In addition, % Ndff in senescent leaves was similar to that of green leaves, though the percentage of total N dropped by over 50% after SP N application.

Table 2
Biomass accumulation (g) in the different components of sweet cherry trees according to the application time of the ^{15}N fertilizer: spring (SP) or summer (SU). Results for each component are expressed as mean and standard deviation (SD). Means within each component followed by the same letter indicate no significant differences ($p > 0.05$) between treatments.

Component	Application time	Biomass (g)	
		Mean	SD
Small roots	SP	56.13 a	38.78
	SU	54.68 a	34.11
Large roots	SP	8890.51 a	5357.69
	SU	9988.73 a	1229.46
Trunk	SP	5650.22 a	1299.10
	SU	4511.55 a	720.90
Current-season branches	SP	376.85 a	74.65
	SU	235.42 a	47.18
1-Year-old branches	SP	705.50 a	238.97
	SU	970.88 a	85.57
Older branches	SP	9617.54 a	8364.13
	SU	8364.13 a	3490.29
Current-season buds	SP	23.33 a	3.25
	SU	15.68 b	1.70
1-Year-old buds	SP	35.02 a	7.74
	SU	41.55 a	9.23
Older buds	SP	80.63 a	30.78
	SU	82.65 a	20.83
Leaves	SP	1139.42 a	64.25
	SU	1148.45 a	64.23
Fruit	SP	1387.10 a	271.47
	SU	1554.26 a	205.28

Table 3
Total N (% N) and N derived from fertilizer (% Ndff) in the different components of sweet cherry trees, according to application time of the ^{15}N fertilizer: spring (SP) or summer (SU). Results for each component are expressed as mean and standard deviation (SD). Means within each component followed by a different letter indicate significant differences ($p < 0.05$) between treatments. In SU application, % Ndff was not measured for fruit since ^{15}N was applied after harvest.

Component	Application time	% N		% Ndff	
		Mean	SD	Mean	SD
Small roots	SP	1.36 a	0.15	11.91 b	0.08
	SU	1.47 a	0.56	25.92 a	3.85
Large roots	SP	0.96 a	0.21	12.92 b	2.02
	SU	0.73 a	0.18	28.17 a	8.47
Trunk	SP	0.19 a	0.02	12.34 b	0.29
	SU	0.14 a	0.06	14.55 a	1.60
Current-season branches	SP	0.62 a	0.10	18.68 a	3.01
	SU	0.56 a	0.07	13.87 b	2.88
1-Year-old branches	SP	1.57 a	0.73	14.77 a	1.43
	SU	0.59 b	0.31	14.45 a	4.27
Older branches	SP	0.32 a	0.07	13.88 a	2.23
	SU	0.14 a	0.13	12.48 a	0.55
Current-season buds	SP	1.23 a	0.18	16.96 a	0.13
	SU	1.16 a	0.22	18.44 a	2.56
1-Year-old buds	SP	0.90 a	0.49	13.89 b	0.92
	SU	1.04 a	0.26	19.53 a	0.43
Older buds	SP	1.15 a	0.03	13.35 b	1.97
	SU	0.84 a	0.18	17.25 a	0.62
Green leaves	SP	1.40 a	0.19	17.89 a	2.18
	SU	1.62 a	0.13	8.08 b	1.38
Senescent leaves	SP	0.67 a	0.12	17.11 a	2.46
	SU	0.60 a	0.09	5.91 b	0.21
Fruit	SP	0.76 a	0.11	16.06	0.83
	SU	0.41 a	0.08	–	–

NUE differed between times of ^{15}N application ($p = 0.0113$): it was 65.7% for SP and 37.44% for SU treatment. Differences were also detected in some organs such as trunk, current-season branches, older branches and leaves (Fig. 2). Nitrogen uptake by large roots was more effective in both treatments, with NUE values over 19%. Total branch NUE was almost 12% for SP and slightly over 3% for SU application but, in both treatments, the percentage was higher for branches located closer to the trunk. In the trunk, NUE reached a level almost 9-fold higher in SP- than in SU-treated trees. In leaves, the SP treatment improved the NUE percentage by 3.45-fold as compared with SU treatment. In fruit, NUE reached 3% in SP, the only treatment fertilized with ^{15}N before harvest.

4. Discussion

No differences between treatments were found in LA, LAI, F:LA ratio and fruit characteristics (Table 1) thus suggesting that cherry trees were uniform. Also, no differences were detected in terms of % N in most of the tree components (small and large roots, trunk, current-season and older branches, all types of buds, leaves and fruit) between trees ^{15}N fertilized in SP and SU (Table 3). In spite of that, significant differences were observed in the N concentration of 1-year-old branches between SP and SU (Table 3). This could be due to some degree of tree-to-tree variation unrelated to treatments since no error was detected in the measurement or calculation of N concentration in those organs.

Nitrogen uptake and distribution within the sweet cherry tree differed when N fertilizer was applied twice (Table 3). When the isotopically-labelled N was applied in SU, higher % Ndff was found in roots; in contrast, the application of isotopically-labelled N in SP brought about higher % Ndff in current-season branches and leaves. In apple trees, Aguirre et al. (2001) found more N and ^{15}N absorbed from SP applications than from those applied during autumn. Furthermore, they suggested that trees cultivated in temperate-cold regions were able to recover more N from the fertilizer applied later in SP in comparison with that applied in autumn, and that

Table 4
Dry weight (kg), N percentage and total N (kg tree^{-1} and kg ha^{-1}) lost from the system, taking into account fruit, senescent leaves and pruned branches. Calculations consider a fruit yield of 5.5 kg tree^{-1} and an orchard density of 1000 plants ha^{-1}.

Component	Dry weight (kg tree^{-1})	N (%)	Total N (kg tree^{-1})	Total N(kg ha^{-1})
Fruit	1.47	0.585	0.00860	8.60
Senescent leaves	1.14	0.635	0.00724	7.24
Current-season branches	0.68	0.590	0.00404	4.04
1-Year-old branches	0.84	1.080	0.00905	9.05
Older branches	1.92	0.230	0.00442	4.42
Total				33.35

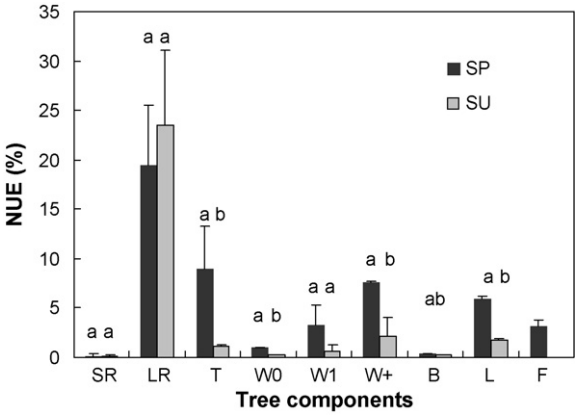

Fig. 2. N uptake efficiency (NUE) in each tree component for two N application times: spring (SP) and summer, after harvest (SU). Tree components are as follows: small roots (SR); large roots (LR); trunk (T); current-season branches (W0); 1-year-old branches (W1); older branches (W+); buds (B); leaves (L) and fruit (F). Each value represents the mean ± SD. Values with a different letter are significantly different ($p < 0.05$). Fruit were not considered in the SU treatment because ^{15}N was applied after harvest.

SP-applied N is immediately used by aerial organs such as leaves and current-year wood. Also in apple trees, Khemira et al. (1998) reported that the N absorbed in late SP was partitioned to fruit and vegetative organs, rather than to storage organs. Thus, N fertilization in late SP and early SU provides N during maximum vegetative growth (Khemira et al., 1998; Sánchez et al., 1995). In pear trees, Sánchez et al. (1992) found that more N was partitioned to roots when applied after harvest, and suggested a 3-week-pre-harvest application in order to accumulate N in flower buds.

Although sweet cherry tree is physiologically active during late SU, it was reported that little or no supplied N was translocated to the leaves after harvest (Sánchez et al., 1995). Our experiment confirms that Ndff in both green and senescent leaves was significantly lower after postharvest fertilizer application (Table 3). Nevertheless, 8% of the ^{15}N applied after harvest was detected in the leaves 1 month after the application (Table 3). Besides, ^{15}N assessment carried out at the dormant bud stage (August) after N application showed significantly higher Ndff levels for the postharvest application, as compared with the SP application, in buds located in both 1-year-old and older branches. Although total Ndff per plant was similar for both treatments (SP: 57.62 ± 9.89 g tree^{-1}; SU: 39.65 ± 5.05 g tree^{-1}), it was preferably allocated to roots and trunk when applied after harvest (Table 3). In contrast, Ndff was preferentially allocated to current branches, leaves and fruit, instead of storage organs when ^{15}N was applied in SP.

The low % Ndff found in our experiment (between 5.91% and 28.17%, depending on the organ considered) may indicate the importance of other sources of N (soil and tree reserves) used for tree growth and fruit production. These values are similar to those found by Khemira et al. (1998) for apple trees but lower than those reported for orange trees (Mattos et al., 2003), apple trees (Nannipieri et al., 1995) and pear trees (Sánchez et al., 1992). This suggests that sweet cherry trees may be capable of making better use of other N sources. Moreover, foliar analysis using leaves from 1-year-old branches sampled in the season previous to ^{15}N application displayed standard N levels, even when no N fertilization was applied that season. In fact, Azarenko et al. (2008) found ^{15}N uptake during the second growing season after the application of the isotopically-labelled N, probably due to temporal immobilization of the applied ^{15}N as soil microbial biomass or soil organic matter. In Los Antiguos, there is no evidence of N immobilization but leaching may account for the 'missing' N.

Nitrogen uptake efficiency is usually low in fruit trees, mainly due to their limited root density in comparison with that of annual crops, and to their higher storage capacity (Mattos et al., 2003; Neilsen et al., 2001; Sánchez et al., 1995; Tagliavini et al., 1996). Efficiency values for N uptake as low as 8.3% in peach trees (Nario et al., 2003), or as high as 60% in young apple trees (Hill-Cottingham and Lloyd-Jones, 1975) are unusual. More frequent values for fruit tree species are near 25–30%, as reported for pecan (Rey et al., 2006; Kraimer et al., 2004), pear (Sánchez et al., 1991) and apple tree (Neilsen et al., 2001). Even in small fruit species such as *Rubus sp.*, which have perennial root systems and biennial canes, efficiency values up to 45% have been reported (Mohadjer et al., 2001; Rempel et al., 2004).

In this work, N uptake efficiency was 37% for ^{15}N applied after harvest but over 65% for the SP application. The latter is similar to those observed in extensively grown crops (Rimski-Korsakov et al., 2009; Sánchez et al., 1995) but much higher than the 21.7% found in 'Royal Ann' sweet cherries after SP application (Azarenko et al., 2008). Differences may be partially explained by the climactic characteristics of the zone. Although no specific comparisons have been made between Los Antiguos and other regions, it is well-known that N uptake efficiency can be strongly influenced by precipitation or irrigation application. Also in cold regions, species like apple tree can take up higher percentages of the N applied in SP rather than in autumn (Aguirre et al., 2001). Those higher percentages are readily available for developing leaves and growing shoots. The more effective use of the Ndff may also be due to the method of application allowing the fertilizer to remain for longer periods near the root area. Thus, N can be intercepted by the low density root system of fruit trees (Neilsen et al., 2001).

Nitrogen reabsorption from the leaves during senescence is the first mechanism contributing to the accumulation of N to storage pools (Tagliavini and Millard, 2005). In apple trees, Neilsen et al. (2001) found that 33–38% of the Ndff, and up to 59% of total N located to leaves were remobilized prior to senescence. These authors suggested a differentiation between spur and shoot leaves, and remarked that N remobilized in SP was comparatively more important for spur leaf growth while N absorbed in SP contributed preferentially to shoot leaf growth. For the apple spur leaves, the Ndff was higher in senescence (26%) than in spring (16%), thus suggesting a preferential withdrawal of N derived from storage in SP. In contrast, N remobilization may be less important for shoot leaves. In fact, Neilsen et al. (2001) and Sánchez et al. (1992) showed that a relatively high percentage of N derived from the current-season uptake was withdrawn from senescent leaves when the canopy as a whole (spur and shoot leaves) was considered.

In our study, net N efflux from senescent leaves to other organs ranged from 52% to 63% of total leaf N accumulation for SP and SU application, respectively. Nevertheless, the % Ndff in senescent leaves was similar to that found in green leaves within each treatment. Moreover, 95.6% and 73% of the Ndff from the SP and the postharvest application, respectively, remained in the leaves at the end of the season. These results contrast with those reported for the pear tree (Quartieri et al., 2002). In that work, senescent leaves had more N derived from the early rather than from the late uptake but the percentage of ^{15}N translocated prior to leaf abscission was similar for both application times (67%).

Our results suggest that the N absorbed in previous seasons is preferentially remobilized from and to perennial organs. If N content in the fruit tree is assumed constant from one year to another (Weinbaum et al., 2001), N removal by fruit harvest could be considered similar to its requirements. If 25–40 kg N ha^{-1} are required for annual growth, a reasonable estimation of total N requirement can be obtained (Neilsen et al., 2001; Ughini and Roversi, 2006; Sánchez et al., 1995).

Nitrogen removal is determined not only by the harvested fruit but also by the N located in the pruned wood and in the leaves, if they are eliminated from the system (Neilsen et al., 2001; Roversi and Monteforte, 2006; Tagliavini et al., 1996). Nitrogen located in removed organs could also be influenced by cultivar, tree age, rootstock, vigour and yield since these factors may influence N uptake (Roversi and Monteforte, 2006; Ughini and Roversi, 2006). In our study, almost 34 kg N ha^{-1} were removed annually from the field. This value is lower than that reported by Roversi and Monteforte (2006), who determined that an average of 47 kg N ha^{-1} is removed by fruit, leaves and pruned wood from 13-year-old trees of 6 cultivars grafted onto 'Mazzard' (*P. avium*), different from the 'Bing'/*P. mahaleb* rootstock used in our study. The main difference between the two studies was harvested fruit N content (1.33% according to Roversi and Monteforte (2006) vs. 0.58% in this work) since the N content level in the pruned material was similar in both works. Fruit yield was low (mean: 5510.84 kg ha^{-1}, SD: 595.37 kg ha^{-1}) in comparison with yields reported elsewhere (Ughini and Roversi, 2006). These two factors (lower fruit N levels and lower yield) may explain the differences between the levels of N removal reported by Roversi and Monteforte (2006) (6.37 kg N ton^{-1}) and those herein observed (2.02 kg N ton^{-1}). Ughini and Roversi (2006) found N removal to increase with increasing fruit yield. For example, 'Droganova', which yields less than 6 ton ha^{-1}, had a N removal of approximately 27 kg ha^{-1}, while 'Ferrovia', with an average annual yield of almost 10 ton ha^{-1}, had a N removal exceeding 43 kg ha^{-1}.

Except for the N removed via the leaves, the production system annually removed almost 34 kg N ha^{-1}. If the senescent leaves are left in the field and they are not blown away, they will decompose releasing N to the soil which will be readily available to the trees. Tagliavini et al. (2007) reported a net immobilization of the N present in the leaves and mowed grass of the orchard during the first year after the incorporation of the plant material to the soil but an important net N release during the second year. In that study, senescent leaves had 1.76% N and almost 60% of that N was eventually recycled to the soil.

5. Conclusion

The uptake efficiency of the applied N for sweet cherry was lower for SU (37%) than for SP (65%) application. The N applied in SU after fruit harvest was stored in structural components (trunk and roots) rather than in removable organs, and also in buds and leaves. The amount of Ndff removed from the field with harvested fruit and pruned wood was greater with the SP (33 kg N ha^{-1}) than with the SU application (18 kg N ha^{-1}), due to the higher N compound levels in 1-year-old branches, leaves and fruit after applying the nitrogenous fertilizer in SP. There could have been differences in soil N content between treatments and SU-applied N could be taken up the subsequent year. On the other hand, soil types and irrigation may contribute to leaching of applied soluble nutrients below the rooting depth of cherry tree. Thus, over applying postharvest N to increase N uptake during the second growing season is not considered the best option since the agricultural use of N fertilizers in excess of crop requirements increases the risk of leaching losses and the concern of potential eutrophication of surface and groundwater.

In cherry tree, the majority of N remobilization occurs before root uptake starts. Thus, N availability in SU should be assured to guarantee appropriate levels of N stored in sweet cherry tree perennial structures. Results suggest that N application after harvest is the best option to increase the level of N stored in sweet cherry tree structural components. Nevertheless, excessive SU applications of N should be avoided since they could lead to decreased cold hardiness of young branches and N leaching through the soil.

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