Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research paper

Seasonal dynamics of root uptake and spring remobilisation of nitrogen in field grown orange trees

Giancarlo Roccuzzo^a, Francesca Scandellari^{b,*}, Maria Allegra^a, Biagio Torrisi^a, Fiorella Stagno^a, Tanja Mimmo^b, Damiano Zanotelli^b, Paola Gioacchini^c, Peter Millard^d, Massimo Tagliavini^b

a Council for Agricultural Research and Agricultural Economics Analysis, Research Centre for Olive, Citrus and Tree Fruit, corso Savoia 190, 95124 Acireale, Italy

^b Free University of Bolzano, Piazza Università 5, 39100, Bolzano, Italy

^c Dept. of Agricultural Sciences, Alma Mater Studiorum – University of Bologna, Italy

^d Landcare Research, PO Box 69040, Lincoln, 7640, New Zealand

ARTICLE INFO

Keywords: Citrus Fruit Labelled nitrogen Nitrogen fertilisation Nitrogen storage Nitrogen remobilisation Nitrogen uptake

ABSTRACT

To be economically and environmentally sound, the nitrogen (N) supply in orchards needs to be finely tuned according to crop needs. This requires knowledge of the amount and the dynamics of N uptake by roots and of its allocation. This paper reports two experiments, carried out on the same set of trees, in order to: 1) study the uptake dynamics of N derived from fertiliser (N_{dff}) and its allocation to tree organs and, 2) quantify its N remobilization in spring from storage organs to new growth. The study was carried out using field-grown adult orange trees (cv. Tarocco nucellare grafted on Troyer Citrange), one of the most important fruit tree crops in the Mediterranean area. Nitrogen was supplied as ammonium nitrate in 10 applications with equal doses evenly distributed from March to November 2009. Four groups of 5 trees each received ¹⁵N-enriched N starting from March, July, September, and November, respectively, while they received unlabelled N in the remaining periods. The results show that trees absorbed roughly 30% of the fertiliser N. Fertiliser-N use efficiency (NUE) was generally low in spite of the fact that readily available N sources were regularly supplied and the trees were irrigated; the later the fertiliser N was supplied, the lower was the NUE. Uptake rate was rather constant from April to November, but relatively less N was partitioned to fruits when the fertiliser was supplied later in the season. Remobilization of N from one-year-old leaves provided more than 60% of the N required by shoots in spring. By the end of April, most of the N had already been transferred from storage to growing organs. We estimated that, during the vegetative season, trees absorbed 94 kg N ha⁻¹ from the soil and half of this amount derived from fertiliser. Spring remobilisation of winter stored N contributed for additional 40 kg N ha⁻¹ to the N needs

1. Introduction

Oranges are an important agricultural commodity worldwide with the annual gross production in the Mediterranean area exceeding 11 million tons (FAO, 2012). Modern agricultural management techniques in citrus orchards, including fertilisation, need to reconcile economic and ecological issues. The nitrogen-fertiliser supply at rates and timings that fit the tree's nitrogen (N) demand, coupled with correct watering scheduling, contributes to minimise N leaching and volatilization losses (Feigenbaum et al., 1987; Mattos et al., 2003; Quiñones et al., 2005; Qin et al., 2016) and increases the nitrogen use efficiency (NUE) (Lea-Cox et al., 2001; Martinez-Alcantára et al., 2012). The rate of N uptake by roots, providing that N is available in the soil solution and there are no internal and external constraints to root uptake, mainly depends on

* Corresponding author. *E-mail address:* francesca.scandellari@unibz.it (F. Scandellari).

http://dx.doi.org/10.1016/j.scienta.2017.08.010

tree N demand, driven by the inherent needs of the growing organs.

Soil, climate, management, and genotype influence fertiliser-N recovery. Mature fruit trees grown in the field have shown fertilizer use efficiencies varying from 25 to 55% (Feigenbaum et al., 1987; Weinbaum et al., 1994; Weinbaum and Van Kessel, 1998). Nitrogen uptake by mature citrus trees can be estimated by the amount of nutrient removed by harvested fruit, abscised fruitlets and flowers, senescent leaves, pruning wood, and root turnover (Feigenbaum et al., 1987; Papanicolaou et al., 1988; Mooney and Richardson, 1992).

Internal cycling of N in both evergreen and deciduous trees, represents an important source of N for seasonal growth (Legaz et al., 1995; Millard and Grelet, 2010; Millard et al., 1996; Quartieri et al., 2002). However, our current knowledge on N uptake and remobilisation by citrus is based mainly on evidence obtained with young trees in





Received 2 May 2016; Received in revised form 27 July 2017; Accepted 7 August 2017 Available online 08 September 2017 0304-4238/ © 2017 Elsevier B.V. All rights reserved.

pots (Legaz et al., 1982; Legaz and Primo Millo, 1988; Legaz et al., 1995) or in the field (Menino et al., 2007), while quantitative estimates for adult, field-grown and fruit-bearing trees are scarce. Therefore, results must be interpreted with caution as the relative contribution of N uptake and remobilization differs depending on rootstock, genotype and age, soil characteristics and agricultural practices.

This study aimed at: 1) studying the uptake dynamics of N derived from fertiliser (N_{dff}) and its allocation to tree organs and 2) quantifying the relative role of spring remobilization of N from storage organs versus the root N uptake in mature field-grown orange trees. During the first year, we provided fertiliser N labelled with ¹⁵N at different timings, to assess fertiliser N uptake dynamics (*dynamics of N uptake*). During the second year, we followed the changes of labelled N in storage organs and its appearance in new organs to quantify storage and spring N remobilization (*N storage and remobilization*).

2. Materials and methods

2.1. Site description

This study was carried out in 2009 and 2010 in a commercial orange [*Citrus sinensis* (L) Osbeck] orchard located in the Plain of Catania (in a site named Scordia, province of Catania, eastern Sicily, Italy; 37° 21' N, 14° 50' E). Trees of the variety Tarocco nucellare (clone 571E-1) grafted on Troyer Citrange [*Poncirus trifoliata* (L.) Raf. x *C. sinensis* (L.) Osbeck] were planted in 1998 at 6×4 m spacing. Troyer Citrange is tolerant to the Tristeza virus and largely adopted in this area as alternative to sour orange. Fruit yield in February 2008 was 37 t per hectare and the average yield the previous three years was 25 t per hectare. In 2009, when the experiment started, the trees were 12-years old.

The orchard soil (average of 0–60 cm layers) had the following characteristics: sand 345, silt 247 and clay 408 g kg⁻¹, total organic carbon 15.1 g kg⁻¹, total N 1.5 g kg⁻¹, total lime 14.8 g kg⁻¹, electrical conductivity 0.26 dS m⁻¹ and pH 8.02 (1:2.5 water).

Annual mean reference evapotranspiration and rainfall in the study area were about 1300 mm and 510 mm, respectively. The citrus orchard was irrigated with micro-sprinklers with a total irrigation volume of approximately 550 mm ha^{-1} (from May to October).

2.2. Dynamics of N uptake

2.2.1. Labelling trees with ¹⁵N-enriched fertiliser

Twenty trees located in five rows were used for this experiment. The selected trees were separated in each direction by one untreated border tree. Trees were uniform in terms of size and productivity. The soil management included the use of a not-residual herbicide to the soil under the canopy of the trees while weeds and grasses present in the remaining soil surface were regularly mowed. Trees in each row were randomly assigned to four treatments differing for the timings of ¹⁵Nenriched fertiliser supply. Nitrogen was provided as ammonium nitrate (NH₄NO₃), either at natural abundance or enriched with ¹⁵N on both N atoms. All trees received a total of 360 g N tree $^{-1}$ year $^{-1}$ in 10 equal monthly applications during 2009 (March 2; April 4, May 6, June 4, July 3, August 3, September 4, October 2, November 3, and November 27) as shown in Fig. 1. Trees in treatments 1 (T1) received only the ¹⁵Nenriched fertiliser; trees in treatment 2 (T2) received seven ¹⁵N-enriched fertiliser applications; trees in treatment 3 (T3) received four ¹⁵N-enriched fertiliser applications; trees in treatment 4 (T4) received two ¹⁵N-enriched fertiliser applications. The total amount of ¹⁵N-enriched fertiliser received by each treatment is shown in Table 1. The labelling was obtained by adding ¹⁵NH₄¹⁵NO₃ (10 atom% ¹⁵N; SIGMA-ALDRICH Co., St. Louis, MO, USA) to NH4NO3 at natural abundance. The ¹⁵N enrichment in the fertiliser varied according to the treatment (from 1.1 atom% in T1 to 5.0 atom% in T4) to ensure an adequate N enrichment of tree organs. When T2, T3 and T4 trees did not receive labelled N, they received the same amount of N as unlabelled NH4NO3.

Each time, the fertiliser was diluted in tap water and applied as uniformly as possible on a 50-cm-wide ring-shaped soil strip (with an area of 4.5 m²), below the canopy at approximately 120 cm from the trunk. The soil area receiving the fertiliser corresponded to the zone wetted by the micro-sprinklers. In March 2009, all trees received the same amount of phosphorus (200 g tree⁻¹ P_2O_5 as single superphosphate equivalent to 87 g tree⁻¹ of P) and potassium (300 g tree⁻¹ K_2O as potassium sulphate equivalent to 249 g tree⁻¹ of K).

2.2.2. Samplings and biomass assessment

Tree organs were sampled between April 2009 and February 2010 approximately at monthly intervals, on the same day of the fertiliser application. At each harvest, a sample of 5 previously tagged shoots (shoot axis and leaves) was collected from each tree from the first (in spring) and, when present, from the second (in summer) shoot flush. A representative sample of leaves grown in 2007 and 2008 (later referred as old leaves) was also collected and weighed after drying. Summer pruning was performed on September 9, 2009 and the pruning material was collected. We assumed that shoots and suckers, although differing in total growth, had a similar growth dynamic; in this way, it was possible to estimate the sucker biomass theoretically present at the samplings of May 4, June 10, July 7 and July 29. The leaves abscised from February 2009 to February 2010 were collected with the aid of two plastic boxes per tree, randomly located at the soil surface and covering $0.34 \text{ m}^2 \text{ tree}^{-1}$ (Roccuzzo et al., 2012). Starting from July, a sample of three fruits per tree was also collected. Fruit harvest occurred on February 23, 2010, when the whole fruit yield was quantified. A sample of wood was collected on the same date from branches of different ages by means of a drill with a sharp head. After oven drying (80 °C until constant weight), the dry weight of samples was determined. A dry subsample from each sample was ground and analysed for total N concentration and ¹⁵N abundance as later described.

To calculate the amounts of total N and ¹⁵N per tree, estimates of tree organ biomass were obtained by the following procedures: the dry weight of individual shoots was multiplied by the number of shoots, counted for each tree. The biomass of woody organs (trunk and branches) and of old leaves was estimated from the circumference of all branches measured in February 2010, using the allometric equation developed by Roccuzzo et al. (2012). On the basis of a destructive analysis carried out in March 2009 aimed at determining the relative proportion of one- and two-year-old leaves, 81% of the estimated old leaf biomass was assumed to derive from the 2007 vegetative season.

Root samples from each tree belonging to T1 treatment were collected on March 26, 2010: twelve soil cores (20 cm \times 20 cm wide, to a depth of 40 cm) per tree were taken in the area around the trees that had received the labelled fertiliser. Fine roots ($\emptyset \le 0.2$ cm) and coarse roots ($\emptyset > 0.2$ cm) were collected and root samples from the different cores of each tree were pooled together. Roots were washed in water, dried and analysed for total N and ¹⁵N abundance. Coarse and fibrous root density (g DW m⁻³) was calculated.

2.2.3. Total N and ¹⁵N analysis

Total N concentration and ¹⁵N abundance of tree and soil samples were determined using a Delta V isotope ratio mass spectrometer equipped with a Flash EA 1112 Elemental Analyzer (Thermo Scientific, Germany). Each sample was analysed in duplicate. The ¹⁵N excess (atom%) in trees and in the fertiliser with respect to the natural level, assumed to be 0.3663 atom% ¹⁵N, was calculated as

 ${}^{15}N_{excess}(atom\%) = {}^{15}N_{sample}(atom\%) - 0.3663(atom\%)$

For each sampled organ, we calculated the percentage of N derived from fertiliser (N_{dff} %) as follows:

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