



Response of Young Hazelnut Trees to Different Nitrogen Fertilization Rates

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Abstract

Nitrogen (N) plays a central role in supporting photosynthesis, vegetative growth, and early productivity in fruit crops. For hazelnut (*Corylus avellana* L.), Italian regional guidelines recommend relatively low N inputs during the establishment phase, particularly under non-irrigated conditions. However, information on optimal N requirements for young, irrigated hazelnut orchards remains limited. This study aimed to identify the optimal N rate for young, irrigated hazelnut orchards and to relate N supply to leaf N concentration, gas exchange, vegetative growth, and early yield. A three-year experiment (2023–2025) was conducted in an irrigated ‘Tonda di Giffoni’ orchard in northern Italy. Four fertilization regimes combining two N doses and two application frequencies were compared with an unfertilized control. Leaf N concentration, gas exchange parameters, vegetative growth, and yield were monitored throughout the study. N supply had limited influence on seasonal physiological patterns, while leaf N concentration showed a clear dose–response relationship. Maximum photosynthetic rates were associated with leaf N concentrations between 20 and 22 g kg⁻¹ dry weight. Growth and yield responses indicated that intermediate–high N applications (approximately 75–100 kg N ha⁻¹ at the 2nd–3rd year) resulted in the greatest trunk growth and highest yield at the 4th year. In the final year, despite fertilizer rates exceeding current recommendations, leaf N concentrations remained below the optimal threshold, suggesting a dilution effect associated with increasing canopy size and structural biomass. The results indicate that young hazelnut trees require higher N inputs than those commonly recommended by regional guidelines. However, increasing N supply beyond the optimal range does not proportionally enhance photosynthesis, vegetative growth, or early yield.

Keywords Nitrogen Fertilization · Hazelnut · Fertilization Rate · Broadcast Fertilization

1 Introduction

Nitrogen (N) is one of the most important essential elements for plant growth, being a fundamental constituent of chlorophyll, amino acids, and proteins (Hawkesford et al. 2012; Taiz et al. 2015). Its availability directly influences photosynthetic efficiency, canopy development, and yield potential. In fruit trees, adequate N supply promotes leaf expansion, shoot elongation, and the establishment of a well-balanced canopy, while both deficiency and excess

can lead to metabolic and structural imbalances affecting productivity and environmental sustainability (Weinbaum et al. 1992; Sun et al., 2018). Leaf N concentration is one of the most reliable indicators of photosynthetic capacity in C3 plants, as it directly reflects the biochemical investment in the photosynthetic apparatus. Across a wide range of woody and herbaceous species, up to 60–70% of total leaf N is allocated to photosynthetic proteins—mainly Rubisco, thylakoid membrane complexes, and electron transport enzymes—which collectively determine the maximum carboxylation and assimilation rates ([https://doi.org/10.1890/0012-9658\(1999\)080\[1955:GOLTRA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[1955:GOLTRA]2.0.CO;2) Reich et al. 1999; Evans and Clarke 2019; Dong et al. 2022). Consequently, a strong linear relationship has been consistently observed between foliar N concentration and photosynthetic performance, largely independent of species or environment. Under N deficiency,

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chlorophyll concentration and photosynthetic rate decline rapidly, leading to reduced specific leaf area, limited carbon assimilation, and lower biomass accumulation (Peng et al. 1995; Zhao et al. 2005). Maintaining leaf N concentration within the physiological sufficiency range is therefore essential to sustain optimal photosynthetic activity, canopy development, and vegetative growth.

In perennial crops such as hazelnut, N plays an even more complex physiological role because it is not only required for current growth but is also stored and remobilized between seasons. Based on long-term field studies conducted in Oregon, hazelnut trees were shown to rely strongly on internal N reserves, which are remobilized during spring bud break and early leaf emergence before new uptake occurs from the soil. Isotopic tracer experiments reported in these studies indicated that only about 28% of applied N fertilizer is recovered by the tree, while a substantial fraction of absorbed N is stored in perennial woody tissues to support subsequent growth cycles. Within the same experimental framework, a reference range for foliar N concentration of 2.2–2.5% (dry weight), based on August leaf sampling, was established as the threshold below which clear N deficiency symptoms occur (Olsen 1997).

Subsequent studies conducted in different hazelnut-growing regions have reported foliar N concentrations that closely align with the range proposed by Olsen (1997), despite substantial differences in climate, cultivar, and soil conditions. In the US Upper Midwest, research on hybrid hazelnuts (*C. avellana* × *C. americana*) highlighted relatively low N requirements. In establishment trials, Braun et al. (2011a, b) found that plant survival and growth decreased with increasing N rate and that satisfactory foliar N concentrations (~2.2%) could be achieved with moderate fertilization, particularly on soils rich in organic matter. Later synthesis confirmed that exceeding this level does not further enhance growth but rather reduces N use efficiency (Braun 2024).

Comparable values have been reported in other traditional, non-irrigated hazelnut production systems. In Chile, recent work on high-yielding orchards of cv. ‘Barcelona’ managed under non-irrigated conditions confirmed that mid-summer leaf N concentrations of 23–24 g kg⁻¹ correspond to optimal conditions for growth and productivity, with a progressive seasonal decline observed towards autumn (Hirzel and Mejias-Barrera 2025). Controlled-environment experiments with juvenile *C. avellana* ‘Tonda di Giffoni’ further demonstrated that a 50% reduction in N supply was sufficient to cause visible chlorosis and reduced biomass accumulation, confirming the crop’s sensitivity to sub-optimal N availability (Voogt et al. 2023). Additional field evidence supports the existence of an optimal N rate beyond which growth efficiency declines. In northeastern China, one-year-old *C. avellana* ×

C. heterophylla seedlings exhibited maximum root biomass, shoot elongation, and photosynthetic capacity at 22 g N plant⁻¹, whereas higher rates reduced root activity and growth (Li et al. 2019). Although this rate cannot be directly translated into a field-scale recommendation, it corresponds to a moderate N supply when expressed on an area basis, depending on planting density, and is of the same order of magnitude around 100 kg N ha⁻¹. Similarly, in Turkey, subsurface application of urea divided into two equal doses significantly increased yield, kernel ratio, and leaf N concentration compared with surface or single-dose applications, demonstrating that timing and placement are as important as the total rate for improving N use efficiency (Özkutlu et al. 2024).

Taken together, these studies indicate that, although recommended N application rates vary widely among regions and production systems, the foliar N concentration associated with optimal physiological performance remains relatively consistent, generally clustering around 22–24 g kg⁻¹. Similar convergence of foliar sufficiency ranges has been reported across perennial woody crops and is widely adopted as a diagnostic threshold in plant nutritional assessment (Olsen 1997; Bryson et al. 2014; Carranca et al. 2018).

Notably, much of the experimental evidence underlying these ranges originates from non-irrigated or traditionally managed orchard systems, suggesting that foliar N concentration is constrained by intrinsic physiological limits rather than by fertilizer input alone. This interpretation is consistent with the concept of biological limits to nitrogen use efficiency, whereby increases in N supply beyond a critical threshold do not result in proportional gains in photosynthesis or vegetative growth (Rotundo and Cipriotti 2017; Tagliavini et al. 2025).

Only recently studies have begun to investigate hazelnut N dynamics under modern irrigated systems. Wiman et al. (2023) demonstrated that fertigation substantially improved N partitioning and yield compared with traditional dryland orchards. Irrigated trees showed higher ¹⁵N enrichment in all organs and greater cumulative production, highlighting the need to reassess N management strategies and reference thresholds originally developed under non-irrigated conditions.

Despite the physiological importance of N, fertilization guidelines for hazelnut vary widely among regions. In Italy, disciplinary standards for integrated production recommend around 70 kg N ha⁻¹ at the third year after planting, while agronomic handbooks suggest contrasting rates—100 kg N ha⁻¹ (Tombsesi 1985) and 56 kg N ha⁻¹ (Botta and Valentini 2019) for the same developmental stage. Long-term monitoring of nutrient removal in Piedmont orchards showed that N is the element most extracted by the crop, with average annual removal of 23–26 kg N ha⁻¹ in producing orchards (Roversi 2014).

Based on this evidence, our hypothesis was that hazelnut responds positively to moderate N supply, whereas excessive fertilization does not proportionally enhance growth or yield and may reduce N use efficiency.

The aim of this experiment was therefore to determine the optimal N dose during the first years after planting that maximizes photosynthetic performance, vegetative growth, and early yield efficiency under irrigated orchard conditions.

2 Materials & Methods

2.1 Site Description and Orchard Management

The experiment was carried out in northern Italy, within the experimental hazelnut orchard of the Università Cattolica del Sacro Cuore, located in Piacenza (45°02'03.2" N, 9°43'51.7" E; 61 m a.s.l.). Piacenza lies in the south-central sector of the Po Valley, an area characterized by a transitional climate between Mediterranean and continental/oceanic regimes typical of Central and Western Europe (Nistor 2016). During the vegetative period (from April to November), average daily air temperatures varied between 10 °C and 27 °C, and the total annual rainfall was approximately 400 mm. More detailed climatic data over the three-year trial are reported in Fig. S1.

The topsoil layer (0–50 cm), corresponding to the main root zone, was classified as silty clay loam according to the USDA system, with 20% sand, 43% silt, and 37% clay, and was characterized by a neutral pH (8.1), an organic matter 2.9%, a total N content of approximately 0.191%. The orchard was established in January 2022, with trees spaced 4 × 4 m apart (625 plants ha⁻¹) and trained to single-stem.

The trial covered three growing seasons (2023–2025). The orchard, cv. *Tonda di Giffoni*, was managed under drip irrigation, with seasonal irrigation volumes of 5, 10, and 16 L plant⁻¹ day⁻¹ in 2023, 2024, and 2025, respectively. These irrigation volumes were determined based on crop evapotranspiration requirements, as described below.

Irrigation scheduling was based on the restoration of 100% of actual evapotranspiration ($ET_a = ET_o \times K_c \times K_r$), where ET_o is the reference evapotranspiration (mm day⁻¹), K_c is the crop coefficient, and K_r represents the ground canopy coverage factor. Irrigation was applied from bud break (April) to leaf fall (October). ET_o was calculated using the FAO Penman–Monteith method (Allen et al. 1998) from measurements of air temperature (T_a), relative humidity (RH), solar radiation (R_s), and wind speed (W_s). These meteorological parameters, together with precipitation (P_p), were continuously monitored by a weather station (Netsens s.r.l., Calenzano, Italy) installed within the orchard. A constant K_c value of 0.8 was adopted throughout the irrigation period.

2.2 Experimental Design and Treatments

The experiment was conducted in a row of the orchard, per each treatment were randomly chosen blocks of 5 trees in which the external ones were used as guards. Measurements were taken on the same 5 trees chosen from the 6 central trees (3+3) in the two blocks. Four N fertilization treatments were applied, in addition to an unfertilized control. Treatments combined two N dose levels (Low and High, defined as N dose per application) with two application frequencies (weekly and bi-weekly), resulting in four fertilization treatments: (i) low dose applied every two weeks (Low Bi-Weekly Dose, LBWD), (ii) low dose applied weekly (Low Weekly Dose, LWD), (iii) high dose applied every two weeks (High Bi-Weekly Dose, HBWD), and (iv) high dose applied weekly (High Weekly Dose, HWD). For each growing season, two N dose levels (Low and High) were defined, and each dose level was applied using the two application frequencies. The terms Low and High therefore indicate relative N dose levels within each year, whereas Weekly and Bi-weekly describe the application frequency. As a consequence of this factorial design, treatments LWD and HBWD received the same total annual N input, while differing in N dose per application and temporal distribution. Annual N rates for each treatment are reported in Table 1. Treatments were arranged in order of increasing N supply per hectare, considering a planting density of 625 plants ha⁻¹. The experiment was carried out during the 2023, 2024, and 2025 growing seasons. N fertilization treatments started after bud break, with the first application performed in late April of each growing season. Doses applied per treatment and year are reported in Table 1.

For each plant, the required amount of fertilizer was weighed and placed in a separate container. The fertilizer was evenly distributed over the soil surface corresponding to the root zone, which was estimated as the canopy projection area on the ground.

2.3 Physiological Measurements

2.3.1 Gas Exchange and Stem Water Potential

Physiological measurements were conducted at midday (11:30–14:30 solar time) during the 2023, 2024, and 2025 growing seasons. Measurements were intentionally conducted at midday to ensure higher representativity of the daily course of gas exchanges. Measurements were carried out from late April/May to late October–early November, covering the entire vegetative period, with at least five measurement campaigns per season. On each measurement date, all treatments were assessed within the same day to ensure comparability under similar environmental conditions.

Table 1 Experimental scheme of N fertilization treatments, including application frequency, fertilizer N content, applied N rate, and estimated cost per hectare (average fertilizer price: 380 € t⁻¹). Treatment codes are defined as follows: LBWD, low N dose with biweekly application; LWD, low N dose with weekly application; HBWD, high N dose with biweekly application; and HWD, high N dose with weekly application. Estimated costs refer exclusively to fertilizer cost and do not include labor, irrigation, or other operational expenses. Treatments differed in N dose per application and application frequency; equal annual N rates may result from different dose–frequency combinations

Year	Dose	Frequency	Treatment code	Fertilizer	N content (%)	Number of Application	g plant ⁻¹	kg N ha ⁻¹	Cost (€ ha ⁻¹)
2023	Low	Weekly	LWD	NO ₃ NH ₄	27%	24	20	75	112.59 €
	High	Weekly	HWD	NO ₃ NH ₄	27%	24	40	150	211.11 €
	Low	Bi-weekly	LBWD	NO ₃ NH ₄	27%	12	20	40	56.30 €
	High	Bi-weekly	HBWD	NO ₃ NH ₄	27%	12	40	75	112.59 €
2024	Low	Weekly	LWD	NO ₃ NH ₄	27%	24	25	100	140.74 €
	High	Weekly	HWD	NO ₃ NH ₄	27%	24	50	200	281.48 €
	Low	Bi-weekly	LBWD	NO ₃ NH ₄	27%	12	25	60	70.37 €
	High	Bi-weekly	HBWD	NO ₃ NH ₄	27%	12	50	100	140.74 €
2025	Low	Weekly	LWD	NO ₃ NH ₄	27%	17	50	130	182.96 €
	High	Weekly	HWD	NO ₃ NH ₄	27%	17	90	250	351.85 €
	Low	Bi-weekly	LBWD	NO ₃ NH ₄	27%	9	50	70	98.52 €
	High	Bi-weekly	HBWD	NO ₃ NH ₄	27%	9	90	130	182.96 €

Midday stem water potential (Ψ_{stem}) was determined using a pressure chamber (Model 3005H07G4P40, Soil Moisture Equipment, Santa Barbara, CA, USA). Fully expanded, mature leaves located on shoots close to the main stem were selected and bagged in aluminum foil at least 30 min prior to measurement.

Stomatal conductance (g_s), transpiration rate (E), and net photosynthetic rate (P_n) were measured simultaneously using a portable gas analyzer (LCpro T, ADC BioScientific, Hoddesdon, UK). Leaf gas exchange measurements were conducted under ambient field conditions during midday and under clear-sky conditions. During measurements, photosynthetic photon flux density (PPFD) at the leaf level typically ranged between 1200 and 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$, air temperature between 28 and 35 °C, and vapor pressure deficit (VPD) between 2.0 and 3.5 kPa, depending on the measurement date. Measurements were performed within a relatively narrow time window to minimize environmental variability among treatments and under ambient CO₂ concentrations, which ranged between 430 and 450 ppm.

2.3.2 Chlorophyll Fluorescence

Chlorophyll fluorescence measurements were performed on fully expanded, sun-exposed leaves using a portable fluorometer (OS5p+, Opti-Science, Hudson, NH, USA). Measurements were conducted under field conditions during midday, on the same leaves used for gas exchange measurements. During the first experimental season, the maximum quantum efficiency of PSII (F_v/F_m) was assessed on dark-adapted leaves. Leaves were dark-adapted for approximately 30 min using leaf clips to ensure full oxidation of QA and opening of PSII reaction centers. Minimum

fluorescence (F_o) was recorded under weak modulated light, while maximum fluorescence (F_m) was induced by a saturating light pulse. The F_v/F_m ratio was calculated as $(F_m - F_o)/F_m$. In subsequent measurements, chlorophyll fluorescence was assessed under light-adapted conditions, focusing on the effective quantum yield of PSII ($Y(\text{II})$) and electron transport rate (ETR). Steady-state fluorescence (F_s) and maximum fluorescence in the light-adapted state (F'_m) were determined using saturating pulses, and $Y(\text{II})$ was calculated as $(F'_m - F_s)/F'_m$. Electron transport rate was estimated as $\text{ETR} = Y(\text{II}) \times \text{PPFD} \times 0.5 \times \alpha$, where PPFD is the incident photosynthetic photon flux density, 0.5 accounts for equal energy partitioning between photosystems, and leaf absorptance (α) was assumed to be 0.84. Calculation and interpretation of fluorescence-derived parameters followed standard ecophysiological approaches (Jones 2014), while instrument settings and measurement protocols followed the manufacturer's guidelines (Opti-Sciences, 2013). Preliminary measurements in the second experimental season confirmed that F_v/F_m showed limited sensitivity under the experimental conditions; therefore, subsequent analyses focused on light-adapted fluorescence parameters ($Y(\text{II})$ and ETR), which are more responsive indicators of photosynthetic regulation under moderate nutritional treatments. Measurements were performed on five replicate plants per treatment ($n = 5$), using one leaf per plant, and the same individuals were monitored throughout the experimental period. In 2023, P_n progressively declined during the season despite relatively stable or slightly increasing intercellular CO₂ concentration (C_i) across treatments (Supplementary Table S1). This pattern indicates that the seasonal reduction in P_n was not primarily driven by stomatal limitations, but rather by non-stomatal factors.

2.4 Vegetative and Reproductive Measurements

The measured variables included trunk cross-sectional area (TCSA), recorded before bud break and after leaf fall; total weight of pruned suckers produced during the current season; length of the longest shoot of both the previous and current years; number of current-year shoots; and number of female flowers. All vegetative and reproductive measurements were performed at complete leaf fall at the end of each growing season, specifically at the end of 2022 (baseline), 2023, 2024, and 2025, depending on the variable considered. Female flower counts were conducted in December 2023 and December 2024. The senescence index was calculated as the ratio between the length of the longest current-year shoot produced on an apical two-year-old shoot and the length of that same two-year-old shoot. Measurements were performed on five replicate plants per treatment ($n=5$), using the same individuals monitored for physiological measurements throughout the experimental period.

2.5 Leaf N Analysis

Leaf samples were collected in August of each growing season (2023, 2024, and 2025) from fully expanded leaves and freeze-dried to constant weight. The samples were then finely ground, and total N concentration was determined using the Kjeldahl method. Lyophilized leaf tissue (0.5 g) was weighed into digestion tubes, to which 7 g of potassium sulfate (K_2SO_4) were added as a digestion catalyst. Under a fume hood, 12 mL of a mixed concentrated sulfuric–phosphoric acid solution (H_2SO_4/H_3PO_4) were carefully added, followed by 7 mL of 30% hydrogen peroxide (H_2O_2). After gentle mixing, a Kjeldahl catalyst tablet was introduced. Samples were placed in a digestion block (SpeedDigester K-425, Büchi, Switzerland) at 390 °C for 15 min to control initial foaming, then the temperature was increased to 700 °C and maintained for approximately 40 min, until the digestate turned emerald green. After cooling, the digested solution was distilled in an alkaline medium (30% NaOH) using a distillation unit (K-355, Büchi, Switzerland), and the released ammonia was captured in 25 mL of 4% boric acid (H_3BO_3) containing methyl red indicator. The absorbed ammonia was then titrated with 0.1 N sulfuric acid (H_2SO_4) to quantify total N.

2.6 Statistical Analysis

All data are expressed as mean \pm SE. Differences among treatments were evaluated using one-way ANOVA model with significance level set at 0.05. Means were separated by Tuckey's *w*-procedure at $P=0.05$ (Sokal and Rohlf, 2012). Graphs were generated using SigmaPlot 10.0 (Systat Software, Inc., San Jose, CA, USA).

3 Results

3.1 Gas Exchange and Photosynthetic Performance

For all growing seasons considered in this study (2023–2025), there were no evident and constant differences among treatments in the seasonal trends of E , P_n , g_s , ETR and $Y(II)$. Concerning P_n , the most representative pattern was observed in 2023 (Fig. 1, panel C), where fluctuations occurred throughout the season, followed by a consistent decrease reaching the minimum at DOY 332. Seasonal mean $Y(II)$ differed among treatments in 2023 and 2024 (Figs. 1 and 2), with the unfertilized control showing higher values than some fertilized treatments. F_v/F_m was assessed during the first experimental season and did not show significant differences among treatments. Preliminary measurements in the second season confirmed a limited sensitivity of this parameter under the experimental conditions; therefore, subsequent analyses focused on light-adapted fluorescence parameters ($Y(II)$ and ETR), which are more responsive to treatment effects.

In 2025 (Fig. 3, panel D), differences among treatments emerged for the seasonal average of P_n . The HWD and HBWD treatments showed significantly higher values compared to the others. Specifically, HWD was +12.18% higher than the Control, +11% than the LBWD, and +4.51% than the LWD. Similarly, HBWD was +14.30% higher than the Control, +13.10% than LBWD, and +6.49% than LWD. In the same year, HBWD also showed the best performance in E , g_s , and $Y(II)$ compared to the other treatments.

Regarding stem water potential (Fig. 4), significant differences among treatments were detected at specific sampling dates, although no consistent treatment effect was observed across the season. The lowest values were recorded in 2025 at DOY 197 (−11.44 MPa for Control) and in 2024 at DOY 206 (−12.96 MPa for LBWD).

3.2 Vegetative Indices and Yields

At the end of 2025, differences in growth rate among treatments were detected. The TCSA (Fig. 5) of LWD was significantly higher than the others, specifically +68.30% compared to Control, +36.18% to LBWD, +17.06% to HBWD, and +34.92% to HWD.

Regarding apical shoot length (Table 2), after the 2024 season, the HBWD treatment had longer shoots compared to other treatments, with a senescence ratio of 1.35, second only to the Control (1.63). After the 2025 season, no significant differences among treatments were observed in shoot length, and LBWD exhibited the highest senescence ratio.

The number of current-year shoots and the number of female flowers were assessed only during the first

Fig. 1 Seasonal trends (Panels A, C, E, G, I, M) and seasonal means (Panels B, D, F, H, L, N) of leaf physiological parameters measured in 2023. A–B: leaf transpiration (E); C–D: net photosynthesis (P_n); E–F: stomatal conductance (g_s); G–H: electron transport rate (ETR); I–L: effective quantum yield of PSII photochemistry ($Y(II)$); M–N: maximum quantum efficiency of PSII (F_v/F_m). Treatments were Control (0 kg N ha⁻¹), low N dose weekly (LWD), high N dose weekly (HWD), low N dose biweekly (LBWD), and high N dose biweekly (HBWD). Values are means \pm SE ($n=5$). Different letters indicate significant differences among treatments within the same sampling date or seasonal mean ($P<0.05$); absence of letters indicates no significant differences

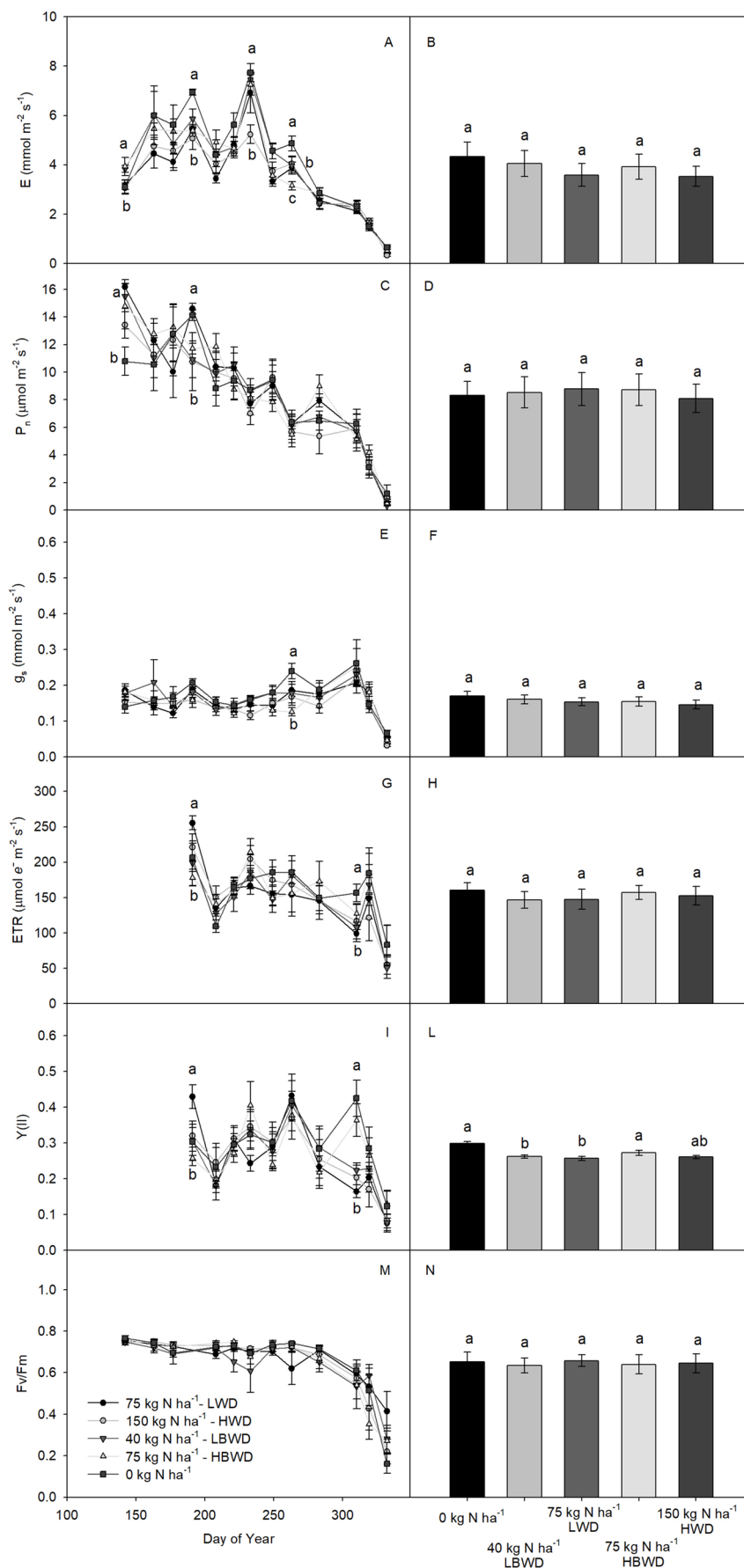


Fig. 2 Seasonal trends (Panels A, C, E, G, I) and seasonal means (Panels B, D, F, H, L) of leaf physiological parameters measured in 2024. A–B: leaf transpiration (E); C–D: net photosynthesis (P_n); E–F: stomatal conductance (g_s); G–H: electron transport rate (ETR); I–L: effective quantum yield of PSII photochemistry ($Y(II)$). Treatments were Control (0 kg N ha⁻¹), low N dose weekly (LWD), high N dose weekly (HWD), low N dose biweekly (LBWD), and high N dose biweekly (HBWD). Values are means \pm SE ($n=5$). Different letters indicate significant differences among treatments ($P<0.05$)

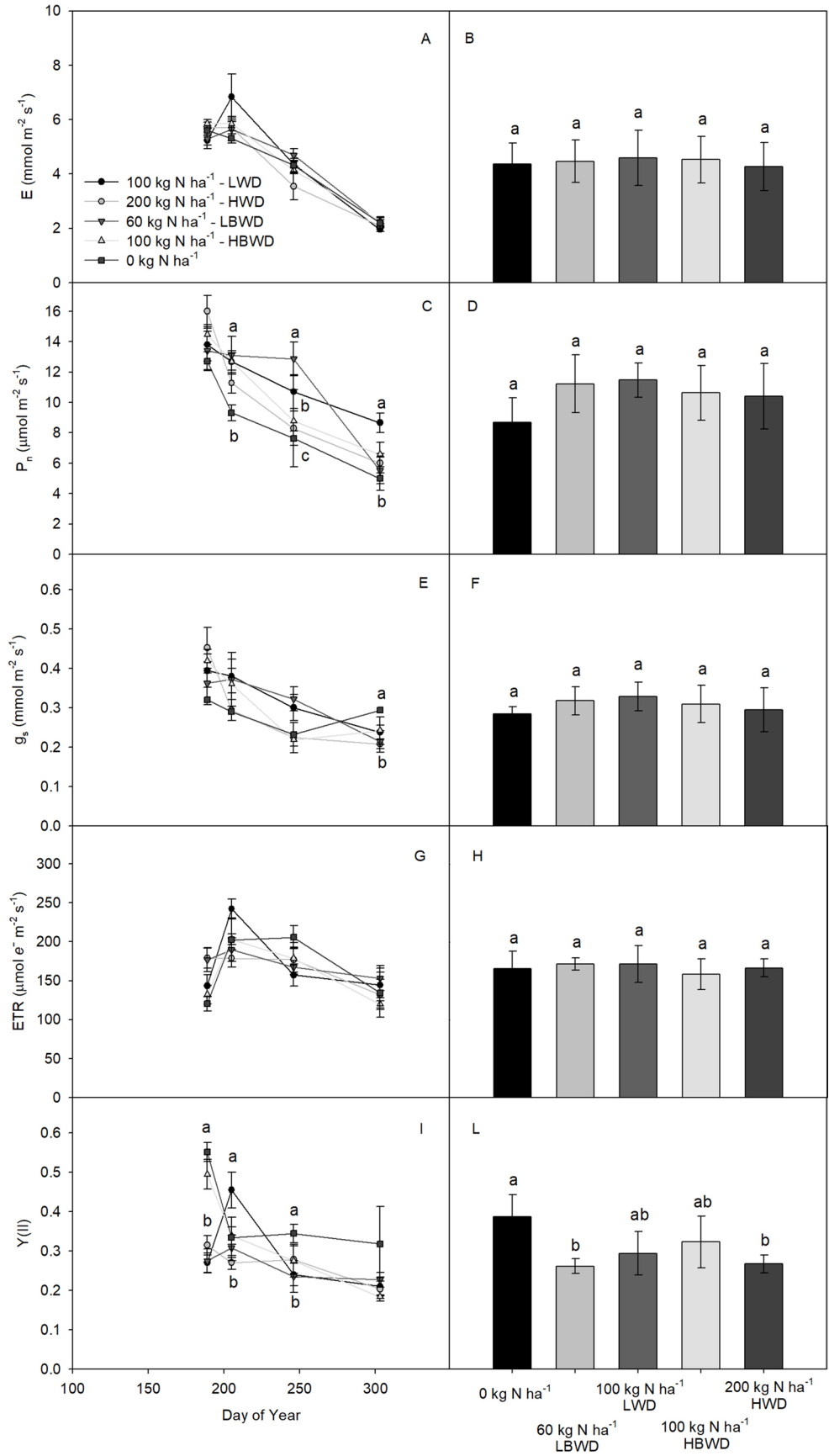
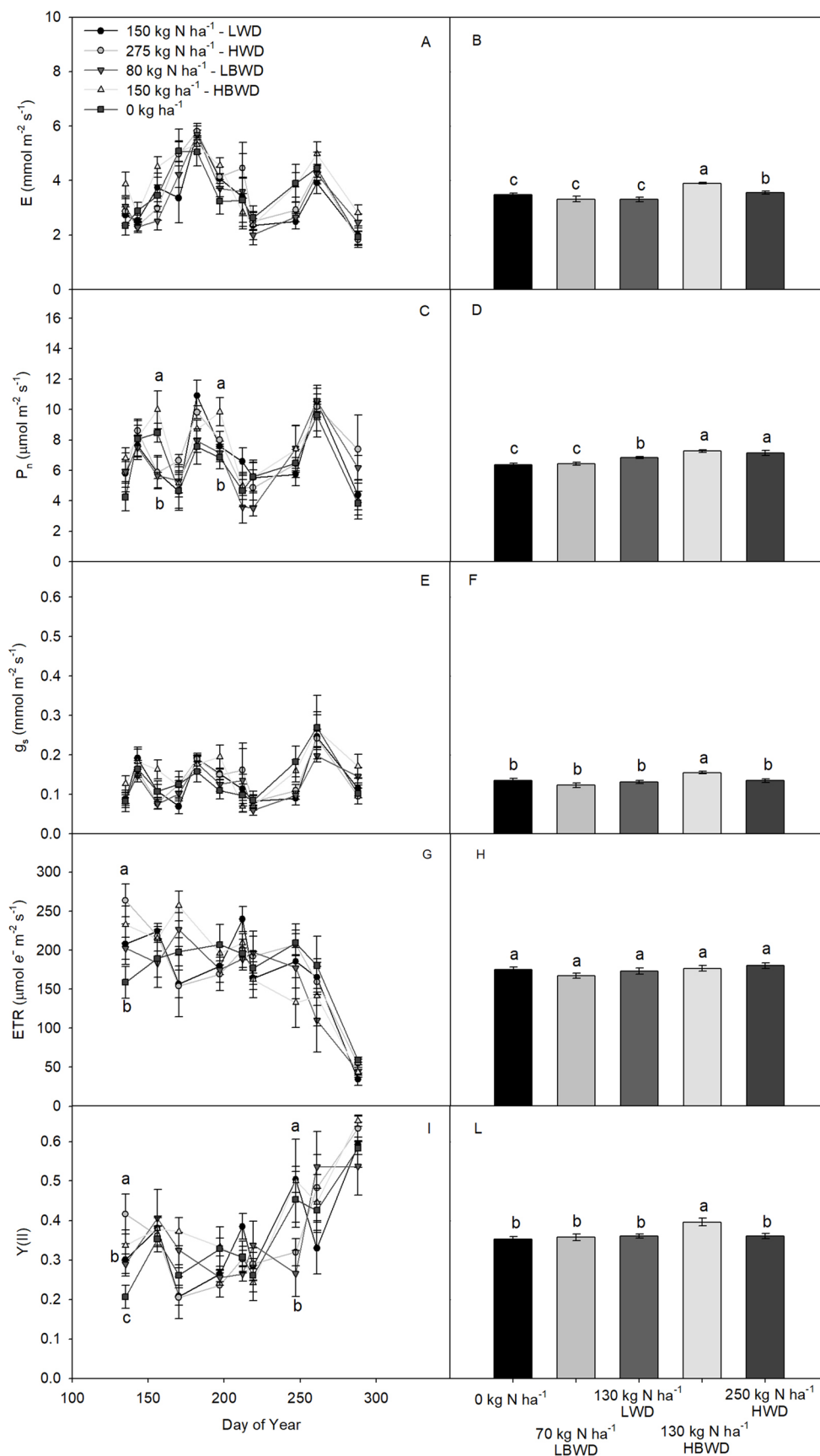


Fig. 3 Seasonal trends (Panels A, C, E, G, I) and seasonal means (Panels B, D, F, H, L) of leaf physiological parameters measured in 2025. A–B: leaf transpiration (E); C–D: net photosynthesis (P_n); E–F: stomatal conductance (g_s); G–H: electron transport rate (ETR); I–L: effective quantum yield of PSII photochemistry ($Y(II)$). Treatments were Control (0 kg N ha⁻¹), low N dose weekly (LWD), high N dose weekly (HWD), low N dose biweekly (LBWD), and high N dose biweekly (HBWD). Values are means \pm SE ($n=5$). Different letters indicate significant differences among treatments ($P<0.05$)



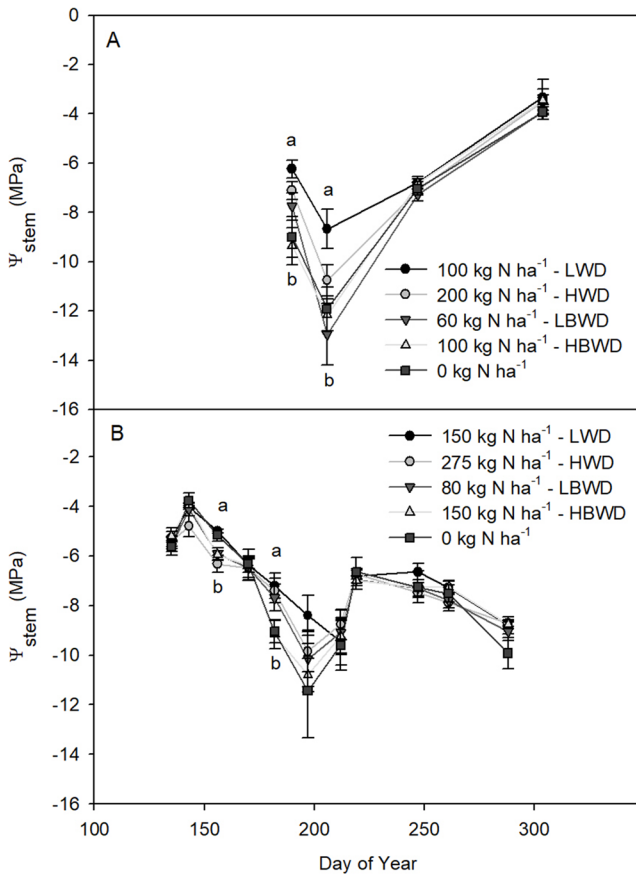


Fig. 4 Seasonal trends of midday stem water potential (Ψ_{stem}) in hazelnut trees under different N rates and application frequencies during 2024 (A) and 2025 (B). Treatments were Control (0 kg N ha⁻¹), low N dose weekly (LWD), high N dose weekly (HWD), low N dose biweekly (LBWD), and high N dose biweekly (HBWD). Values are means \pm SE ($n=5$). Different letters indicate significant differences among treatments at the same sampling date ($P<0.05$)

experimental season (2023) as early indicators of vegetative and reproductive potential. These measurements were not repeated in subsequent years due to the increasing structural complexity of the canopy and the limited additional information provided relative to the substantial sampling effort required.

Regarding yield (Fig. 6), at 3rd leaf stage (2024), no differences among treatments were observed. However, in 2025 (4th leaf stage), HBWD and LWD produced the highest yields, both significantly higher than the Control.

3.3 Leaf N Concentration

Leaf N concentration increased with N supply in all years (Fig. 7), although the intensity of the response varied across seasons. In 2023 and 2024, leaf N concentration displayed a clear quadratic response to increasing N rate, reaching a maximum at intermediate inputs and slightly decreasing at higher rates. In contrast, in 2025 the response was less

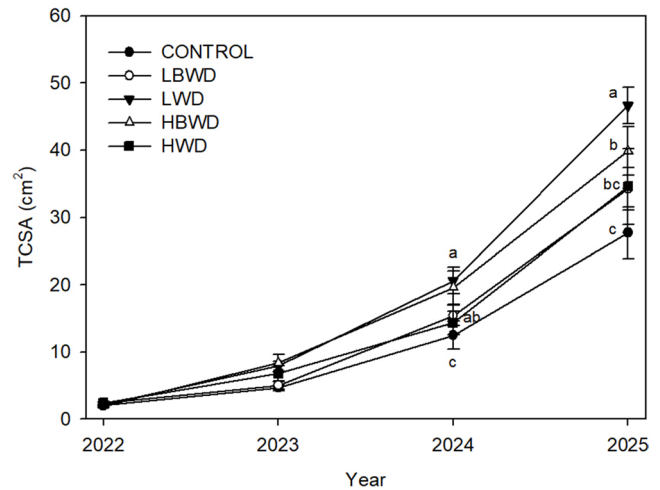


Fig. 5 Annual trend of trunk cross-sectional area (TCSA) of hazelnut trees from 2022 to 2025 under different N rates and application frequencies. Treatments were Control (0 kg N ha⁻¹), low N dose weekly (LWD), high N dose weekly (HWD), low N dose biweekly (LBWD), and high N dose biweekly (HBWD). Values are means \pm SE ($n=5$). Different letters indicate significant differences among treatments within the same year ($P<0.05$)

pronounced, with overall lower leaf N values and a more gradual increase across treatments. The control consistently showed the lowest leaf N concentration, while fertilized treatments ranged between 18 and 24 g kg⁻¹, depending on the year.

A significant relationship was found between leaf N concentration and P_n (Fig. 8) during July and August, with peak photosynthetic rates occurring at approximately 22 g kg⁻¹ leaf N, corresponding to an applied N rate of about 100 kg N ha⁻¹. As observed in Figs. 7 and 8 also highlights the differences in leaf N concentration between 2023, 2024 and 2025, which are reflected in the photosynthetic performance.

4 Discussion

The primary objective of this study was to identify optimal N application rates during the establishment phase of irrigated hazelnut orchards under commercial field conditions. Despite N inputs reaching up to three times the rates recommended for integrated crop management in Italy (Regione Lazio, 2025) and exceeding those suggested in technical handbooks for hazelnut cultivation (Tombesi 1985; Botta and Valentini 2019), measured leaf N concentrations consistently remained near or only slightly above the minimum threshold of the optimal sufficiency range reported in the literature (Olsen 1997; Bryson et al. 2014). This apparent discrepancy between fertilizer input and foliar N status highlights a critical limitation of broadcast fertilization during orchard establishment: as trees develop, maintaining

Table 2 Effect of nitrogen (N) fertilization rate and application frequency on vegetative and reproductive parameters of hazelnut trees during 2022–2025. In 2022, all trees received a uniform nitrogen application (25 kg N ha⁻¹) prior to the initiation of fertilization treatments, and these data represent pre-treatment baseline measurements. Treatment codes are defined as follows: LBWD, low nitrogen dose with biweekly application; LWD, low nitrogen dose with weekly application; HBWD, high nitrogen dose with biweekly application; and HWD, high nitrogen dose with weekly application. Values are mean ± SE (*n* = 5). Different letters indicate significant differences among treatments within each year (*P* < 0.05)

Treatment	N (kg ha ⁻¹)	Length apical shoot (cm)	Weight pruned suckers (g)	Number one-year shoots	Senescence ratio	N. female flower
Control	25	43.40 ± 10.56	-	-	-	-
LBWD	25	51.40 ± 5.12	-	-	-	-
LWD	25	65.20 ± 5.99	-	-	-	-
HBWD	25	52.50 ± 2.14	-	-	-	-
HWD	25	64.00 ± 4.75	-	-	-	-
Treatment	N (kg ha ⁻¹)					
Control	0	43.10 ± 4.61	354.88 ± 19.56	26.50 ± 5.39	0.35 ± 0.02	6.60 ± 3.66
LBWD	40	50.00 ± 4.11	370.31 ± 41.78	26.25 ± 1.11	0.37 ± 0.04	14.00 ± 2.08
LWD	75	55.90 ± 3.65	220.86 ± 15.41	15.67 ± 0.67	0.22 ± 0.02	22.00 ± 1.53
HBWD	75	52.10 ± 4.48	727.06 ± 123.40	25.00 ± 5.22	0.73 ± 0.12	34.33 ± 9.70
HWD	150	54.80 ± 7.93	451.18 ± 110.87	29.50 ± 1.04	0.45 ± 0.11	7.67 ± 1.67
Treatment	N (kg ha ⁻¹)					
Control	0	47.33 ± 3.53	718.00 ± 127.13	-	1.63 ± 0.39	-
LBWD	60	42.80 ± 5.42	1290.00 ± 56.79	-	0.91 ± 0.19	-
LWD	100	57.25 ± 1.89	781.25 ± 91.43	-	1.12 ± 0.09	-
HBWD	100	69.00 ± 7.76	975.83 ± 146.55	-	1.35 ± 0.15	-
HWD	200	47.00 ± 8.19	718.33 ± 28.92	-	1.15 ± 0.33	-
Treatment	N (kg ha ⁻¹)					
Control	0	29.10 ± 0.91	-	-	0.55 ± 0.12	-
LBWD	70	32.00 ± 3.51	-	-	0.78 ± 0.09	-
LWD	130	26.70 ± 2.17	-	-	0.48 ± 0.04	-
HBWD	130	30.42 ± 3.54	-	-	0.41 ± 0.02	-
HWD	250	26.70 ± 2.17	-	-	0.55 ± 0.09	-

Fig. 6 Nut yield per plant in 2024 and 2025 as affected by N rate and application frequency. Treatments were Control (0 kg N ha⁻¹), low N dose weekly (LWD), high N dose weekly (HWD), low N dose biweekly (LBWD), and high N dose biweekly (HBWD). Values are means ± SE (*n*=5). Different letters indicate significant differences among treatments within each year (*P*<0.05)

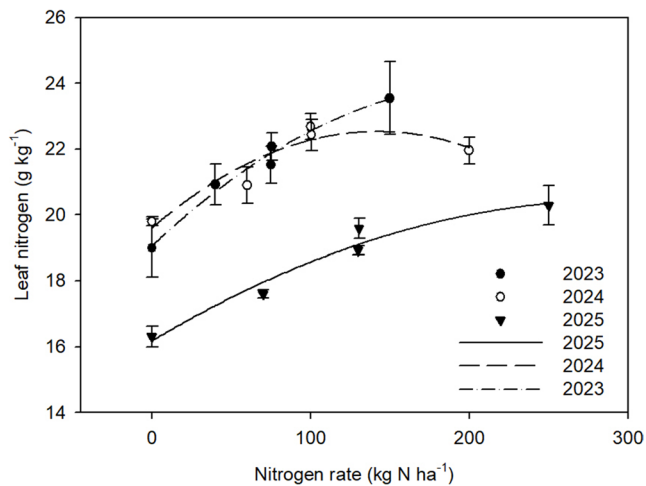
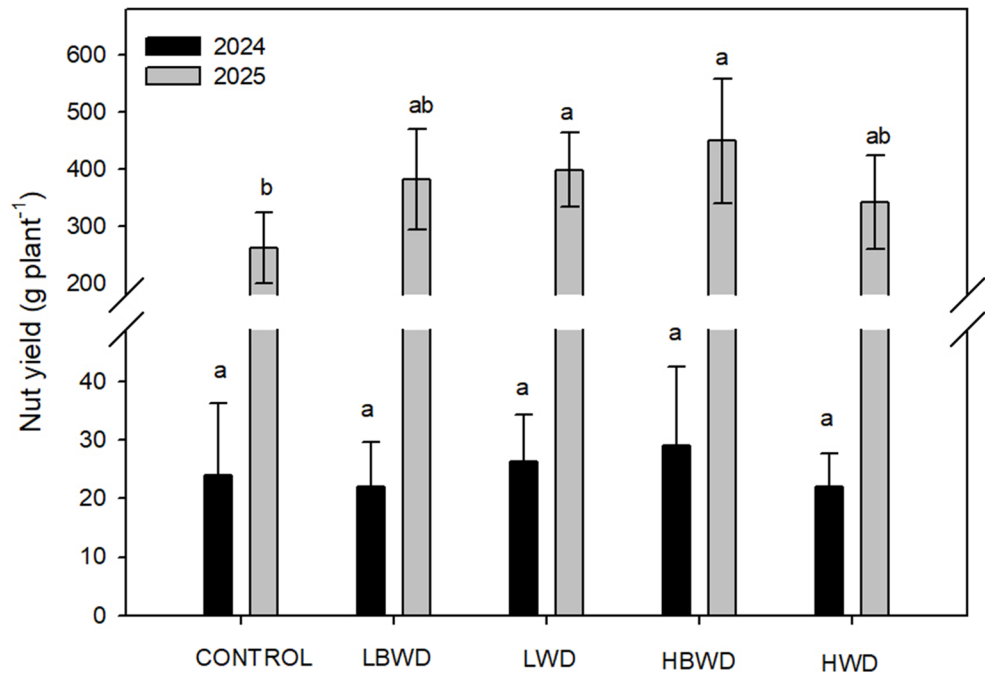


Fig. 7 Relationship between N rate and leaf N concentration in 2023, 2024, and 2025

Each point represents the mean ± SE (*n*=5). Nonlinear regression curves are shown for each year and were significant at *P*<0.05

adequate leaf N concentrations requires progressively higher fertilizer inputs, raising both economic and environmental concerns. These findings indicate that, although irrigation modifies N availability at the soil–root interface, intrinsic physiological constraints and growth-related dilution effects impose upper limits on achievable foliar N concentrations regardless of N supply.

The relationship between N supply and leaf N concentration followed a non-linear pattern during the first two seasons, consistent with previous studies reporting a maximum attainable foliar N concentration of approximately 22 g kg⁻¹ in hazelnut (Braun et al. 2011a, b). In contrast, this saturation

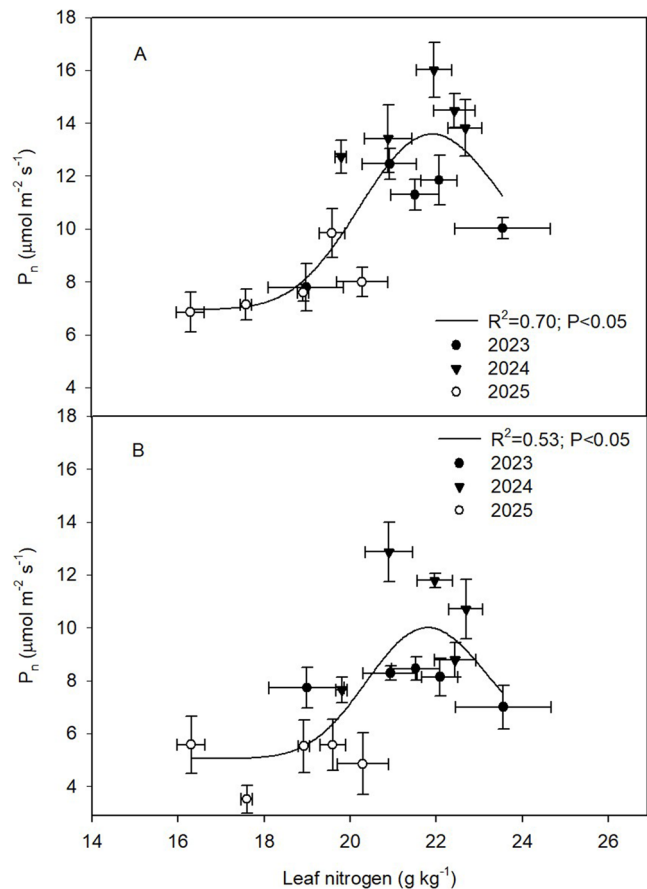


Fig. 8 Relationship between leaf N concentration and net photosynthesis (P_n) in hazelnut leaves measured in July (A) and August (B) across three growing seasons (2023–2025). Each point represents the mean ± SE (*n*=5). Nonlinear regression curves were significant at *P*<0.05

response was not observed in the third season, when foliar N concentrations remained below 20 g kg^{-1} despite relatively high N inputs. This deviation can be attributed to the progressive expansion of canopy and root systems, leading to a dilution of N across increasing structural biomass. The so-called N dilution effect is well documented in perennial crops, where biomass accumulation outpaces N uptake, resulting in declining tissue N concentrations even under sustained fertilization (Evans 1989; Greenwood et al. 1990). The progressive decline in foliar N concentration observed across years in this study may therefore reflect the combined effects of canopy and root system expansion and associated changes in soil N distribution. As the root system explores an increasing soil volume, the effective fertilizer N available per unit of root biomass may decrease, reducing apparent N uptake efficiency despite sustained or increased N inputs (Greenwood et al. 1990; Marschner 2012).

Seasonal patterns in leaf gas exchange provided insight into the physiological regulation of photosynthetic performance under irrigated conditions. In 2023, the progressive decline in net photosynthesis was not accompanied by a corresponding decrease in intercellular CO_2 concentration, indicating that photosynthetic limitations were predominantly non-stomatal in nature. This pattern—characterized by stable or increasing C_i values concurrent with declining CO_2 assimilation—is widely interpreted as evidence for increasing contributions of non-stomatal limitations under field conditions (Flexas and Medrano 2002; Lawlor and Tezara 2009). Such limitations may include reductions in biochemical capacity for CO_2 fixation, constraints on electron transport, and increasing importance of mesophyll conductance limitations under high atmospheric demand and elevated temperatures (Grassi and Magnani 2005). Although C_i alone does not allow quantitative partitioning among stomatal, mesophyll, and biochemical limitations without additional analyses such as A/C_i response curves (Deans et al., 2020), the consistency of this response across measurement dates strongly suggests that internal physiological processes exerted primary control over photosynthetic performance during the latter portion of the growing season.

Inter-annual differences in stomatal conductance and electron transport rate between 2023 and 2024 likely reflect a combination of climatic variability and plant developmental progression. Higher values of g_s and ETR in 2024 suggest enhanced photochemical capacity under more favorable environmental conditions and greater physiological acclimation as trees aged. Similar year-to-year variability in stomatal behavior and fluorescence-based parameters has been widely reported in perennial woody species under field conditions and is commonly attributed to inter-annual variability in climate and seasonal acclimation processes (Grassi and Magnani 2005; Flexas and Medrano 2002).

These findings emphasize the importance of multi-year field trials to correctly interpret N effects on photosynthetic performance under variable climatic conditions.

A central outcome of this study is the identification of a clear functional relationship between leaf N concentration and net photosynthesis in field-grown hazelnut, with peak photosynthetic rates occurring at approximately $20\text{--}22 \text{ g kg}^{-1}$ leaf N. This represents one of the first field-based demonstrations directly linking foliar N status to photosynthetic capacity in hazelnut orchards. The strong correlation observed supports the well-established biochemical role of N in determining photosynthetic capacity in C3 plants (Evans 1989; Evans and Clarke 2019), where N is a major constituent of Rubisco and components of the electron transport chain. The observed plateau in photosynthetic response beyond this threshold aligns with the concept of diminishing returns in N fertilization, whereby N use efficiency declines once photosynthetic machinery approaches saturation (Evans and Clarke 2019; Niinemets et al. 2015). From a practical standpoint, this threshold provides a physiologically meaningful target range for leaf analysis and N management decisions in hazelnut.

Treatment effects on vegetative growth and early yield corroborated the physiological responses. Intermediate N treatments (HBWD and LWD), corresponding to approximately 75 and 100 kg N ha^{-1} in the second and third years, respectively, produced the largest trunk cross-sectional area and the highest early yield while maintaining leaf N concentrations within the identified optimal range. These N rates exceed traditional recommendations for hazelnut under Mediterranean conditions (Tombesi 1985; Botta and Valentini 2019), reflecting the increased N demand of irrigated orchards where enhanced water availability accelerates growth. The stronger response of trunk growth compared to apical shoot length suggests preferential allocation of N-derived resources to structural development, which may have long-term implications for orchard productivity.

The progressive reduction in treatment responsiveness observed over successive years may also be linked to changes in soil N distribution and root system architecture. As root systems expanded into deeper and wider soil volumes, the effective fertilizer concentration per unit of root biomass likely declined, reducing apparent uptake efficiency despite increasing total inputs. Soil N dynamics in orchards are influenced by mineralization–immobilization processes, leaching losses, spatial heterogeneity of nutrient distribution, and irrigation-induced redistribution of N within the soil profile (Havlin et al. 2014; Marschner 2012). Under these conditions, broadcast fertilization becomes increasingly inefficient as trees mature, highlighting the importance of integrating soil and plant-based diagnostics when designing N management strategies for perennial crops.

5 Conclusions

This study establishes, for the first time under field conditions, a direct quantitative relationship between leaf N concentration and photosynthetic performance in hazelnut, identifying 20 to 22 g kg⁻¹ as the functional optimum that maximizes carbon assimilation. This physiology related parameter transforms leaf tissue analysis from a descriptive indicator into a mechanistic tool for N management decisions. Under our experimental, to achieve this optimal range during orchard establishment, N application rates of 75 and 100 kg N ha⁻¹ in the second and third years after planting are required under irrigated conditions—substantially exceeding standard agronomic recommendations but necessary to overcome growth-related dilution effects as trees develop. However, the economic reality is sobering: fertilizer costs at these rates exceed 200 euros per hectare annually by the third year, representing a significant operational expense, while broadcast application becomes progressively less efficient as canopy size increases and N becomes distributed across expanding root systems and structural biomass. This pattern of diminishing returns reveals a fundamental limitation of conventional fertilization strategies in intensive hazelnut production. The broader implication is clear: irrigation fundamentally alters N dynamics in orchard systems, creating both opportunities and challenges that demand rethinking traditional nutrient management approaches. Fertigation emerges as the critical next step, offering the potential to synchronize N delivery with plant demand, improve uptake efficiency, reduce total inputs and costs, and minimize environmental losses, transforming what is currently an expensive and inefficient practice into a sustainable production strategy. Beyond the immediate practical applications, this work demonstrates that understanding the mechanistic linkage between nutrient supply, tissue concentration, and physiological function provides the foundation for evidence-based management systems applicable across perennial crop production, where the challenge of maintaining optimal nutrition during rapid vegetative growth phases remains a universal constraint on establishment success and long-term productivity.

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Data Availability The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare that there are no competing interests related to this work.

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