Original Research Paper

Foliar and root uptake of N deriving from simulated atmospheric N depositions in potted apple (*Malus domestica*) trees

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Abstract: A significant human-driven increment of the available reactive nitrogen (Nr) forms has occurred during the past century at the global scale, which in turn has increased the amount of Nr deposition. Grafted apple trees (Gala / M.9 strain T337) were used in a pot experiment conducted in semi-controlled conditions, where the ^{15}N-labelling technique allowed to trace the fate of N from ammonium nitrate ($^{15}NH_4^{15}NO_3$, isotopic enrichment: 10.3 atoms %) distributed at three increasing rates (N1, N2, N4, where N2 is the double of N1 and N4 is the double of N2) either to soil or to canopy (foliar application) to simulate atmospheric N depositions. At the end of the experiment, plants were destructively sampled, and N derived from depositions (N_{dfd}), total N, and biomass of above and belowground organs were determined. Uptake rates ranged from 21% to 57% and the N_{dfd} recovery was higher for soil than for foliar application. Foliar-supplied plants showed a higher N_{dfd} in leaves and shoots than soil-supplied ones, while the latter showed a higher N_{dfd} in roots than the former. Moreover, total N in trunk, shoot axes and leaves increased with the N rates up to the level N2, with no further increase in N4. Increasing tree N availability, regardless the supply mode, increased the shoot:root N content. The fact that the N uptake rate was rather stable at increasing N rates suggests that if N from atmospheric depositions becomes increasingly available at the canopy or soil level, it will actively contribute to apple tree nutrition and account for a significant fraction of the apple tree N needs.

Keywords: nitrogen deposition; biomass production; N and C allocation; ^{15}N; fertilization; fruit tree nutrition; stable isotope

1. Introduction

Nitrogen (N) is an essential element of organic tissues and has a pivotal role in controlling the primary production of terrestrial ecosystems. Despite the N abundance in the atmosphere in its molecular form (N_2), this element needs to be broken down into other forms known as reactive nitrogen (Nr) before it can be effectively absorbed by plants (UNEP and WHRC, 2007). A massive increase of the worldwide circulating Nr occurred in the past century, boosted by the enormous growth of food production to sustain the increase of global population (Erisman et al., 2011). The so-called great acceleration of Nr cycle is mainly caused by the industrial synthesis of mineral fertilizer, the increased land surface devoted to biologically N-fixing crops and the combustion of fossil fuels (Gruber and Galloway, 2008; UNEP and WHRC, 2007; Vitousek et al., 2013, 1997). The increase of the Nr pool in the atmosphere, has caused an increase of Nr deposition (Galloway et al., 2008; Schwede et al., 2018) and contributed to global warming (Erisman et al., 2011; Reis et al., 2009). Both wet and dry Nr depositions can occur in chemically oxidized forms, such as nitrogen dioxide (NO_2), nitrogen monoxide (NO), nitrite (NO_2^-), nitrate (NO_3^-), and nitric acid (HNO_3), or in reduced forms, such as ammonia (NH_3) and ammonium (NH_4^+) (Li et al., 2016). Nr depositions at the global scale may range from 1 to 50 kg N ha^{-1}yr^{-1}

(Flechard et al., 2020; Li et al., 2016; Schwede et al., 2018; Wen et al., 2020).

The effects of Nr depositions depend on the soil N availability (de Vries et al., 2009; Erisman et al., 2011; Galloway et al., 2008; Phoenix et al., 2012; Pregitzer et al., 2008), i.e. under N-limited conditions they might stimulate growth (de Vries et al., 2009), while under large soil N availability they either have no effect or decrease the net primary productivity in the long period (Schmitz et al., 2019). While there is an extensive literature on this topic for forest trees (Tomaszewski et al., 2003), only few studies have addressed the effects of Nr depositions on crop productivity. For example, in a 15-years experiment in a maize-wheat rotation conducted in Northern China, it was estimated that 17-21% of the total N input derived from N depositions, which suggests that depositions significantly contributed to the total biomass production and grain yield of both crops (Liu et al., 2019).

Plants can modulate their biomass allocation pattern under the effect of environmental drivers, such as the availability of nutrients. According to a recent global meta-analysis, trees growing in soils well-endowed with N, increase the allocation to foliage and aboveground woody biomass, but not to roots (Li et al., 2021). Similarly, grapevines C allocation shifted from shoots to roots with decreasing N availability (Grechi et al., 2007), while an increase of the root:shoot ratio in the presence of N deficit was reported for several herbaceous species (Gastal et al., 2012; Kang and Iersel, 2004; Lynch et al., 2011). The response of root biomass allocation and root respiration to nutrient availability, however, depends on the species (Müller et al., 2000), the plant developmental stage, the plant size and the initial N availability (Ceccon et al., 2011; Hartmann et al., 2018; Panzacchi et al., 2012; Zhang et al., 2019). In the main Italian apple growing district (Trentino Alto Adige, North of Italy), Nr deposition, estimated in the range 10-20 kg N ha^{-1}y^{-1} depending on the proximity with the sources of N emission (Marchetti et al., 2003; Schwede et al., 2018), could contribute to the N nutrition of the apple trees, the main crop in such area.

Indeed, plants can take up Nr derived from atmospheric depositions both through the foliage, that intercepts the depositions, and through the roots, once the deposited Nr enters the soil. In agricultural systems, Nr canopy uptake could be crucial to biomass production during phenological stages with high nutrient demand, when root uptake alone is not enough, or under environmental conditions that are limiting root activity. In many horticultural systems, foliar fertilization, i.e. with urea solution, is already a widespread technique (Dong et al., 2005a; Toselli et al., 2004; Wargo et al., 2003), and proved to be effective in reducing NO_3^- losses via leaching, normally occurring with soil fertilization (Dong et al., 2005b). However, it is unclear if foliar uptake could lead to different patterns of carbohydrates allocation with respect to root uptake.

Considering the importance of N depositions and the role that foliar N supply has on apple management, we have undertaken a study to mimic increasing rates of N deposition, reaching either the canopy or the soil using potted apple trees. We wanted to test the hypothesis that apple trees can take up N from the canopy and from the roots at different rates, and that increasing canopy or root N uptake could differentially affect shoot and root growth and N partitioning.

2. Materials and Methods

2.1. Plant material and experimental setup

Two-year-old apple trees (cv. Gala Schniga® SchniCo© grafted in summer 2013 on M9, strain T337, rootstock) were transplanted in April 2015 in 23-L pots, filled with a previously sieved sandy soil collected from the topsoil of an apple orchard belonging to the Laimburg Research Center (Ora, South Tyrol, Italy). Before transplanting, the height, the diameter, and the fresh weight of each tree was measured in order to select for the experiment only trees characterized by uniform size. The plants were kept outdoors under a transparent plastic shelter to prevent rain from reaching the potted trees. Soil moisture was maintained close to field capacity by a drip irrigation system. In July, when trees reached their maximum leaf area, they started receiving a water solution of labelled ammonium nitrate ($^{15}NH_4^{15}NO_3$, iso-

topic enrichment: 10.3 atoms % ^{15}N), either by foliar spray (foliar application, hereafter F) or by soil supply (hereafter S). Three doses of N application (9, 18 and 36 kg N ha^{-1}) were compared to simulate three levels of yearly N depositions. To convert N rates per hectare into amounts of N per plant, we considered that each small tree, when growing in a nursery, occupies an area of 0.28 m^2, accounting for a tree density of approximately 35700 trees ha^{-1}. Nitrogen concentration in the sprayed solution used for foliar supply was 0.82, 1.64 and 3.28 g L^{-1}, for low (N1), medium (N2) and high (N4) N rates, respectively. The N solution was sprayed to the foliage from the top, simulating the presence of a mist and the amount of sprayed solution measured. A total of 225, 450 and 900 mg N tree^{-1} was supplied from 15 July to 9 October, split into seven applications, to N1, N2 and N4 treatments, respectively. Five trees were randomly assigned to each of the six N treatments (hereafter F1, F2, F4, S1, S2, and S4) and to the unfertilized control. Leaf chlorophyll concentration was estimated on 9 October by the portable instrument SPAD 502 Plus Chlorophyll Meter, (Spectrum technologies, Inc.), measuring five leaves per tree. On 23 November, when leaf abscission was almost complete, total tree shoot length was measured, then all trees were destructively harvested and divided into the following parts: roots, tree stem below the grafting point (later Wood$_{BG}$), tree stem above the grafting point (later Wood$_{AG}$), shoot axes and leaves. Each organ was separately oven-dried to constant weight at 65 °C, weighted and milled to prepare it for the analysis of total C, total N and ^{15}N concentration.

2.2. Analysis of labeled N

After drying, samples were milled with a ball mill. An aliquot of each sample of approximately 2-5 mg was weighted in a tin capsule. Total carbon (C), nitrogen (N) and ^{15}N abundance (^{15}N in sample) of each sample were measured by CF-IRMS (Continuous Flow-Isotope Ratio Mass Spectrometry, Thermo Fisher Scientific). The percentage of nitrogen derived from depositions was calculated following the equation:

$$N_{dfd} = \frac{(^{15}N\ abundance\ in\ sample\ -\ ^{15}N\ natural\ abundance)}{(^{15}N\ abundance\ in\ fertilizer\ -\ ^{15}N\ natural\ abundance)} \times 100$$

where *^{15}N abundance in fertilizer* and *^{15}N natural abundance* were equal to 10.3 and 0.3672 atoms % (average of control samples before the application of the labelled fertilizer), respectively (Curetti et al., 2013; Scandellari and Tagliavini, 2018; Petrillo et al., 2020). Hereafter, the ^{15}N recovered in the plants will be referred to as *N derived from depositions* (N$_{dfd}$).

The N and C content in roots and shoots (shoot axes + leaves) were determined multiplying the average dry biomass times the correspondent concentration of the element.

2.3. Statistical analysis

The assumptions for the ANOVA of normal distribution and homogeneity of variances were verified with Shapiro-Wilks and Levene test, respectively. After that, data were subjected to a factorial ANOVA adapted to a completely randomized design, considering as factors the N dose (N1, N2, N4) and the application mode (foliar, soil). In case of significant differences (P<0.05), LSD test was performed for multiple comparisons. Statistical analysis and plots were made using R software version 3.6.3 (R Core Team, 2020).

3. Results

3.1. N uptake and partitioning

As general trend, the trees took up more N from simulated deposition when N was supplied to the soil than when applied to the foliage, although such effect was statistically significant only when the trees received the highest N rate (Table 1). As expected, regardless the organ that took up the labeled N (foliage or roots), increasing the N rate resulted in increasing N uptake rates (Figure 1). When N was

applied to the soil, increasing N rate also increased the fraction of N recovered in the plants (Table 1). Differently, higher amounts of foliar-supplied N did not cause a significant increase in the percentage of N recovered in the plant (Table 1).

Table 1. Actual amount of labelled nitrogen (mg tree^{-1}) supplied by foliar or soil application by the three N supply rates and the corresponding fraction recovered in plants (% of the amount supplied). For the recovered N percentage, different lowercase and uppercase letters indicate statistically significant differences (p<0.05) between N supply rates (N1, N2, and N3) and application modes (foliar *vs.* soil application), respectively.

Nitrogen supply rate	Supplied N (mg tree^{-1})	Recovered N (%)	
		Foliar	Soil
N1	233	21	33 b
N2	488	26	48 ab
N4	908	26 B	57 Aa
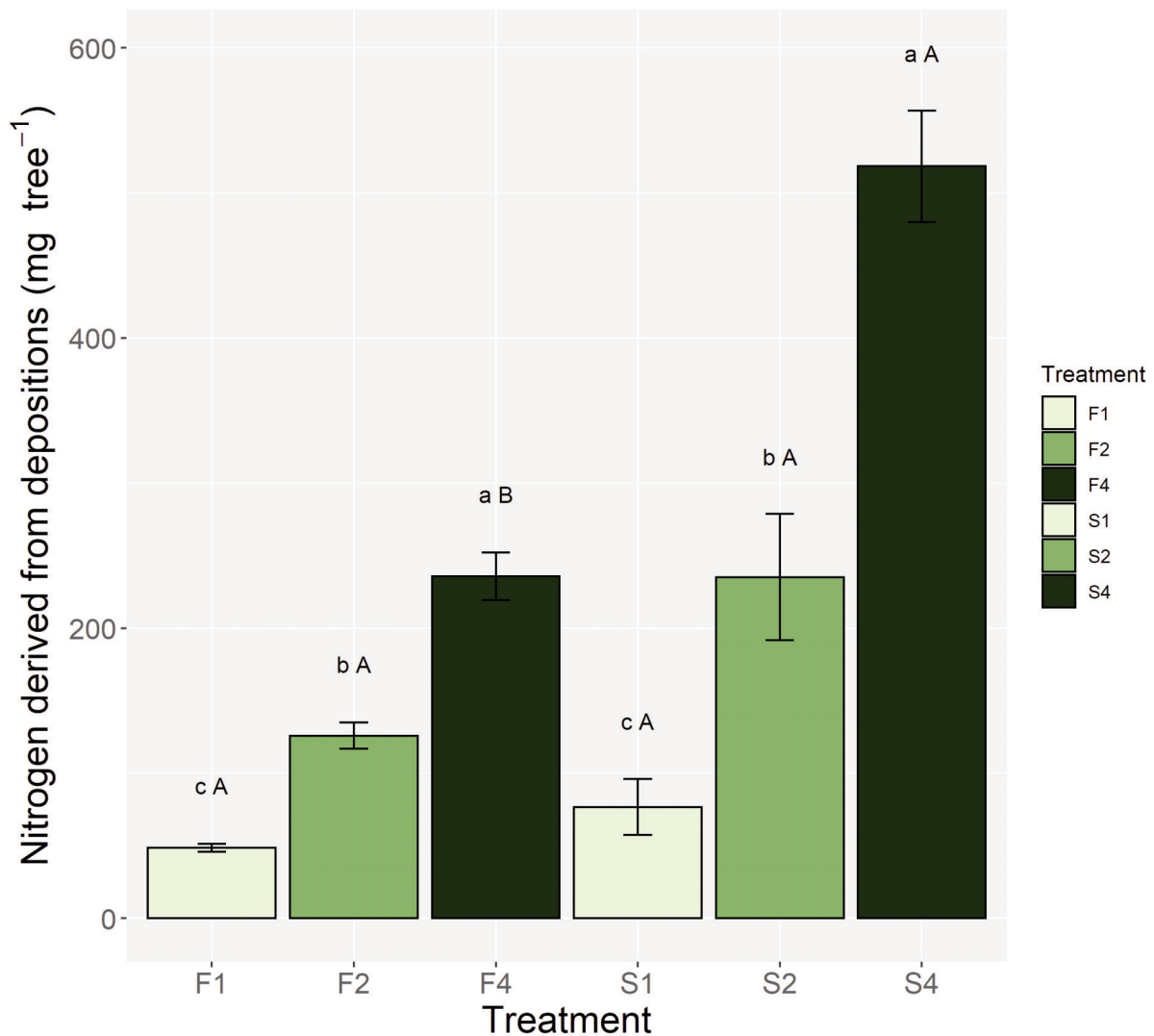
Figure 1. Nitrogen derived from depositions (mg tree^{-1}) recovered in plants at the end of the experiment. Lowercase letters indicate the results of LSD post-hoc test after one-way ANOVA (p-value < 0.05), performed separately for the foliar and for soil. Uppercase letters show the results of pair-wise comparison performed with t-test between foliar and soil treatment with equal N supply rate (i.e. F1 *vs.* S1, F2 *vs.* S2, and F4 *vs.* S4, p-value < 0.05). Error bars indicate the standard error of the means (n=5).

The labeled N (N_{dfd}) was differentially partitioned among the tree organs depending on whether it was absorbed by the roots or by the leaves (Figure 2). A relatively higher fraction of the absorbed N_{dfd} was found in leaves and shoot axes when the labeled N was supplied to the foliage (approx. 20-35%) than to the soil (approx. 5-10%). On the contrary, when N was supplied to soil, more N was partitioned to the roots (approx. 35-40%) than when supplied to the foliage (approx. 10%) (Figure 2). In both sets of trees and regardless of the N rate, most N_{dfd} was found in the woody organs (Figure 2); however, most N_{dfd} was recovered in the above ground organs (leaves + shoot axes + wood$_{AG}$) when N was supplied to the foliage, whereas it was found more in the below ground organs (wood$_{BG}$ + roots) when it was supplied to the soil.

In October, leaf chlorophyll concentration, that is usually linearly correlated with total leaf N concentration, was highest in N4 trees irrespective of the supply mode (Table 2). At the end of the experiment, the N concentration of the trees treated with N1 rate was generally similar to that of control trees (Table 2). The factorial ANOVA applied to root N concentration data showed a significant interaction between the rate and the supply mode. Indeed, only plants receiving soil N were affected by the N rate
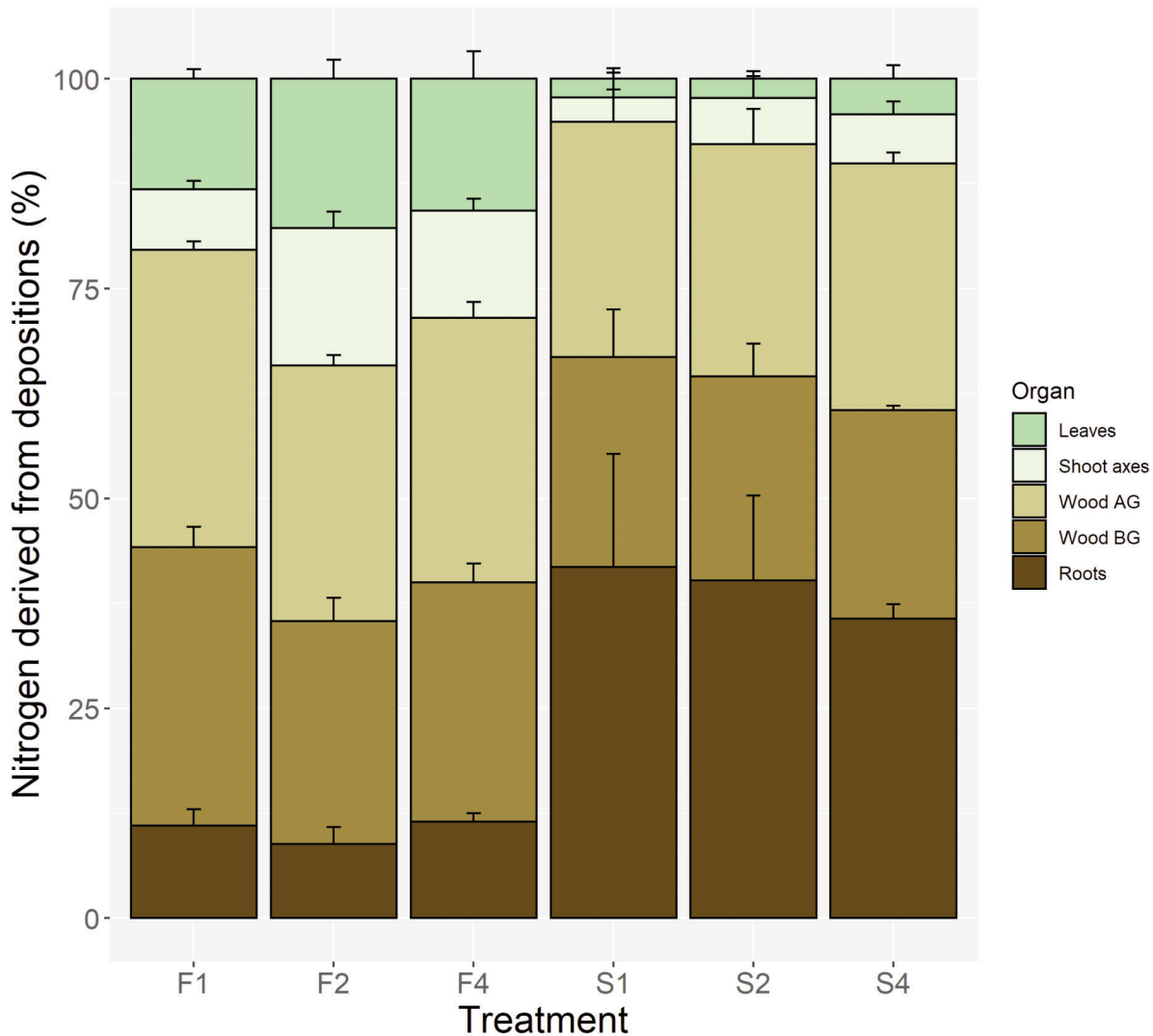

Figure 2. Relative contribution of different plant organs to the total amount of nitrogen derived from depositions (N_{dfd}), recovered in plants at the end of experiment. Wood$_{AG}$ is the aboveground portion of wood (trunk), and wood$_{BG}$ is the belowground portion (rootstock). Error bars indicate the standard error of the means (n=5).

(Table 2), which increased from N1 to both N2 and N4. Moreover, when the two highest N rates (N2 and N4) were applied, root N concentration was higher when the N was supplied to the soil than to the foliage. Trunk N concentration progressively increased with the N rate irrespective of the N supply mode. Regardless of the N supply mode, total N concentration of leaves and shoot axis increased from N1 to N2, but not from N2 to N4. It should be noticed that the leaf N concentration is generally low, probably as a consequence of the ongoing senescence of these organs and of the concurrent N remobilization (Tagliavini et al., 1998). While trunk N was slightly higher when the N was given to the soil than to the foliage, the opposite effect was recorded for the N concentration in the shoot axes.

Table 2. Leaf SPAD values and total nitrogen concentration (% DW) in leaves, roots, shoot axis and trunk (Wood$_{AG}$) as affected by N rate and application type (supply mode) and their interaction.

Source of variation	SPAD units	Nitrogen (%)				
		Root		Trunk	Shoot axis	Leaves
		(F)	(S)			
Supply rate						
N1	50.6 ± 0.6 b	0.83 ± 0.06 Aa	0.84 ± 0.01 Ab	0.60 ± 0.02 b	0.91 ± 0.05 b	0.62 ± 0.02 b
N2	51.9 ± 0.7 b	0.81 ± 0.02 Ba	1.05 ± 0.03 Aa	0.64 ± 0.03 ab	1.23 ± 0.10 a	0.78 ± 0.06 a
N4	55.1 ± 0.6 a	0.83 ± 0.08 Ba	1.15 ± 0.07 Aa	0.70 ± 0.04 a	1.30 ± 0.08 a	0.82 ± 0.4 a
p-value	*	ns	*	*	*	*
Supply mode						
Foliar (F)	53.0 ± 0.5			0.61 ± 0.02 b	1.30 ± 0.07 a	0.75 ± 0.03
Soil (S)	52.2 ± 0.6			0.68 ± 0.03 a	0.98 ± 0.07 b	0.73 ± 0.05
p-value	ns			*	*	ns
Interaction						
p-value	ns	*		ns	ns	ns
Control (not fertilized)	45.2 ± 1.0	0.82 ± 0.01		0.58 ± 0.04	0.95 ± 0.07	0.64 ± 0.07

Data are averages ± standard error of the means (n=5). The significance levels correspond to the result of a factorial ANOVA. Wherever the main effects were significant (* = p-value < 0.05), letters correspond to multiple comparisons performed with LSD post hoc test. Wherever interaction was significant, values are given for each combination of dose and mode. Uppercase letters correspond to significant differences after pairwise comparison (t-test, p-value < 0.05) between the two supply modes; lowercase letters correspond to the result of multiple comparisons performed with LSD post hoc test among N rates. Average values of control trees are reported in the last row. ns indicates no statistically significant differences.

3.2. Effects on growth

Shoot biomass followed the trend N1 < N2 < N4, both when N was supplied to the soil and to the foliage, although only rate N1 was significantly lower than the rates N2 and N4. Moreover, only trees treated with N2 and N4 had shoot biomass significantly higher than in N1-treated plants (Table 3). No significant effect of treatments on shoot length was recorded. Average root biomass was always lower in treated (fertilized) than in control plants, even though this difference was not statistically significant. The N root:shoot ratio was highest under no or low nitrogen application (control and N1, respectively), while it decreased in N2 and N4 trees, independently of the supply mode. The effect of increasing the N rate on the C root:shoot ratio was similar to the N root:shoot ratio, but it was not statistically significant (Table 3).

Table 3. Average values (± standard error) of total shoot length, shoot (sum of leaves and shoot axes) and root biomass, and C and N root: shoot ratio, as affected by N rate and application type (supply mode).

c	Total shoot length (cm tree^{-1})	Biomass (g DW tree^{-1})		Root:shoot	
		Shoots	Roots	C tot	N tot
Supply rate					
N1	242.1 ± 15.5	46.51 ± 2.96 b	55.65 ± 3.47	1.17 ± 0.12	1.50 ± 0.21 a
N2	298.7 ± 20.4	56.51 ± 2.92 a	49.47 ± 6.15	0.86 ± 0.09	0.97 ± 0.19 b
N4	279.3 ± 20.7	58.15 ± 3.72 a	60.69 ± 5.10	0.90 ± 0.08	1.03 ± 0.14 b
p-value	ns	*	ns	ns	*
Supply mode					
Foliar (F)	284.8 ± 15.7	55.41 ± 3.51	57.10 ± 5.12	1.01 ± 0.11	1.01± 0.10
Soil (S)	265.3 ± 18.2	52.03 ± 2.08	53.44 ± 2.97	0.95 ± 0.06	1.33 ± 0.12
p-value	ns	ns	ns	ns	ns
Interaction					
p-value	ns	ns	ns	ns	ns
Control (not fertilized)	264.2 ± 31.4	52.91 ± 9.11	70.53 ± 21.90	1.20 ± 0.20	1.59 ± 0.41

The significance levels (* = p-value <0.05; ns = not statistically significant) correspond to the result of factorial ANOVA, where supply dose, supply mode, and their interaction were considered. Whenever the effects were significant, letters correspond to multiple comparisons performed with LSD post hoc test. Average values of control trees are reported in the last row.

4. Discussion

4.1. Root vs. foliar N uptake

Nr depositions occurring during the growing season in the apple orchard either reach the soil directly or are intercepted by the canopies of the apple trees. The fate of N intercepted by the canopies includes leaf uptake and N leaching after rain or overhead irrigation events. Additionally, some N can be deposited on the leaf surfaces and reach the soil after leaf abscission. As the N from atmospheric depositions can be taken up directly from the tree canopies, a quantitative assessment of the uptake efficiency is important to fine-tune N supply in orchards. In this study, the N deposition taken up by the leaves were lower than that taken up from the roots (Table 1 and Figure 1). This result could have been affected by the young age of the trees and by their relatively low leaf area and ability to intercept the N depositions (Bowman and Paul, 1992).

4.2. Allocation of N derived from depositions and effects on plant growth

The allocation pattern showed that a higher share of N$_{dfd}$ was retrieved in shoots (axes and leaves) when the depositions were taken up by the canopy, while soil applications caused a higher share of N$_{dfd}$ in roots (Figure 2). Similar results were found by Dong et al. (2005a), especially when they applied the N late in the season. The foliar supply mode strongly improved the allocation of newly absorbed fertilizers to new aboveground organs with respect to new belowground organs (AG/BG ratio of N$_{dfd}$ was 2.3 and 12.9 with soil and leaf distribution, respectively). Regardless the supply mode, increasing the N availability, shifted in part both the labelled (Figure 2) and total N (Table 3) from roots to shoots as a result of both an increase in the leaf and shoot N concentration, and a higher shoot biomass, obtained when the rate of simulated N depositions increases. Conversely, N allocation to roots was in most cases inversely related to the N rate, regardless the supply mode. A decrease in ^{15}N allocation to the roots of apple trees at increasing levels of plant N was also shown by Cheng et al. (2002).

In general, the largest fraction (i.e. ~50%) of N_{dfd} was found in the trunk and in the rootstock, possibly as a consequence of internal translocation of part of the nutrients from the annual aboveground organs to woody organs occurred late in the season, during leaf senescence.

The effect of N treatments on total N accumulation and plant growth was rather limited. However, a significant effect on shoot growth at increasing N depositions was observed independently from the supply mode and starting from N2 rate. On the contrary, N depositions did not affect the belowground biomass (Table 3). The decreasing trend of the C root:shoot ratio at increasing N level is not supported by statistics and cannot confirm the general assumption of a greater C allocation to root under low N availability observed in several plant species (Gastal et al., 2012; Kang and Iersel, 2004; Lynch et al., 2011).

4.3. N deposition effects on apple tree nutrition

Studies at global and local scale indicate that Nr depositions largely varies depending on the location; they may reach even 50 kg N ha^{-1} in areas close to large N emission sources (Flechard et al., 2020; Marchetti et al., 2003; Schwede et al., 2018; Wen et al., 2020). Extrapolating useful knowledge from a manipulative potted experiment, to be used for orchard N management, is not easy. Our experiment did not include the dry depositions and we could not differentiate between the uptake from reduced and oxidized N forms. Moreover, the simulated deposition covered a short time scale and included N concentrations in the applied solution probably higher than those generally occurring in nature. However, interestingly, we found that at the highest N depositions level, the N uptake rate at the leaf level was not reduced, while it even increased when the deposited N reached the soil becoming available for root absorption. This suggests an increasing role of N deposition in the N nutrition of apple trees if N depositions increase.

It has to be considered that the hedgerow nature of intensive apple orchards (Dragoni et al., 2005; Volschenk, 2017; Zanotelli et al., 2019) intrinsically limits their ability to intercept the totality of the bulk N deposition. The N deposition that falls directly on the tree canopy or on the portion of soil explored by apple roots, can be approximated to 2/3 of the orchard surface, the remaining part being represented by the central part of the orchard alleys. When apple trees are grafted on dwarfing rootstocks, few apple roots are present underneath the alley soil (Scandellari et al., 2015; Zanotelli et al., 2013), however, the atmospheric N deposition may eventually contribute to the N nutrition of the grasses covering the interrow. Assuming yearly Nr depositions in the range of 15-20 kg N ha^{-1} in the main Italian apple growing districts (Marchetti et al., 2003; Schwede et al., 2018), based on the uptake rate we have recorded, we can speculate that 5-6 kg of N from atmospheric depositions can be available to apple trees in the same year they fall to the ground, therefore reducing the fertilizer needs. The remaining fraction of the deposited N could remain, at least in part, available in the soil for future root uptake.

5. Conclusions

When N depositions occur, they are partially intercepted by the tree canopy while the remaining fraction reaches the soil surface. In this study, we have separated the effects of both leaf and root uptake of the deposited N. Regardless of the N rate, the N absorbed by the leaves mainly remained in the canopy and was less partitioned to the roots as compared to the soil deposited N. The supply rate had a strong effect on total N allocation, with a general significant reduction of the root:shoot N at increasing N rates. The fact that the N uptake rate was rather stable when N rates increased suggests that if atmospheric N depositions get bigger, they will become increasingly important in the nitrogen nutrition of apple trees. Further studies, to be conducted in open field conditions, are anyhow needed to reinforce the soundness of the indications provided by this study carried out with model plants.

Conflicts of Interest: the authors declare no conflict of interest.

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