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Growth, Critical N Concentration and Crop N Demand in Butterhead and Crisphead Lettuce Grown under Mediterranean Conditions

Giulia Conversa * **and Antonio Elia**

Department of the Science of Agriculture, Food and Environment (SAFE), University of Foggia, via Napoli 25, 71122 Foggia, Italy; antonio.elia@unifg.it

* Correspondence: giulia.conversa@unifg.it

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Abstract: Excessive nitrogen (N) fertilizers are applied in lettuce causing both environmental issues and N crop luxury consumption. In order to improve the N use efficiency (NUE) by defining optimal crop growth and N requirements of butterhead and crisphead lettuce, two field experiments were conducted using 0, 50, and 100 kg ha^{-1} of N fertilizer to study (i) the growth and productivity, (ii) the NUE, (iii) the critical N dilution curve, and (iv) the N demand. Nitrogen supply enhanced dry weight (DW) accumulation in the butterhead (from 295 to 410 g m^{-2}), but not in the crisphead type (251 g m^{-2}). The NUE indices underlined the poor ability of the crisphead type in absorbing soil N and also in the utilization of the absorbed N for producing DW. The critical N dilution curves %Nc = 3.96 $DW^{-0.205}$ and %Nc = 3.65 $DW^{-0.115}$ were determined for crisphead and butterhead lettuce, respectively. Based on these type-specific %Nc curves, the estimated N demand was 125 kg ha^{-1} in the butterhead and 80 kg ha^{-1} in the crisphead lettuce for producing 4.3 and 2.5 Mg ha^{-1} of DW, respectively, under Mediterranean climate. Neither N fertilization nor genotype affected crop productivity.

Keywords: growth; specific leaf nitrogen; nitrogen use efficiency; critical nitrogen uptake

1. Introduction

Among leafy vegetables, lettuce (*Lactuca sativa* L.) is the most important species, grown on over 1.86 million hectares around the world [1]. The crop is characterized by low efficiency in nitrogen (N) recovery, with the highest N absorption occurring during the last phase of the growing cycle [2], when a sub-optimal N availability may result in a decrease in head yield and quality. In order to avoid N deficiency, N fertilizers are frequently applied in excess compared with the crop demand [3], causing both environmental issues (e.g., nitrate contamination of aquifers and eutrophication of surface water) and N crop luxury consumption, the latter resulting in excessive nitrate accumulation in the plants. Nitrate-contaminated freshwater and leafy vegetables with a high concentration of nitrate are considered potentially dangerous for human health [4].

In order to improve N use efficiency (NUE) the determination of the patterns of crop growth and of N demand during the crop cycle are at the basis of optimal N fertilization planning, timing, and management [5–7].

The study employing a growth analysis of the effects of different N rates on dry matter production and N accumulation during the crop cycle may be useful to define optimal crop growth and N requirements [8]. The N crop demand, which can be defined as the amount of N necessary to sustain the potential growth of a crop at any time of the cycle [9], is frequently modeled using the concept of the critical N plant concentration. It is the minimum N concentration in dry biomass (critical N—%Nc) required for maximum dry weight (DW) accumulation under specific climatic conditions and agronomic practices [10]. Critical N concentration decreases during the crop cycle according to the equation %Nc = a DW^{-b} [11] (%Nc dilution curve) with a crop-specific pattern. Using the critical N curve, the crop's N demand can be obtained from the crop biomass accumulation during the crop cycle, which is in turn determined by environment and genotype [12]. The availability of a crop-specific critical N dilution curve is therefore of key importance for deriving N uptake and improving the dynamic assessment of N status in a crop.

Tei and coworkers [13] found the critical N dilution curve for the butterhead type (%Nc = 4.56 $DW^{-0.357}$) grown in Central Italy. In California (USA), Bottoms and coworkers [14] working on a large dataset of experimental and commercial field data of both crisphead and romaine lettuce types suggested the empirical linear regression %Nc = 4.20 − 2.8 DW to distinguish between N-deficient and N-sufficient conditions across the entire season, underlining the unsuitability of %Nc dilution curve proposed by Tei et al. [13] for the butterhead. Nevertheless, considering that the environment as well as the large variability in physio-morphological traits between lettuce typologies strongly affect the dry mass production and N uptake [15,16], a type-specific critical %N dilution curve may need to be locally calibrated [6,17] to tune N crop demand.

To the best of our knowledge, no such studies are available for modeling N crop nutrition and optimizing N fertilization of lettuce grown under Southern Italian/Mediterranean climatic conditions. Therefore, the main aims of the present paper were to define (i) the growth and productivity, (ii) the nitrogen use efficiency, (iii) the critical N dilution curve, and (iv) the N demand of the most widely cultivated lettuce typologies (butterhead and crisphead), grown at three N fertilization levels.

2. Materials and Methods

2.1. Field Experimental Site and Climatic Conditions

Two open field lettuce (*Lactuca sativa* L., var. *capitata*) trials were carried out in 2008 (exp. 1) and 2009 (exp. 2) on a commercial farm located in Foggia Province, Puglia Region (Italy) (latitude 41°46′ N, longitude 15°5′ E, 74 m above mean sea level). The site is within an area dominated by a Mediterranean climate with a mild winter and dry-and-warm summer; mean minimum and maximum temperatures are 10.8 ± 1.7 °C and 19.9 ± 2.2 °C, respectively, and the mean temperatures of the coldest (January) and hottest (August) months are 7.1 and 24.5, respectively. The average annual rainfall is 537 mm. The weather conditions during the two growing seasons are reported in Figure 1.

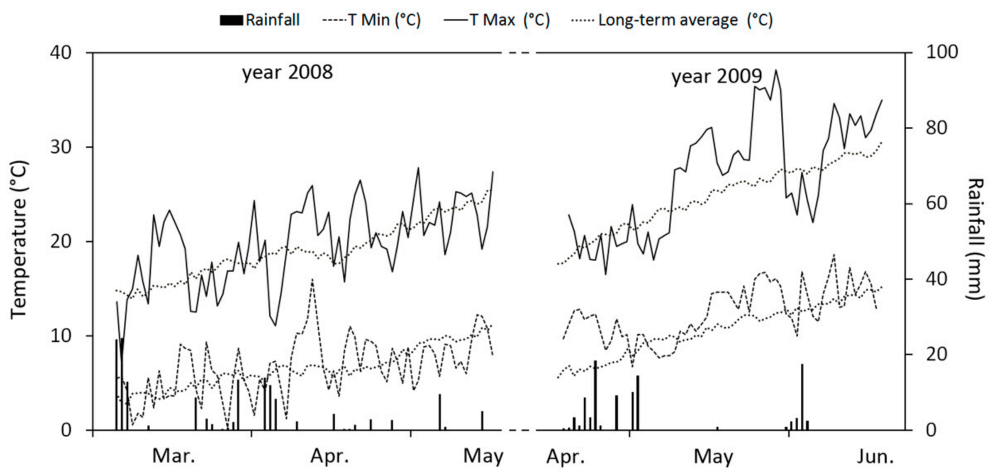

Figure 1. Climate data for the two lettuce trials (5 March–14 May 2008 and 16 April–12 June 2009).

The area falls within a nitrate vulnerable zone; therefore, it is subjected to the European Directive 109 91/676/EEC prescriptions.

Both trials were carried out in the same field and the soil characteristics were 24% clay, 34% silt, 42% sand, pH 7.52 (soil:water 1:2.5), 2.2% organic matter, 1.52‰ total N, 382 ppm NH_4OAc-extractable K, 24 ppm Olsen P, and 7% active carbonate.

Seedlings with 4–5 true-leaves were transplanted 30 cm apart in 30 cm spaced rows (11.1 plants m^{-2}) on 5 March 2008 (exp. 1) and 16 April 2009 (exp. 2). Both trials included three nitrogen fertilization rates: 0, 50, and 100 kg ha^{-1} (indicated as N_0, N_{50}, N_{100}) and two types of capitata lettuce: the butterhead (cv. Faustina—ISEA) and crisphead (cv. Silvinas—Rijk Zwaan) type. Each experiment was arranged in a split-plot design with four replications and with N rate as the main factor and cultivar as sub factor. The experimental plot unit included 6 rows and was 1.8 m wide and 5 m long (9 m^2).

In both years, the preceding crop was broccoli and crop residues were incorporated into the soil one month before the lettuce transplanting. In each growing cycle, the level of PK fertilization was adjusted to the plant nutritional requirements; a sprinkler irrigation system was used to satisfy the water requirements of the crops, which were assessed through the water balance method using the Penman–Monteith approach to estimate the reference evapotranspiration. A total of 1460 and 2010 m^3 ha^{-1} of irrigation water was supplied in the first and the second cycle, respectively.

Nitrogen fertilizer was applied in surface strips as NH_4NO_3 (34:0:0, N:P:K) (Yara International ASA, Oslo, Norway). Forty percent of the N was applied at transplanting and the remaining 60% at the 20th true-leaf stage (7 April 2008 and 11 May 2009 for exp. 1 and 2, respectively).

Starting from approximately 2 weeks after transplanting until harvest, 9 (exp. 1) and 5 (exp. 2) destructive samplings (picking three plants in the four central rows of each experimental plot) were carried out, with a one-week interval in the first year (14, 21, 28, 35, 42, 49, 56, 64, and 70 DAT (days after transplant)) and a two-week interval in the second (19, 26, 33, 40, and 57 DAT). At each sampling, shoot and root fresh (FW) and dry weight (DW), number of leaves, leaf area, and (only at 14, 28, 42, 49, 64, and 70 DAT in 2008; 19, 26, 33, 40, and 57 DAT in 2009) shoot N concentration were determined. Harvest was carried out on 13 May 2008 in exp. 1 (70 days after transplant—DAT) and 11 June 2009 in exp. 2 (57 DAT), respectively. Twenty lettuce heads per replication were randomly selected at the optimal stage for fresh consumption to determine fresh yield, chlorophylls, and nitrate concentration.

Leaf area was measured by LI-COR 3100 (LICOR, Lincoln, NE, USA). Root samples were washed through two sieves to remove soil and weighed after drying up water using filter papers.

For DW determination, plant material was dried in a ventilated oven at 65 °C until the achievement of constant weight. Dry mass concentration was calculated as DW/FW (g kg^{-1}). Dried shoot material was then milled through a 1.0 mm sieve (IKA Labortechnik, Staufen, Germany) and the nitrogen (N) concentration was determined using the Kjeldahl method (Kjeltec model 1035—Foss Tecator). Nitrates were extracted from 0.5 g of dried shoots with 50 mL solution containing 3.5 mM sodium carbonate (Na_2CO_3) and 1.0 mM sodium bicarbonate (Na_2HCO_3). Nitrates were measured by ion chromatography (Dionex^{TM} ICS 3000, Thermo Scientific, Waltham, MA, USA) with a conductivity detector, using the pre-column Dionex^{TM} IonPack AG23 and the Dionex^{TM} IonPack AS23 (4 mm × 250 mm, 5 µm) separation column, according to the method reported in Bonasia et al. [18]. Reduced N was calculated as the difference between total N content and N-nitrate content. The chlorophyll a and b concentrations were spectrophotometrically determined as reported by Conversa et al. [19].

Shoot dry weight accumulation, leaf area index (LAI), and N crop uptake during the crop cycles were plotted using days after transplant. At harvest, specific leaf area (SLA) was calculated as leaf dry weight/leaf area ratio (g m^{-2}); specific leaf nitrogen (SLN) was calculated as leaf N content/leaf area ratio (g m^{-2}); and specific leaf-reduced N (SLNred) was calculated as leaf reduced N content/leaf area ratio (g m^{-2}).

2.2. Nitrogen Use Efficiency Indices

The nitrogen use efficiency (NUE) and NUE components (expressed as kg kg^{-1}) were calculated following Conversa et al. [17]. Briefly, the NUE components were:

Partial factor productivity of applied N (PFP), which represents the kilogram of product (heads' fresh weight—HFW) harvested per kilogram of N fertilizer applied (NA), also called simply NUE, can be used as an index of total economic output relative to the use of all N sources (soil N and applied fertilizer N):

$$PFP = HFW/NA\ (kg\ kg^{-1}) \quad (1)$$

Agronomic efficiency of N (NUEa), which represents the kilogram of yield (in terms of heads dry weight) increase per kilogram of N fertilizer applied (NA), is calculated as:

$$\text{NUEa} = (\text{HDWf} - \text{HDWc})/\text{NA} \ (\text{kg kg}^{-1}) \quad (2)$$

where HDWf = heads dry weight of fertilized treatment, HDWc = heads dry weight of control treatment, and NA = dose of applied N.

Apparent N recovery efficiency (REC) by the crop, which represents the kilogram increase in N uptake per kilogram of N applied, is calculated as:

$$\text{REC} = (\text{TNf} - \text{TNc})/\text{NA} \ (\text{kg kg}^{-1}) \quad (3)$$

where TNf = total N uptake in aboveground biomass at maturity (kg ha^{-1}) when an amount NA is applied, and TNc is the corresponding total plant N uptake in aboveground biomass at maturity (kg ha^{-1}) when no N-fertilizer is applied.

Physiological efficiency of N (NUEp), which represents the kilogram of yield (in terms of heads dry weight) increase per kilogram increase in N uptake from fertilizer, is calculated as:

$$\text{NUEp} = (\text{HDWf} - \text{HDWc})/(\text{TNf} - \text{TNc}) \ (\text{kg kg}^{-1}) \quad (4)$$

2.3. N Critical Curve

The critical N concentration was determined following Justes and coworkers [20]. The critical N curve indicates the minimum N concentration observed at a given time among all N treatments that had given, to that date, the maximum amount of the aboveground DW. For each trial, sampling date, and lettuce type, the observed aboveground DWs, obtained with the different N rates, were compared with the corresponding total N concentrations (%N). The Students' two-tail *t*-test was used to test the hypothesis of means equality at $p \leq 0.1$. All the values identified as critical (%Nc, in g 100 g^{-1} DW) were related to the aboveground dry biomass (DW, Mg ha^{-1}) according to the equation of Lemaire and Salette [21]:

$$\%\text{Nc} = \text{a DW}^{-\text{b}} \quad (5)$$

where a represents the critical N concentration in the dry biomass when DW ≥ 0.9 Mg ha^{-1}, and b is a statistical parameter governing the slope of the relationship.

The fitting of the Equation (5) was performed for %N pooled data and for each lettuce type. Additionally, all the observed %N values were compared with the critical N concentrations predicted by the curves proposed for butterhead lettuce (%Nc = 4.56 DW$^{-0.357}$) by Tei and coworkers [13] and for crisphead and romaine lettuce by Bottoms and coworkers [14] to distinguish between N-deficient and N-sufficient conditions (%Nc = 4.20 − 2.8 DW).

2.4. Statistical Elaboration of Data

All the data were submitted to analysis of variance by using the GLM Procedure of SAS software [22]. Mean separations were based on Fisher's protected Least Significant Difference (LSD) test at the 0.05 probability level.

The Gauss–Newton method of the NLIN procedure of SAS software was used for the non-linear regression fittings of Equation (5) on %Nc against the DW accumulation. The evaluation of model accuracy was performed using adjusted R^2 (adjR^2), root mean square error (RMSE), and relative root mean square error (RRMSE). The last two indices were calculated as follows:

$$\text{RMSE} = \sqrt{\sum_{i=1}^{n} (\text{S}_i - \text{O}_i)^2/\text{n}} \quad (6)$$

$$RRMSE = RMSE\ 100/\bar{O} \quad (7)$$

where S_i and O_i are simulated and observed values, respectively, and $\bar{O}$ is the observed mean value. RMSE describes the difference between model simulations and observations in the units of the variable. Its value close to zero indicates a perfect fit; however, a value less than half of the standard deviation of the observations may be considered low [23]. RRMSE provides a measure (%) of the relative difference between simulated and observed data. Adjusted R^2 and RMSE and RRMSE indices were also used to evaluate the goodness of prediction of plant critical N concentration by the N curve proposed by Tei et al. [13] and by the equation proposed by Bottoms et al. [14].

3. Results

3.1. Weather Conditions

Minimum and maximum averaged daily temperatures were 6.7 °C and 19.8 °C, respectively, for the 2008 cycle (5 March–14 May), while for the 2009 cycle (16 April–15 June) they were 12.2 °C and 26.5 °C, respectively. In 2009, both maximum and minimum temperatures were higher than the long-term averages (10.5/24.9 °C, respectively) and particularly during the period from the 4th to the 7th week after transplanting and in the last 10 days before harvest. Total rainfall was 157 and 99 mm in 2008 and 2009, respectively (Figure 1). The cumulated thermal time (700 versus 942 day degrees (°C)) and global radiation (1222 versus 1308 MJ m^{-2}) were lower in the first than in the second trial.

3.2. Yield, Dry Weight Accumulation, and Partitioning

Neither N fertilizer application nor genotype affected the shoot fresh weight (yield) (Table 1). At transplanting, the crisphead and butterhead lettuce had similar dry weight of seedlings (1.164 ± 0.089 and 1.110 ± 0.039 g m^{-2}, respectively). During both crop cycles, the butterhead typology (Figure 2A,C) exhibited a higher accumulation of shoot DW than the crisphead one (Figure 2B,D).

Table 1. Effect of N fertilization rate, lettuce genotype, and year of cultivation on crop yield and plant dry weight partitioning.

Treatments	Shoot Fresh Weight (Yield)	Shoot Dry Weight Content	Shoot Dry Weight Concentration	Root Dry Weight	Root/Shoot Ratio
	(Mg ha^{-1})	(g m^{-2})	(g kg^{-1})	(g m^{-2})	
N rate (kg ha^{-1}) (N)					
0	79.2 a [(1)]	267 b	32.1 b	32.1 a	0.11 a
50	84.8 a	304 a	34.3 ab	32.9 a	0.11 a
100	86.9 a	329 a	36.1 a	32.8 a	0.11 a
Genotype (G)					
Butterhead	86.6 a	350 a	38.7 a	49.0 a	0.14 a
Crisphead	80.7 a	251 b	29.6 b	16.1 b	0.08 b
Year (Yr)					
2008	67.4 b	180 b	26.5 b	19.5 b	0.11 a
2009	99.9 a	420 a	41.8 a	51.4 a	0.11 a
Significance [(2)]					
N	ns	**	*	ns	ns
G	ns	**	***	***	***
Yr	***	***	***	***	ns
Yr*N	ns	ns	ns	ns	ns
Yr*G	ns	ns	ns	***	ns
N*G	ns	*	*	ns	*
N*G*Yr	ns	ns	ns	ns	ns

[(1)] Means in columns not sharing the same letters are significantly different according to Least Significant Difference (LSD) test (p = 0.05). [(2)] ns, *, **, and ***, not significant or significant at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively.

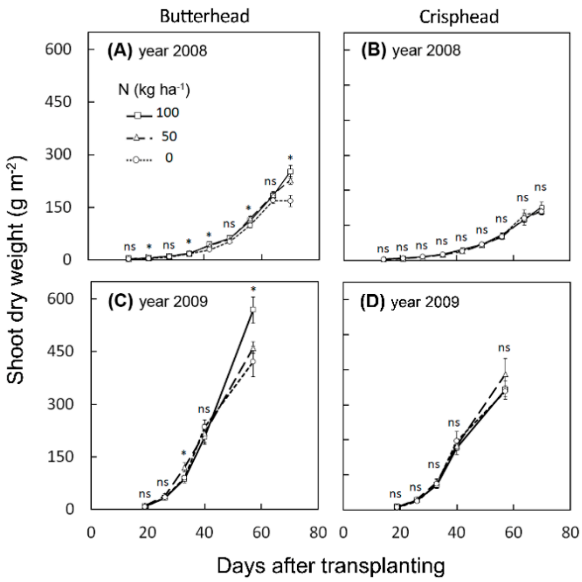

Figure 2. Shoot dry weight (DW) accumulation during the growing seasons (2008: **A**,**B**; 2009: **C**,**D**) of butterhead (**A**,**C**) and crisphead (**B**,**D**) lettuce as affected by N level. Vertical bars represent standard error (n = 4) with notes indicating a significant (*: p = 0.05) or not significant difference (ns) between N levels, according to the LSD test.

The DW accumulation was particularly evident three weeks before the harvest during the linear growth phase, with butterhead type also showing a positive response to N supply. Specifically, by increasing N fertilization rate at harvest, shoot DW rose from 295 to 410 g m^{-2} in the butterhead lettuce, while it was 251 g m^{-2}, on average among N rates, in the crisphead one (Figure 3A).

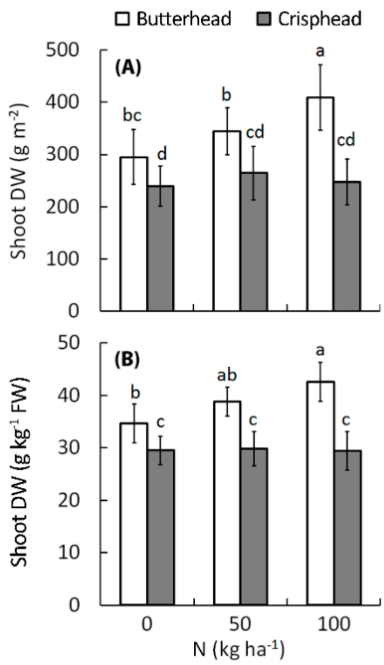

Figure 3. Shoot dry weight accumulation (**A**) and concentration (**B**) at harvest, in butterhead and crisphead plants as affected by N fertilization level. Vertical bars (± Standard Error (SE) of mean; n = 8] with different letters are significantly different according to the LSD test (p = 0.05).

Similarly, dry weight concentration was only enhanced in the butterhead lettuce by the dose of N fertilizer, and it was higher than the crisphead one (29.6 g kg^{-1} DW, on average) (Figure 3B). The root dry weight was also higher in butterhead than in crisphead lettuce (Table 1), especially in the 2009 trial. In the butterhead, a greater root:shoot DW ratio was also detected (Table 1), with the highest values in the unfertilized plants.

In both lettuce types, growth and yield were much lower (−32% and −57%, respectively) in first compared with the second experiment ($p \leq 0.001$) (Table 1).

3.3. Shoot Morpho-Physiological Traits

Approaching the harvest, N_{50} and especially N_{100} treatment significantly improved leaf area index (LAI) in both lettuce types (Figure 4) as confirmed by the final values (Table 2). They were slightly higher in butterhead (Figure 4A,C) than in crisphead lettuce (Figure 4B,D).

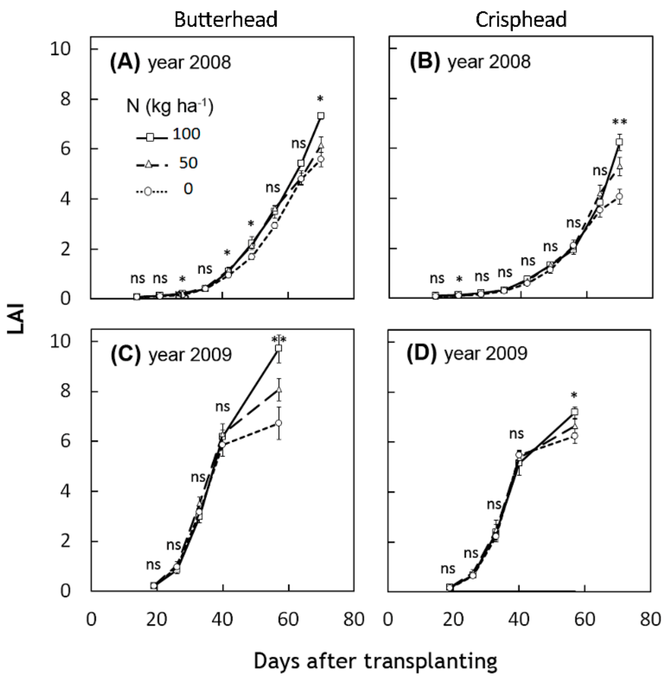

Figure 4. Changes in leaf area index (LAI) during the 2008 (**A**,**B**) and 2009 (**C**,**D**) crop cycles as affected by lettuce type (butterhead: **A**,**C**; crisphead: **B**,**D**) and N level. Bars indicate ± SE of mean (n = 4). The notes: ns, *, **, indicate differences not significant or significant at $p \leq 0.05$, $p \leq 0.01$, respectively.

N fertilization did not substantially modify the number of leaves, specific leaf area (SLA), specific leaf nitrogen (both SLNtot and SLNred), and the total chlorophyll content (on a leaf area basis) (Table 2). All these characteristics were affected by the genotype, with the butterhead type showing a greater leaves number ($p \leq 0.001$), LAI ($p \leq 0.001$), SLA ($p \leq 0.05$), SLNtot ($p \leq 0.05$), SLNred ($p \leq 0.05$), and chlorophyll concentration ($p \leq 0.05$) (Table 2). As for the yield and DW accumulation, they were higher in the 2009 than 2008 experiment ($p \leq 0.001$) (Table 2).

Table 2. Effect of N fertilization rate, lettuce genotype and year of cultivation on head morpho-physiological characteristics.

Treatments	Leaves (no.)	LAI ($m^2\ m^{-2}$)	SLA [1] ($g\ m^{-2}$)	SLN_{tot} [2] ($g\ m^{-2}$)	SLN_{red} ($g\ m^{-2}$)	Total Chlorophyll ($\mu g\ cm^{-2}$)
N rate ($kg\ ha^{-1}$) (N)						
0	38.6 a [3]	5.62 c	45.9 a	1.51 a	1.38 a	18.6 ab
50	39.2 a	6.54 b	44.9 a	1.45 a	1.30 a	20.4 a
100	41.1 a	7.61 a	41.6 a	1.36 a	1.20 a	17.1 b
Genotype (G)						
Butterhead	48.5 a	7.26 a	46.8 a	1.53 a	1.39 a	22.3 a
Crisphead	30.8 b	5.92 b	41.5 b	1.35 b	1.21 b	15.1 b
Year (Yr)						
2008	35.8 b	5.78 b	31.6 b	1.17 b	1.13 b	14.5 b
2009	43.5 a	7.41 a	56.7 a	1.71 a	1.47 a	19.2 a
Significance [4]						
N	ns	**	ns	ns	ns	*
G	***	***	*	*	*	*
Yr	*	***	***	***	**	*
Yr*N	ns	ns	ns	ns	ns	ns
Yr*G	ns	ns	ns	ns	ns	ns
N*G	ns	ns	ns	ns	ns	ns
N *G*Yr	ns	ns	ns	ns	ns	ns

[1] SLA = Speficic Leaf Area. [2] SLN = Specific Leaf Nitrogen. [3] Means in columns not sharing the same letters are significantly different according to LSD test ($p = 0.05$). [4] ns, *, **, and ***, not significant or significant at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively.

3.4. Crop N Uptake, Nitrogen, and Nitrate Shoot Concentration

The highest N removal rates occurred starting from 25 days before harvest in both years (Figure 5) during the phase of rapid growth (Figure 2), when 68–74% of the total N uptake was detected. The daily crop N uptake (averaged over the years) peaked in the most fertilized plants of butterhead lettuce (2.7, 3.0, and 3.7 $kg\ ha^{-1}\ d^{-1}$ with N_0, N_{50}, and N_{100}, respectively), while in the crisphead one it averaged 2.4 $kg\ ha^{-1}\ d^{-1}$.

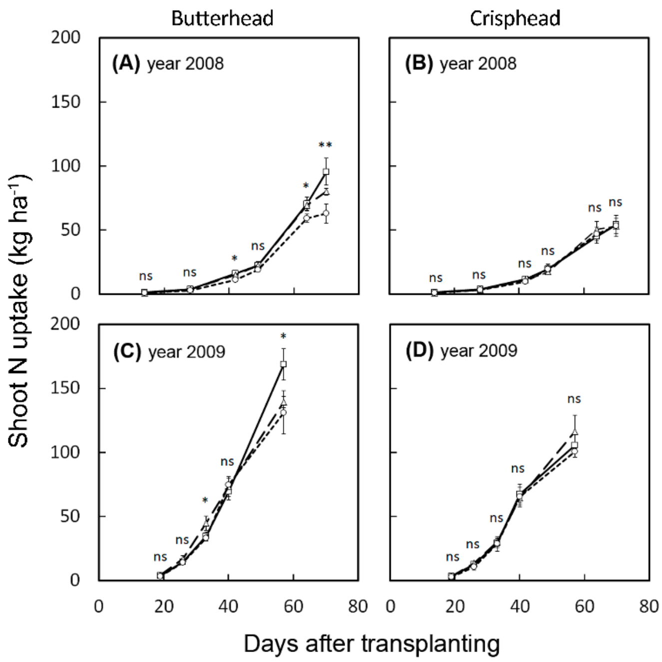

Figure 5. Changes in aboveground N uptake during the 2008 (**A** and **B**) and 2009 (**C** and **D**) crop cycles as affected by lettuce type and N level. Bars indicate ± SE of mean ($n = 4$). The notes: ns, *, **, indicate differences not significant or significant at $p \leq 0.05$, $p \leq 0.01$, respectively.

As a consequence, in the butterhead type the final N uptake rose from 97.0 in N_0 to 132.1 kg ha^{-1} in N_{100} treatment, while in the crisphead it was lower and almost unchanged between the three N levels (80.5 kg ha^{-1}, on average) (Table 3; Figure 6).

Table 3. Effect of N fertilization rate, lettuce genotype, and year of cultivation on N crop uptake, N tissue concentration, and nitrate content on a DW and a fresh weight (FW) basis.

Treatments	N Crop	Reduced N	N Concentration	NO_3	
	Uptake (kg ha^{-1})	(g 100 g^{-1} N Uptake)	(g kg^{-1} DW)	(g kg^{-1} DW)	(g kg^{-1} FW)
N rate (kg ha^{-1}) (N)					
0	87.0 b [1]	92.1 a	33.8 a	10.9 b	324 b
50	97.2 ab	91.5 b	33.2 a	11.9 ab	371 ab
100	106.0 a	90.4 b	33.6 a	13.3 a	424 a
Genotype (G)					
Butterhead	112.9 a	91.6 a	33.6 a	11.7 a	397 a
Crisphead	80.6 b	91.0 a	33.6 a	12.4 a	348 a
Year (Yr)					
2008	66.5 b	97.0 a	36.9 a	4.7 b	131 b
2009	127.0 a	85.7 b	30.3 b	19.2 a	615 a
Significance [2]					
N	***	***	**	***	***
G	*	*	ns	*	*
Yr	**	ns	ns	ns	ns
Yr*N	ns	ns	ns	ns	ns
Yr*G	ns	ns	ns	ns	ns
N * G	*	ns	ns	ns	ns
N *G*Yr	ns	ns	ns	ns	ns

[1] Means in columns not sharing the same letters are significantly different according to LSD test ($p = 0.05$). [2] ns, *, **, and ***, not significant or significant at $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$, respectively.

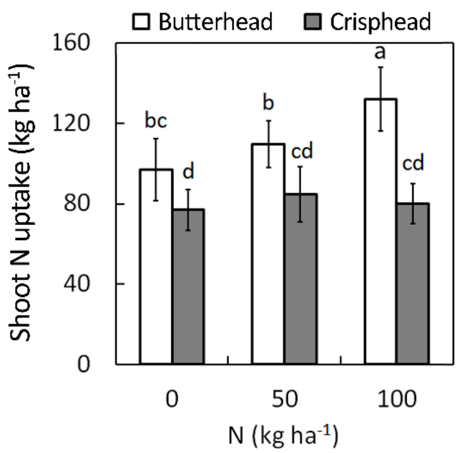

Figure 6. Effect of N fertilization rate and lettuce type on the shoot N uptake. Vertical bars (± SE of mean; $n = 4$) with different letters are significantly different according to the LSD test ($p = 0.05$).

On average, the fraction of reduced N over total N accumulated by the heads at the harvest decreased slightly in fertilized compared with unfertilized plants ($p \leq 0.05$) (Table 3). Nitrogen fertilizer application did not affect final nitrogen concentration in lettuce tissues although it resulted in an increase ($p \leq 0.05$) in nitrate concentration (both on a DW and a FW basis) passing from N_0 to N_{100}. Neither the concentration of total N and nitrate nor the incidence of reduced N on the total N taken up by the crop changed in the two lettuce types (Table 3). In 2009, crop N uptake was 48% higher than in the 2008 cycle ($p \leq 0.001$), despite the total N concentration being lower ($p \leq 0.01$). However, a greater nitrate concentration in lettuce tissues and a decrease in N-reduced percentage was observed (Table 3).

3.5. Nitrogen Fertilization Use Efficiency Indices

In both experiments, neither the agronomical N use efficiency (NUEa) nor its component, the apparent nitrogen recovery (REC), were affected by N fertilization and they were much greater in butterhead than in crisphead lettuce ($p \leq 0.05$) (Table 4). A significant interaction N*G was found for the second component of NUEa namely the physiological nitrogen use efficiency (NUEp). Irrespective of N fertilizer rate, lower NUEp values were detected in the crisphead type, while NUEp rose in the butterhead, particularly with the lower N rate (Figure 7). Partial factor productivity (PFP) was higher with the N_{50} than the N_{100} rate particularly in the 2009 cycle (Table 4), but it did not differ between lettuce types.

Table 4. Effect of year of cultivation, N fertilization rate, and lettuce genotype on N use efficiency indices.

Treatments	REC	NUEp	NUEa	PFP
	(kg kg^{-1})			
N rate (kg ha^{-1}) (N)				
50	0.20 a [(1)]	30.9 a	7.5 a	1696 a
100	0.19 a	21.8 a	6.2 a	869 b
Genotype (G)				
Butterhead	0.30 a	37.8 a	10.7 a	1326 a
Crisphead	0.09 b	14.8 b	2.9 b	1239 a
Year (Yr)				
2008	0.17 a	31.6 a	5.4 a	1033 b
2009	0.22 a	21.1 a	8.3 a	1532 a
Significance [(2)]				
N	ns	ns	ns	***
G	*	*	*	ns
Yr	ns	ns	ns	***
Yr*N	ns	ns	ns	*
Yr*G	ns	ns	ns	ns
N*G	ns	*	ns	ns
N*G*Yr	ns	ns	ns	ns

[(1)] Means in columns not sharing the same letters are significantly different according to LSD test ($p = 0.05$). [(2)] ns, * and ***, not significant or significant at $p \leq 0.05$ or $p \leq 0.001$, respectively.

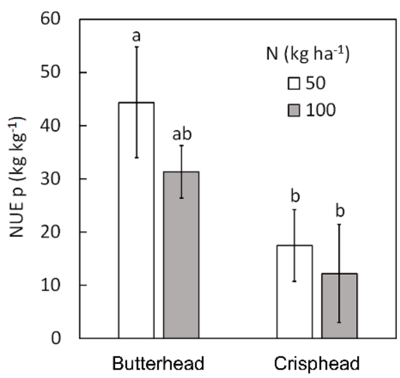

Figure 7. Physiological nitrogen use efficiency (NUEp) as affected by N levels and lettuce type. Vertical bars (± SE of mean; $n = 12$) with different letters are significantly different according to the LSD test ($p = 0.05$).

3.6. Critical Nitrogen Dilution Curves

The levels of critical N concentration (%Nc) were determined for each lettuce type during both cropping cycles following the procedure suggested by Justes et al. [18]. The critical dilution curves were applied for aboveground dry weight (DW) values ranging from 0.9 to 3.7 Mg ha^{-1} for the crisphead and from 0.9 to 5.7 Mg ha^{-1} for butterhead lettuce. This fraction of DW accumulation occurred in the last 25 days of the crop cycle (from about 40 to 63 DAT, averaged over both years). During this period, both growth (Figure 2) and LAI (Figure 4) exhibited a linear increase, suggesting that the competition for light among plants was already occurring with LAI values higher than 2. In the previous phase, N concentration rose from 2.7%, observed at 16 DAT, to 3.7%. The fitting of the power function %Nc = aDW^{-b} performed both on type-pooled %N data and on the type-specific ones showed a high goodness (Table 5). However, when the type-specific N critical values were considered, the fitting gave slight lower RMSE and RRMSE with adjR^2 showing the highest values especially in crisphead lettuce (Table 5). The type-specific power function for the butterhead (%N_{BH}) and crisphead (%N_{CH}) had a similar "*a*" coefficient, while the "*b*" parameter was statistically lower in butterhead than in crisphead lettuce (Table 5; Figure 8). In general, by evaluating the prediction performance of the N-critical functions proposed by Tei et al. [13] (%N_{Tei}) and by Bottoms et al. [14] (%$N_{Bottoms}$) against the %Nc observed data, the adjR^2 values were lower, and the RMSE and RRMSE higher than those obtained by type-specific modeling, particularly with the %N_{Tei} modeling and for the butterhead type (Table 5, C–D).

Table 5. Indices of model performance evaluation, estimates and standard errors of parameters, after fitting the N power function (%N = a DW^{-b}) on the critical N concentration with the shoot dry weight. Our modeling fits were performed, considering the experimental %N data all together (A) or pooling them by lettuce type (B). The prediction performance of the Tei et al. [13] and Bottoms et al. [14] functions were also evaluated (C, D).

Model Evaluation	$_{adj}R^2$ (1)	RMSE	RRMSE	Function Parameters	
				a	***b***
(A) with all %N_C data	0.835 **	0.11	3.17	3.778 ± 0.056	−0.152 ± 0.022
(B) by lettuce type					
Butterhead (%N_{BH})	0.823 **	0.09	2.69	3.654 ± 0.067	−0.115 ± 0.024
Crisphead (%N_{CH})	0.940 **	0.09	2.41	3.964 ± 0.145	−0.205 ± 0.041
Significance (2)				ns	*
(C) using Tei et al. critical N					
Butterhead	0.806 **	0.31	8.45		
Crisphead	0.925 **	0.19	4.81		
(D) using Bottoms et al. equation					
Butterhead	0.789 *	0.18	5.01		
Crisphead	0.938 **	0.09	2.45		

(1) $_{adj}R^2$ values with * and **, indicate that the ANOVA for the regression between the predicted and observed values is significant at $p \leq 0.05$ or $p \leq 0.01$, respectively. (2) ns and *, not significant or significant at $p \leq 0.05$, respectively, between the lettuce types.

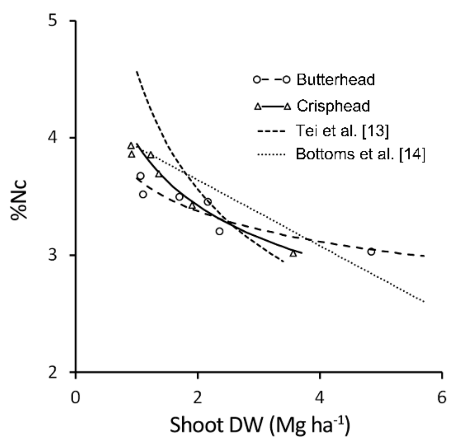

Figure 8. Plot of the critical dilution curves found for butterhead and crisphead lettuce over the 2008 and 2009 field trial data. The dilution N curves for lettuce as indicated by Tei et al. [13] and by Bottoms et al. [14] are also reported. Symbols: Observed data.

4. Discussion

4.1. Crop Growth and Production

Nitrogen effect on crop growth depends on the advantage in terms of carbon gain resulting from both improvement of light interception via leaf area growth and the enhancement of the photosynthesis rate at leaf level [24]. The latter is reported to be strictly linked to the specific leaf N (SLN) and chlorophyll content per unit leaf area [25,26].

In this research, the effect of N fertilization was related to lettuce typology, with a clear enhancement of growth in the butterhead and a negligible response in the crisphead (Figures 2 and 3A). By considering the potential effect of nitrogen on photosynthesis rate, it can be observed that in neither lettuce type did N fertilization affect the content either of the total (SLNtot) or the organic nitrogen (SLNred), which is directly related to the photosynthetic machinery [27]. Moreover, the SLA accounting for the amount of dry biomass accumulated per unit leaf area, remained unchanged in N-fertilized compared to unfertilized plants, and the chlorophyll content even showed a slight reduction when the largest N rate was applied (Table 2). Overall these results show that both in butterhead and crisphead lettuce, the photosynthesis rate remained fairly constant in N_0, N_{50}, and N_{100} leaves so it appears not to be involved in the growth response to N fertilization of the two lettuces. Consequently, it is reasonable to suppose that the observed difference in growth between fertilized butterhead and crisphead typology was mainly mediated by light interception level. Although the intercepted radiation has not been measured in this research, it is likely that leaf area expansion prompted by N fertilization (Figure 4; Table 2) resulted in an increase in light interception and so in growth, mainly in the butterhead type. Whereas, the lack of growth response in fertilized crisphead lettuce could have been caused by a very limited enhancement of the light interception due to the specific head shape of this type, where most of the leaves are in a hidden or occluded position with respect to the light. Regardless of the genotype features, the present data seem to be in agreement with results reported by other authors [3,28] who underline that N fertilization in lettuce affects leaf area, and hence the light interception, more than leaf photosynthesis.

Irrespective of N fertilization, butterhead lettuce showed higher efficiency in terms of dry biomass accumulation, as can also be inferred by comparing the N_0 plants (Figure 3A). In the butterhead type, the higher leaf number and expansion along with the greater SLN and level of chlorophylls may suggest a higher photosynthetic activity at the canopy level. Moreover, its greater root apparatus (higher root DW, higher root/shoot ratio) resulting in a larger acquisition of soil-N (Figure 6) contributes to explain the higher shoot dry weight accumulation as suggested by the findings of Kerbiriou et al. (2014) [29].

Crop productivity was not significantly enhanced by N fertilization, despite the positive evidence on butterhead growth (Figure 3A). However, in this type the better N crop nutrition improved the commercial quality due to the higher dry mass concentration of leaves (Figure 3B). In both years, the two lettuce types had a very similar yield (Table 1), since the higher water content (lower dry mass concentration) of the crisphead type compensated for its lower dry biomass accumulation (Figure 3B).

In the plant tissues, the fraction of total N content as organic form (reduced N) was very high even in fertilized plants (close to 91%, on average). Therefore, nitrate concentration, both on a dry (11.9 mg kg^{-1}) and fresh weight basis (373 mg kg^{-1}) (Table 3), was always far below the maximum limits imposed by the European Communities (EC) for lettuce grown in an open field and harvested from March to October (2.500 mg kg^{-1} of FW; EC Regulation No. 1258/2011) [30].

Better growth and yield performances were obtained in the second growing season, when temperatures were closer to the optimal values for lettuce [31] and the mean daily solar radiation was higher than in the 2008 cycle (22.5 versus 17.0 MJ m^{-2} day^{-1}) mainly due to the second trial being carried out later in the spring.

4.2. Plant N Uptake and Nitrogen Use Efficiency

The aboveground N uptake (Figure 5) closely followed the pattern of crop growth (Figure 2) showing that the highest N requirement of lettuce occurs in the last three weeks of the crop cycle as also reported by Sosa et al. [32] who observed 60% of the biomass and N accumulation in the 22 days before the harvest of lettuce. The nitrogen taken up by the butterhead plants was higher than that of the crisphead, particularly in the most fertilized ones (+65%) (Figure 6). However, in both lettuces the nitrate concentration detected by raising the N fertilization rate (Table 3) linkable to a decreasing trend in NUEp (Figure 7) can be considered very low. This suggests no large luxury N consumption even at the highest N rate.

The marginal increase in head dry biomass for each unit of nitrogen supplied (NUEa) was very low irrespective of N fertilizer rate (6.5 kg kg^{-1}, on average). It was clearly restricted by the low apparent N recovery (REC) with only 19% of the applied N recovered by the crop. REC remained unchanged with increasing N rate, suggesting that the highest N supply did not greatly exceed the crop N demand [33], also confirmed by the low increase in nitrate concentration in the N_{100} plant tissues. The observed REC was comparable with that reported by Greenwood et al. [33] (~15%), and Bottoms et al. [14] (16%) for crisphead and romaine lettuce grown with much higher N rates (from 175 to 236 kg ha^{-1}) than the N fertilizer applied in this study. Di Gioia et al. [16] have reported a higher REC (32%) in lettuce for N rates ranging from 60 to 180 kg ha^{-1}, while Tei et al. [5,34] observed a decrease in REC from ~70 to ~35% when increasing the N rate from 50 to 200 kg ha^{-1}. The variability in the fraction of N-fertilizer taken up by lettuce could be explained by the changes in the contribution of sources of soil N other than the applied fertilizer (e.g., from organic matter mineralization). Bottoms et al. [14] found a very low REC (7%) with 150 kg ha^{-1} of N fertilization and a concentration of 20 mg kg^{-1} of native NO_3–N in the soil. The low REC values in our research could be due to the quite high N availability deriving both from soil organic matter and/or residues of the previous broccoli crop. It was confirmed by the high N uptake in unfertilized crop (87 kg ha^{-1}) which represented 80% of the N taken up by the fertilized ones. Vegetable crop residues are considered a potential major source of N for the subsequent crop as they often have a small harvesting index, with broccoli in particular leaving up to 180 kg ha^{-1} of N in the residues [35].

Furthermore, this study highlighted a clear difference in N-fertilizer use efficiency according to lettuce typology with noticeably low REC, NUEp, and so NUEa by the crisphead type (Table 4; Figure 8). All these results confirm the difference between the lettuce typologies in their ability to acquire/absorb N and in their use efficiency of the absorbed N for producing dry biomass due to the higher efficiency of both root and photosynthetic apparatus. Di Gioia et al. [16] have also reported differences in NUE indices between romaine (REC = 32%; NUEp = 22 kg^{-1} kg^{-1}; NUEa = 6.5 kg^{-1} kg^{-1}) and oak-leaf lettuce types (REC = 27%; NUEp = 15 kg^{-1} kg^{-1}; NUEa = 3.5 kg^{-1} kg^{-1}).

In our trials, the difference in climatic trend during the two trials did not affect NUEa, REC or NUEp (Table 4). However, the NUEp, averaged over years (27 kg kg^{-1}, Table 4), was much greater than that reported for fall–winter cycles in the same region (~14 kg^{-1} kg^{-1}; [16]), probably due to the higher global radiation in spring cycles compared with the fall–winter ones.

The efficiency with which N supply is converted into economic yield (PFP) is the most important index for growers because it integrates both indigenous and applied N uptake efficiency and a decrease with increasing fertilization rate is expected [36]. In these trials, PFP almost halved with 100 kg ha^{-1} of N (0.90 Mg kg^{-1}) compared with the lower N rate (1.70 Mg kg^{-1}) justifying the negligible response, in terms of fresh yield, to the increase in N fertilization observed in both cultivars.

4.3. Nitrogen Critical Curve and Critical Uptake

By considering the type-specific critical N dilution curve, the fitting was excellent, highlighting a faster reduction in plant N concentration during the cycle in the crisphead ($b = -0.205$) than in butterhead ($b = -0.115$) lettuce (Table 5; Figure 8). These results suggest that the butterhead type, consistently with its greater dry mass production (Figure 3) and N requirement (Figures 3 and 6), maintains its photosynthetically active compartment longer with higher N concentration than in the structural pool, as reported in [36].

The critical N dilution curve of Tei et al. [13] (%N_{Tei}), which was defined on a butterhead lettuce grown under sub-continental/continental climate, proved its limits in predicting the %Nc for this type, mainly due to the much higher values in the initial growth (Figure 8) confirming the results of Bottoms et al. [14]. Furthermore, although the %N_{Tei} covers the range of DW yield up to 3.4 Mg ha^{-1}, its trend over this threshold would underline a large underestimation of %Nc values compared to the %N_{BH} (Figure 8) due to the greater difference in *b*parameter (−0,357 versus −0.115). This result highlights a marked effect of the climate condition on the growth pattern of the buttered lettuce, confirming the need for the local re-calibration of the critical %N curve. A closer pattern was found by comparing the %N_{BH} and %N_{CH} with the empirical equation proposed by Bottoms et al. [13] (N‰ = 42-2.8 DW (Mg ha^{-1})) (%$N_{Bottoms}$) for romaine and crisphead lettuce grown under the Californian Mediterranean-like climate. In particular, for the crisphead lettuce, the %N_{CH} and %$N_{Bottoms}$ functions are mostly overlapping, with very similar adjR^2, RMSE, and RRMSE values of their predictions. For the butterhead type, the %$N_{Bottoms}$ equation gives a clear underestimation of N concentration, when DW is in the range between 4 and 6 Mg ha^{-1} (Figure 8), likely due to the differences in plant growth and physiology between butterhead and the lettuce typologies used by Bottoms et al.

In our case, based on the type-specific %Nc functions, the N demand ranged from 80 to 170 kg ha^{-1} of N for the butterhead, and from 50 to 110 kg ha^{-1} of N for the crisphead for sustaining a dry biomass production varying in the two seasons from 2.5 to 6.0 Mg ha^{-1} in the butterhead and from 1.4 to 4.0 Mg ha^{-1} in the crisphead lettuce (Figure 9A). Taking into consideration the fresh biomass production (yield) (Figure 9B), the N demand diverges more between the two types, with the butterhead type having a higher DW concentration than the crisphead.

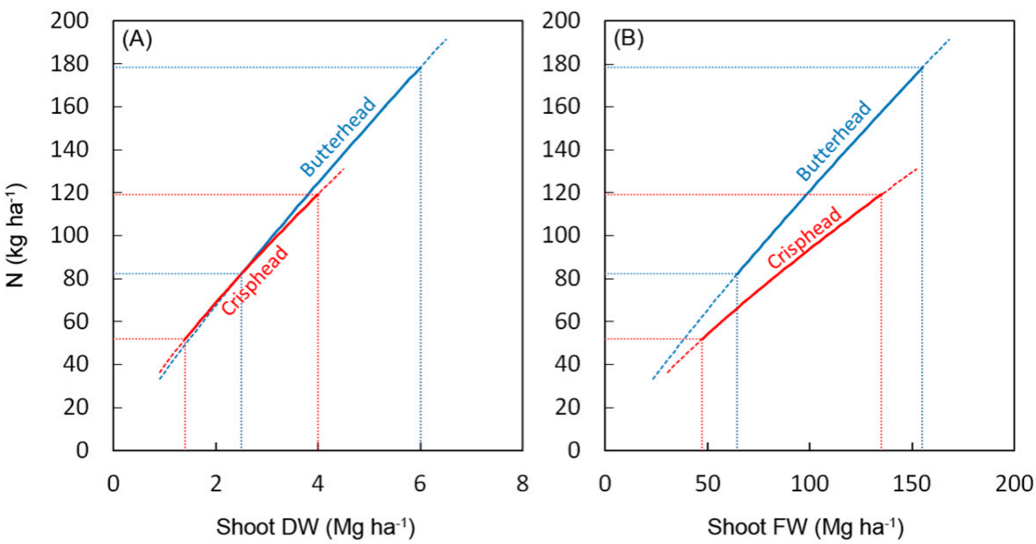

Figure 9. N demand (kg ha^{-1}) for butterhead and crisphead lettuce (**A**) as a function of dry biomass yield (in the range used for critical N curve validation—solid line) and (**B**) of the corresponding trend when using fresh yield as the independent variable.

5. Conclusions

The study provides evidence that growth and N uptake in lettuce are affected by genetic characteristics, with the butterhead type having a higher ability to uptake nitrogen and higher efficiency in using nitrogen for dry mass production than the crisphead one. The calibration of the specific N critical dilution curve performed for the two lettuce typologies may optimize their N nutrition, accounting for their own potential in dry mass production linked to the genetic characteristics and the interaction with climatic conditions.

Under the Mediterranean climate, the critical N dilution curves %Nc = 3.96 $DW^{-0.205}$ and %Nc = 3.65 $DW^{-0.115}$ are suggested for crisphead and butterhead lettuce, respectively. On an average, the optimal N uptake ranges from 80 kg ha^{-1} in crisphead to 125 kg ha^{-1} in butterhead lettuce to produce 2.5 and 4.3 Mg ha^{-1} of dry biomass, respectively, which correspond in terms of fresh biomass to an average yield of between 90 and 110 Mg ha^{-1} for both lettuce types.

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