



Species, Cultivar and Seasonal Effects on Nodulation and Nitrogen Utilization of Spring Mediterranean Grain Legumes

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Abstract

Information on nitrogen economy of grain legumes during seed filling is critical for their sustainable cultivation in rainfed agroecosystems, but a comparable assessment of different legumes under the same growth conditions is lacking. The purpose of this study was to examine species, cultivar and seasonal effects on nodulation and nitrogen accumulation, remobilization, and utilization. Five grain legumes [common vetch (*Vicia sativa* L.), red pea (*Lathyrus cicera* L.), lentil (*Lens culinaris* Medik), chickpea (*Cicer arietinum* L.), and field pea (*Pisum sativum* L.)], each represented by two cultivars, were grown for two years in a semiarid Mediterranean environment. There were considerable environmentally induced differences among species in nodulation, with chickpea maintaining a sufficient number of nodules (16 nodules plant⁻¹) and nodule dry weight (57 mg plant⁻¹) even in the drier year (2015). Pre-podding nitrogen accumulation varied among species, particularly in the wetter year (2014). Nitrogen translocation from vegetative parts to seeds was correlated with pre-podding nitrogen accumulation ($r=0.99$, $P<0.01$ in 2014 and $r=0.98$, $P<0.01$ in 2015). Interspecific differences in nitrogen translocation efficiency were enlarged under drought, with red pea exhibiting the highest (84%) and lentil the lowest value (72%). Chickpea produced the highest seed nitrogen accumulation in both years and had the highest nitrogen utilization efficiency (21 kg seeds per kg N) the drier year. Nitrogen utilization efficiency was positively correlated with nitrogen harvest index and negatively with nitrogen concentration in vegetative parts at maturity. Most trait differences were less evident within species than between species. Overall, chickpea was found to outperform other legumes in semiarid Mediterranean conditions in terms of nitrogen accumulation in seeds and nitrogen utilization efficiency.

Keywords Nitrogen accumulation · Nitrogen translocation · Nitrogen utilization efficiency · Climate change

1 Introduction

Crop diversification is necessary to ensure food and nutritional security for the world's growing population, particularly in the light of major emerging concerns such as climate change. In this context, incorporating grain legumes into cropping systems can contribute to environmentally friendly intensification of agricultural production by providing several advantages, the majority of which are associated with their ability to fix atmospheric nitrogen (N). In this sense, grain legumes can boost total soil N and soil water content while

minimizing atmospheric N emissions and reactive inorganic N inputs. However, the soil N balance after grain legumes may occasionally be neutral or even negative (Walley et al. 2007; Koutroubas et al. 1998) depending on identity of crop species and the levels of N fixation (Döring 2015). The importance of grain legumes as a preceding crop is well understood, particularly in cereal rotations. Nitrogen fertilization can be lowered by 23–31 kg ha⁻¹ after grain legumes, and cereal yields are typically 0.5–1.6 Mg ha⁻¹ greater than after cereal pre-crops (Preissel et al. 2015). Moreover, the N yield of unfertilized wheat following faba bean was 27% greater than that after barley (Guinet et al. 2020). In addition to N contribution, grain legumes can play an important role in weed, pest and disease management, enhancing overall farming profitability. Moreover, when opposed to cereal residues, legumes' low C/N ratio leads in faster rate of residue decomposition, favoring the increase of soil organic matter. This is especially important

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for soils in the Mediterranean region of Europe, which are frequently deficient in organic matter (Zdruli et al. 2004).

The biological N fixation of legumes is positively associated with nodule development and nodule characteristics such as dry weight (Pampana et al. 2018). The ability of plants to form root-nodules varies greatly between and within grain legumes (Oladzad et al. 2020; McCauley et al. 2012; Ferguson et al. 2019), and is strongly influenced by soil conditions, particularly mineral N content (Virk et al. 2022). Indeed, when there is enough N in the soil, legume plants limit nodule numbers and size, as well as N fixation activity in order to conserve resources, as forming and maintaining nodules requires a lot of energy (Ferguson et al. 2019). Experiments with soybean, on the other hand, revealed that even when soil N supply was not a constraint, symbiotic N was still necessary to obtain high yield, most likely because fixed atmospheric N is already in the organic reduced form and is therefore more easily accessible to plant metabolism (Koutroubas et al. 1998). Besides soil properties, abiotic stresses, like heat and water stress, which are very common in Mediterranean environments, suppress nodule growth and activity (Ferguson et al. 2019). From a practical standpoint, farmers can mitigate environmental stresses by adjusting the sowing time of grain legumes. Autumn sowing is preferred in locations with warm winters because it provides a yield advantage over spring sowing. However, in chilly locations, the sowing of grain legumes is delayed until early spring because autumn or winter-planted crops show low stands due to poor germination and crop loss due to weather (Koutroubas et al. 2009; Chen et al. 2006).

The developing seeds of grain legumes have substantially high N requirements because of their high N concentrations, providing the advantage of rapid seedling establishment and growth even when N levels in the soil are low (Del Pozo et al. 2000). Seed N demands are typically poorly supported by current soil N uptake and symbiotic N fixation, both of which decline progressively during seed filling (Salon et al. 2001). Therefore, internal N remobilization is required to support seed growth. Nitrogen remobilization occurs in all vegetative parts, but the rate and the efficiency of remobilization vary depending on the plant part and its N content (Salon et al. 2001). Species differences in N remobilization to seeds have been reported in grain legumes (Pampana et al. 2016). Moreover, genotypic differences in N translocation have been linked to several physiological traits such as seed filling duration (Turner et al. 2005), as well as variations in N translocation efficiency (Pampana et al. 2016), which is the proportion

of pre-podding N that has been translocated to seeds. Apart from the genotypic constitution, the environment plays an important role in N translocation to seeds, which is generally greater under abiotic stress (Turner et al. 2005). Nitrogen remobilization has been recognized as a physiological trait influencing genetic variation in N utilization efficiency (i.e., the ratio of grain yield to the total N accumulation), especially in non-legume crops that are depended solely on N fertilization (Bouchet et al. 2016; Nehe et al. 2020). In leguminous plants, N utilization has received less attention, with results depending on environmental conditions (Fotiadis et al. 2019; López-Bellido et al. 2004). Palmero et al. (2022) reported subtle differences in nitrogen utilization efficiency (NUE) among grain legumes, and Neugschwandtner et al. (2015) found that chickpea demonstrated a consistently high NUE even under drought conditions.

In dryland agriculture, grain legume adaptation is quite variable as it strongly depends on season rainfall. Information on species differences in overall N economy during seed filling and its response to environmental fluctuations is limited for grain legumes. The aim of the present study was to determine species, cultivar, and seasonal effects on nodulation and N accumulation, remobilization and utilization and their associations with grain N yield of Mediterranean spring legumes. Such information is essential to support management decisions, including the selection of the appropriate species and cultivars, in order to improve the sustainability as well as the seasonal adaptation of grain legumes in semiarid Mediterranean agroecosystems.

2 Materials and Methods

2.1 Location Description and Weather Conditions

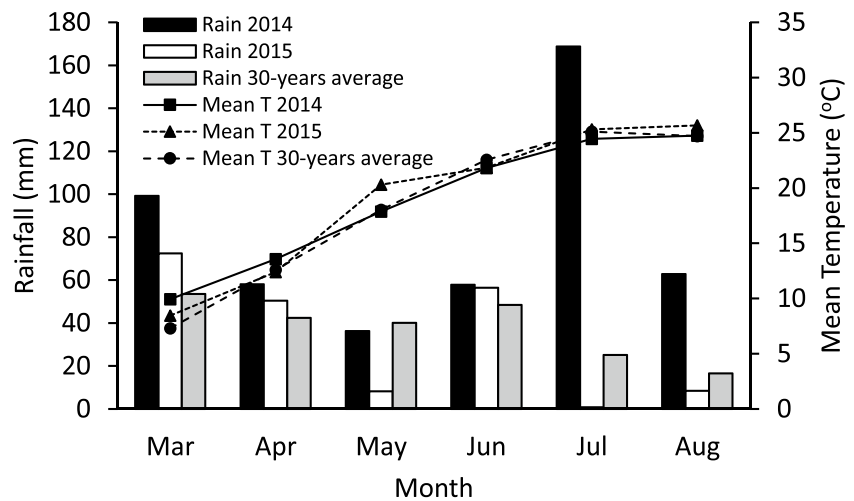
The experiments were carried out in 2014 and 2015 at the farm of Democritus University of Thrace in Orestiada (41°33'N, 26°31'E, altitude 33 m), Greece, on a silty clay (Typic Xerofluvent) soil, the main properties of which are listed in Table 1. Common wheat (*Triticum aestivum* L.) was grown in the field the previous year. Rainfall and temperature recordings were collected from the Hellenic Sugar Industry's meteorological station (Fig. 1).

Weather conditions during the growing season of legumes differed greatly between years (Fig. 1). The growing season in 2014 was notably wetter, with total seasonal rainfall

Table 1 Soil properties (0–30 cm) of the experimental site

pH (1:2 water)	Electrical conductivity (S m ⁻¹)	Organic matter (g kg ⁻¹)	NO ₃ -N (mg kg ⁻¹)	Total N (g kg ⁻¹)	Olsen P (mg kg ⁻¹)	Available K (mg kg ⁻¹)
6.68	0.04	10.1	9.0	1.11	10.2	495.3

Fig. 1 Total monthly rainfall and mean monthly temperature during the growing season in 2014, 2015, and long-term (30-years) mean



(483 mm) being substantially higher than that of 2015 (197 mm). Compared with the long-term average (226 mm), total seasonal rainfall in 2014 was 114% higher, while that of 2015 was 13% lower. The total amount of rainfall in June and July, when the grain filling of legumes occurred, was 227 mm in 2014 but just 57 mm in 2015. The average temperature of each growing season was similar across years (18.7 °C in 2014 and 18.9 °C in 2015). However, the mean monthly temperature during the early plant growth (March and April) was slightly higher in 2014 than in 2015. On the contrary, from the beginning of seed growth onwards, the average monthly temperature in 2015 was slightly higher than that in 2014.

2.2 Treatments and Experimental Design

Treatments included five legume species: common vetch (*Vicia sativa* L.), red pea (*Lathyrus cicera* L.), lentil (*Lens culinaris* Medik), chickpea (*Cicer arietinum* L.), and field pea (*Pisum sativum* L.). These species were chosen to

represent the most commonly grown cool-season grain legumes in the area. Each legume was represented by two cultivars developed by the Institute of Industrial and Forage Crops, Hellenic Agricultural Organization (Table 2). A randomized complete block design with four replications was established for the experiment. Both years, spring planting was applied (17 March 2014, and 6 March 2015). Fertilization management followed common practice for the studied species in the area. The experimental area was fertilized with 26 kg P ha⁻¹ yr⁻¹ in the form of superphosphate, which was hand broadcasted and incorporated into the soil before planting. Due to the presence of indigenous rhizobia populations in the soil, no inoculation was applied. The plot size was 12 m² (3 by 4 m) and consisted of 12 rows spaced 25 cm apart. Seeds were sown by hand and the seedlings were thinned after emergence to achieve a uniform population of 40, 60, 150, 50, and 100 plants per m² for common vetch, red pea, lentil, chickpea, and field pea, respectively. Weeds were controlled by hand weeding when necessary.

Table 2 Common name, Latin binomial, cultivars, flowering time, and seed weight of grain legume species used in the study

Species		Cultivar	Flowering time	Thousand seed weight (g)
Common name	Latin binomial			
Common vetch	<i>Vicia sativa</i> L.	Pigasos	Early	Middle (690–690)
		Tembi	Early	Small (600–650)
Red pea	<i>Lathyrus cicera</i> L.	Rodos	Early	260–280
		Argos	Early	260–280
Lentil	<i>Lens culinaris</i> Medik	Thessalia	Middle-early	Large (60–65)
		Samos	Early	Large (40–45)
Chickpea	<i>Cicer arietinum</i> L.	Andros	Early	Small (205–230)
		Kassos	Middle-late	Small (200–230)
Field pea	<i>Pisum sativum</i> L.	Vermio	Late	130–140
		Ithomi	Middle-early	130–140

2.3 Crop Samplings, Measurements and Calculations

Crop phenology was assessed two times per week. The time from sowing to seedling emergence, flowering [growth stage (GS) R2], onset of seed growth (GS R5), and maturity (GS R8) was recorded. At GSs R5 and R8, plants from a 1-m-long row from each plot were cut at ground level and then they were divided into individual plant organs (i.e., leaf and stem, pod walls, and seeds). Samples were dried in an oven at 70 °C until constant weight, weighed and ground in a Wiley mill (Glen Creston Ltd.,

Stanmore, Middlesex, UK). The concentration of total N in plant tissues was analyzed using the standard macro-Kjeldahl method (Nelson and Sommers 1973). Nitrogen accumulation rate for a given time period was determined as the ratio between the change in total aboveground N accumulation and the number of days in that period (Hunt et al. 2002). The number of nodules per plant was counted at flowering on ten consecutive plants dug from an inner row of each plot after gently removing soil by washing. Nodules dry weight was determined after drying at 70 °C until constant weight. Parameters associated with N recycling and partitioning within aboveground plant parts were calculated using the following formulae (Hocking and Pate 1977; Koutroubas et al. 2023):

Nitrogen translocation (NT, kg ha^{-1}) = [Total aboveground N accumulation at GS R5 (kg ha^{-1})]
– [N accumulation of vegetative parts at GS R8 (kg ha^{-1})]

Nitrogen translocation efficiency (NTE, %) = $\frac{\text{NT (kg ha}^{-1}\text{)}}{\text{Total aboveground N accumulation at the GS R5 (kg ha}^{-1}\text{)}} \times 100$

Nitrogen utilization efficiency (NUE, kg kg^{-1}) = $\frac{\text{Seed dry weight at the GS R8 (kg ha}^{-1}\text{)}}{\text{Total aboveground N accumulation at the GS R8 (kg ha}^{-1}\text{)}}$

Nitrogen harvest index (NHI) = $\frac{\text{Seed N accumulation at the GS R8 (kg ha}^{-1}\text{)}}{\text{Total aboveground N accumulation at the GS R8 (kg ha}^{-1}\text{)}}$

2.4 Statistical Analysis

Statistical analysis of data was performed using MSTAT-C (Michigan State University, East Lansing, MI, MSTAT-C 1989). A year-combined ANOVA was used to analyze differences among species when averaged across all cultivars within each species. In addition, an over years combined ANOVA was conducted separately for each species to compare cultivars within a particular species. Year, species, and cultivars as well as the interactions between them were considered as fixed effects. Year was regarded as a fixed effect, because of the considerable variation in rainfall between the research years. Mean comparisons were done using the Fisher's protected least significant difference (LSD). Relationships between traits were evaluated by simple correlation analysis.

3 Results

3.1 Phenological Development of Plants

No differences among species concerning time to crop emergence were observed in either year. On average, crop emergence was delayed by 10 d in 2014 compared with 2015 (35 DAS vs 25 DAS, respectively). In 2014, the beginning of seed growth occurred earlier for common

vetch, red pea, and field pea (75 DAS) compared with lentil and chickpea (89 DAS). The overall crop duration was 120 d for common vetch, red pea, lentil, and field pea and 141 d for chickpea. In 2015, there were no differences among species concerning time required for the beginning of seed growth or the overall crop duration, which averaged 74 and 116 DAS, respectively.

3.2 Nodule Characteristics

Nodule number and dry weight was significantly affected by year, species, and year by species interaction (Table 3). Except for the lentil, which maintained consistent values across years, the number of nodules and their dry weight decreased in 2015 compared with 2014. Chickpea showed the smallest year-to-year decrease in nodulation characteristics (58% for the nodule number and 60% for the nodule dry weight), maintaining an adequate nodule number and weight in both years.

3.3 Nitrogen Accumulation, Translocation and Utilization

Nitrogen accumulation rate up to the beginning of seed growth differed between years and species (Fig. 2). Specifically, the average N accumulation rate in 2014 ($3.3 \text{ kg ha}^{-1} \text{ day}^{-1}$) was more than twice that in 2015

Table 3 Nodule number and nodule dry weight as affected by legume species and year of the experimentation

Year/Species	Nodules per plant (no.)	Nodule dry weight (mg plant ⁻¹)
2014		
Common vetch	22.1 ± 3.16 c	75.3 ± 12.96 b
Red pea	67.7 ± 1.72 a	72.2 ± 9.96 b
Lentil	4.3 ± 0.06 e	11.2 ± 1.72 c
Chickpea	38.3 ± 0.83 b	145.1 ± 10.73 a
Field pea	18.0 ± 1.78 cd	61.9 ± 21.03 b
Mean	30.1 ± 5.04 A	73.1 ± 1.07 A
2015		
Common vetch	0.3 ± 0.33 e	2.4 ± 2.40 c
Red pea	5.6 ± 2.77 e	12.9 ± 4.88 c
Lentil	0.2 ± 0.15 e	2.4 ± 2.43 c
Chickpea	16.1 ± 3.42 d	57.5 ± 14.54 b
Field pea	4.5 ± 0.76 e	9.3 ± 3.55 c
Mean	5.3 ± 1.60 B	16.9 ± 5.59 B
Source of variation	df	Mean squares
Year (Y)	1	6481**
Species (S)	4	1593**
Y × S	4	950**

Within each column, means ± standard error followed by the same lowercase or uppercase letter are not significantly different at $P=0.05$

** Significant at $P=0.01$

(1.6 kg ha⁻¹ day⁻¹). Species differed in N accumulation rate only in 2014, with field pea having the highest value (3.7 kg ha⁻¹ day⁻¹) and red pea having the lowest (2.8 kg ha⁻¹ day⁻¹).

Except for red pea, which showed similar values across years, total aboveground N accumulation before the onset of seed filling was substantially higher in 2014 than in 2015 (Table 4). Concerning the species performance, chickpea and lentil showed higher N accumulation at GS R5 in 2014, while in 2015 the differences among species were marginal. Cultivar differences within a particular species in N accumulation at R5 were significant only for field pea, with cv. Vermio accumulating, on average, more N than cv. Ithomi (Fig. 3a). At maturity, chickpea had the highest total aboveground N in both years, owing primarily to its higher seed N accumulation compared with the other legumes (Table 4). Nitrogen accumulation at GS R5 was significantly correlated with dry matter accumulation at this stage (Fig. 4a). In addition, total N accumulation at maturity was significantly correlated with seed N yield (Fig. 5b). Species differed in NHI, but the differences varied over time (Tables 5 and 6). Nitrogen harvest index ranged from 0.773 (chickpea) to 0.845 (lentil) in 2014 and from 0.715 (lentil) to 0.860 (chickpea) in 2015. In 2015, NHI decreased in common vetch and lentil, remained unchanged in red pea and field pea, and increased in chickpea compared with 2014. Nitrogen harvest index was

significantly correlated with seed N yield in 2015 ($r=0.90$, $P<0.01$) but not in 2014 ($r=-0.10$, $P>0.05$).

Nitrogen translocation from storage tissues to seeds was significantly influenced by year, species, and year by species interaction (Table 6). In most species, NT decreased in 2015 compared with 2014, with the percentage of reduction ranging from 21% (red pea) to 74% (lentil). Species differences in NT were evident in 2014, with lentil and chickpea exhibiting more NT than the other legumes, whereas in 2015, no species differences in NT were observed. Cultivar differences in NT within a particular species were significant for common vetch, where cv. Pigasos translocated on average more N than cv. Tembi, and for red pea, where cv. Rodos translocated on average more N than cv. Argos (Fig. 3b). In both years, NT was significantly correlated with total N accumulation at the beginning of seed growth (Fig. 4b). When both years' values were considered in the analysis, NT was significantly correlated with the amount of N in seeds at maturity ($r=0.63$, $P<0.01$).

Nitrogen translocation efficiency was substantially greater in 2014 (84%) than in 2015 (78%) (Table 6). In 2015, NTE decreased by 20% for lentil, 7% for common vetch, and 7% for field pea compared with 2014, while NTE remained unchanged across years for red pea and chickpea. Nitrogen translocation efficiency was significantly correlated with NT ($r=0.69$, $P<0.05$ in 2014 and $r=0.82$, $P<0.01$ in 2015).

The NUtE differed among species, but the differences were not consistent across years (Table 6). Common vetch in

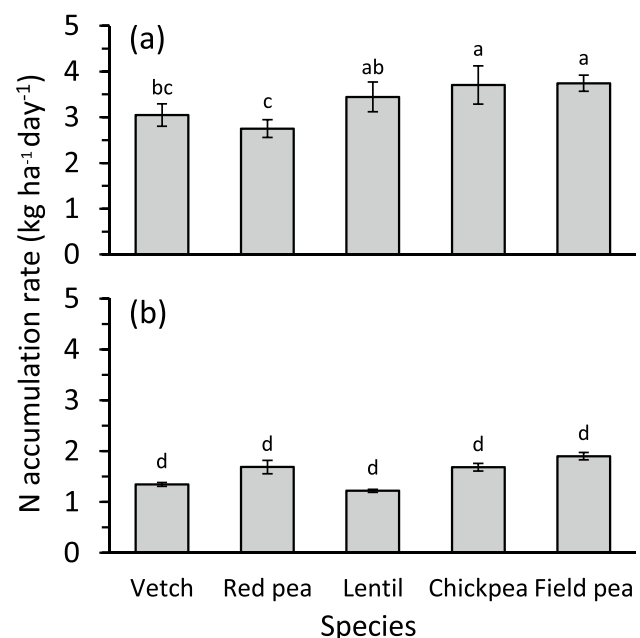


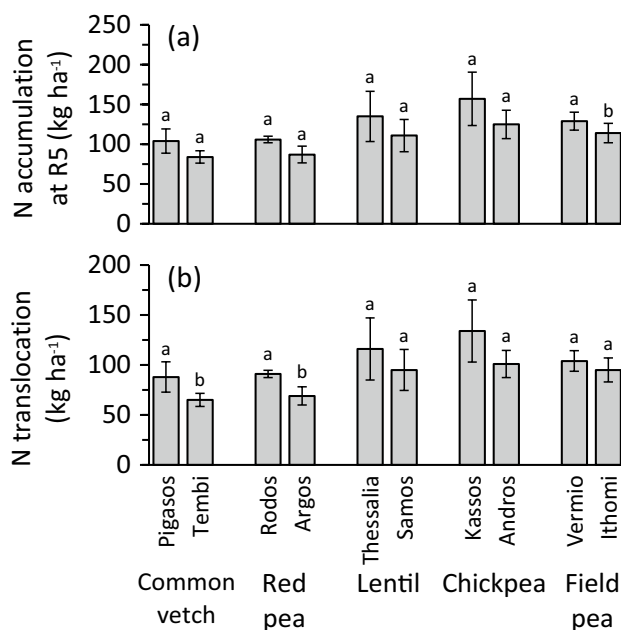
Fig. 2 Nitrogen accumulation rate up to the beginning of seed growth (GS R5) as affected by legume species and year of the experimentation (a, 2014; b, 2015). Vertical bars represent the standard errors of the means. Bars with the same letter indicate means that are not significantly different at $P=0.05$

Table 4 Nitrogen accumulation at the beginning of seed growth (GS R5) and at maturity (GS R8) as affected by legume species and year of the experimentation

Year/Species	N accumulation (kg ha ⁻¹)				
	GS R5	GS R8			
		Veg. parts	Seeds	Total	
2014					
Common vetch	122 ± 6.07 bc	20 ± 0.76 bcd	90 ± 2.44 c	110 ± 2.24 c	
Red pea	110 ± 8.65 cd	21 ± 0.61 bc	96 ± 5.80 bc	117 ± 6.30 bc	
Lentil	186 ± 16.95 a	19 ± 0.87 cde	105 ± 3.61 ab	123 ± 3.15 b	
Chickpea	200 ± 19.73 a	33 ± 2.04 a	112 ± 4.27 a	145 ± 2.81 a	
Field pea	150 ± 9.15 b	24 ± 1.03 b	92 ± 1.68 c	116 ± 2.21 bc	
Mean	154 ± 9.60 A	23 ± 1.24 A	99 ± 2.41 A	122 ± 3.11 A	
2015					
Common vetch	66 ± 2.10 ef	15 ± 1.54 ef	43 ± 2.78 e	58 ± 3.01 e	
Red pea	83 ± 1.26 def	13 ± 0.87 f	65 ± 4.60 d	77 ± 5.42 d	
Lentil	60 ± 0.79 f	17 ± 1.77 de	47 ± 2.53 e	64 ± 3.19 e	
Chickpea	82 ± 4.37 def	15 ± 1.88 ef	91 ± 4.80 c	106 ± 5.80 c	
Field pea	93 ± 1.88 cde	21 ± 1.19 bcd	63 ± 2.89 d	84 ± 3.98 d	
Mean	77 ± 2.95 B	16 ± 0.86 B	62 ± 4.15 B	78 ± 4.24 B	
Source of variation	df	Mean squares			
Year (Y)	1	59,005**	526**	14,063**	19,847**
Species (S)	4	3,174**	83**	1,334**	1,893**
Y × S	4	3,699**	81**	438**	243*

Within each column, means ± standard error followed by the same lowercase or uppercase letter are not significantly different at $P=0.05$

*, ** Significant at $P=0.05$ and $P=0.01$, respectively

**Fig. 3** Nitrogen accumulation at the beginning of seed growth (GS R5) and nitrogen translocation of legume species as affected by cultivar. Each bar represents means over two years. Vertical bars represent the standard errors of the means. Within each species, cultivar means followed by different letter are significantly different at $P=0.05$

2014 and chickpea in 2015 had the highest NUtE. Compared with 2014, NUtE in 2015 decreased for common vetch and lentil, remained unchanged for red pea and field pea and increased for chickpea. Differences in NUtE between cultivars within a particular species were observed for lentil and field pea, but these differences were not consistent across years (Table 5). In particular, there were no NUtE differences between lentil cultivars in 2014, but NUtE was significantly higher in cv. Samos than in cv. Thessalia in 2015. Similarly, no cultivar differences were observed for field pea in 2014, while in 2015 cv. Vermio showed better NUtE than cv. Ithomi. Nitrogen utilization efficiency was significantly correlated with NHI (Fig. 5a). In addition, NUtE was negatively correlated with N concentration of vegetative tissues at maturity ($r=-0.52$, $P<0.05$).

4 Discussion

In rainfed farming systems, where most grain legumes are typically cultivated, crop growth is highly dependent on total seasonal rainfall and distribution. This was evident in the present study, in which five grain legumes were grown over two years under different weather conditions. Almost all the traits evaluated showed considerable year-to-year variation, which was attributed primarily to seasonal differences in

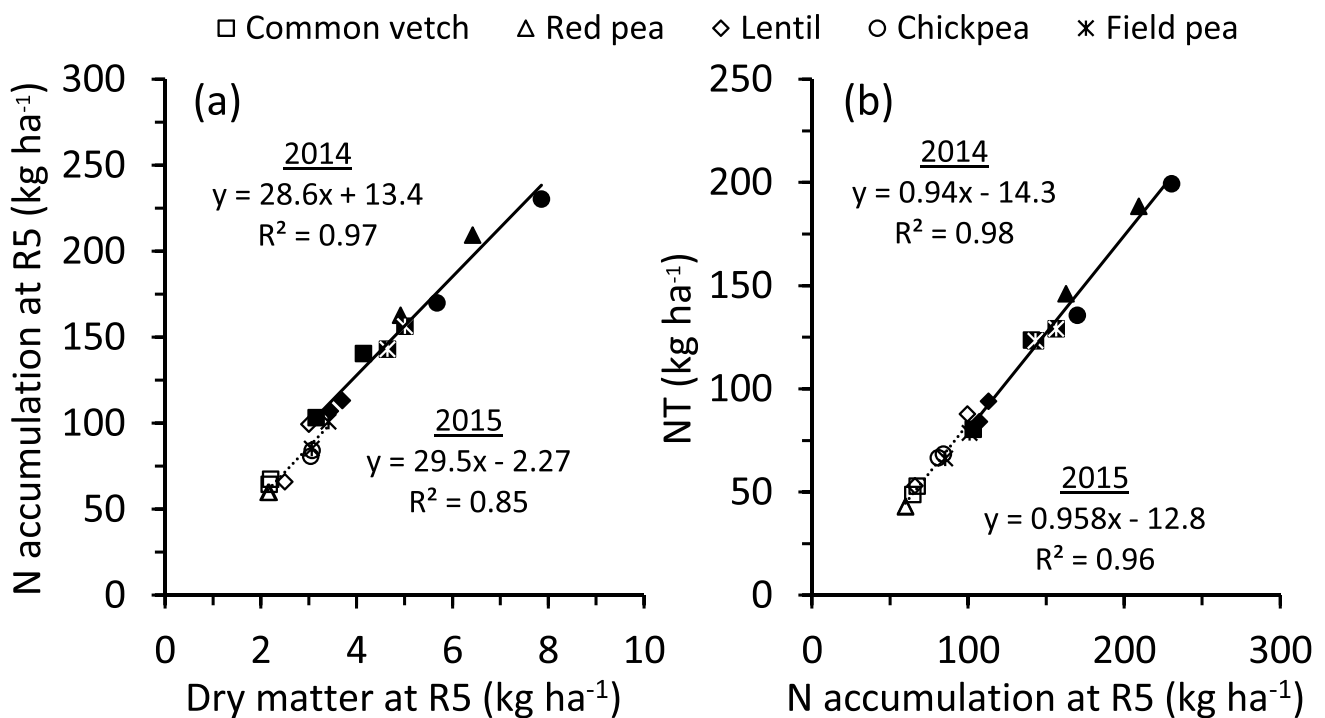


Fig. 4 Relationships between (a) dry matter and nitrogen accumulation at the beginning of seed growth (GS R5), and (b) between nitrogen accumulation at GS R5 and nitrogen translocation (NT) in 2014 (black symbols, solid line) and 2015 (open symbols, dashed line).

Each relationship was based on means derived from five legume species (common vetch, red pea, lentil, chickpea, and field pea) and two cultivars for each species ($n = 10$)

water availability. Specifically, the drought conditions in 2015 inhibited the nodule development, in terms of both nodule number and nodule biomass per plant, compared with the wetter 2014. However, the degree to which each legume responded to environmental changes varied, with chickpea maintaining adequate nodulation, even in the year with the least rainfall, as evidenced by the presence of most nodules (16 plant⁻¹) and the highest nodule weight (57 mg plant⁻¹) when compared to the other species. These findings support previous studies that reported environmental-induced differences in grain legumes nodulation patterns, with more nodules developing in wetter years than in drier years (Hossain et al. 2017). Apart from nodulation, the growing environment had a substantial impact on parameters related to N accumulation, translocation, and utilization, as it is well known that both soil N uptake and biological N fixation are highly sensitive to abiotic stresses such as drought (Ferguson et al. 2019). However, due to the year by species interaction, legumes' performance in terms of most trait measurements was not consistent across years, as discussed in the section below.

In most grain legumes, N accumulation typically peaks at the early stages of reproductive growth. In chickpea, for example, it has been reported that N fixation maximized between flower bud initiation and flowering (Turner et al.

2005), while the rate of N accumulation maximized near flowering, reaching up to 0.74 g N m⁻² day⁻¹ under Mediterranean conditions (Fotiadis et al. 2019). In the present study, there was a considerable variation in N accumulation among species, the extent of which was dependent on the growing season. Specifically, legume differences in total aboveground N accumulation prior to the onset of grain filling were more pronounced when the environmental conditions favored the manifestation of genetically determined differences in N assimilatory processes (i.e., in 2014). Under these conditions, plants exhibited high early N accumulation rate, which, along with the longer growth duration of chickpea and lentil, resulted in more pre-podding N accumulation compared with the other legumes. On the other hand, drought in 2015 reduced early N accumulation, with the reductions ranging from 25% in red pea to as high as 68% in lentil compared with 2014. In both legume and non-legume crops, N accumulation during vegetative growth has been linked to crop mass growth (Marrou et al. 2018; Koutroubas et al. 2012). In the current study, the strong correlation between early accumulation of N and dry matter in both years confirmed these findings, implying that N uptake was associated with robust growth, regardless of the growing environment. As far as the maturity stage is concerned, chickpea consistently outperformed the other legumes in

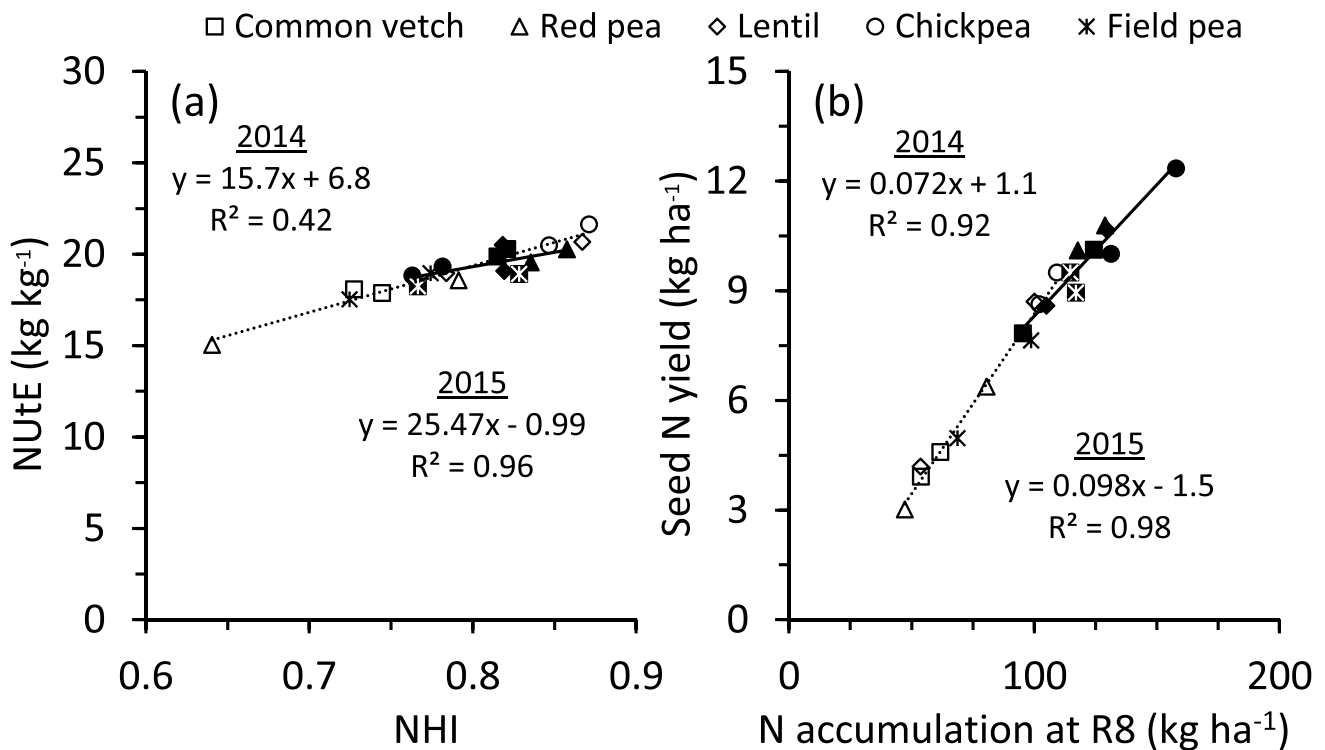


Fig. 5 Relationships between (a) nitrogen harvest index (NHI) and nitrogen utilization efficiency (NUE), and (b) between nitrogen accumulation at maturity (GS R8) and seed nitrogen yield in 2014 (black symbols, solid line) and 2015 (open symbols, dashed line). Each rela-

tionship was based on means derived from five legume species (common vetch, red pea, lentil, chickpea and field pea) and two cultivars for each species ($n = 10$)

Table 5 Nitrogen utilization efficiency of legume species as affected by cultivar and year of the experimentation

Species/cultivar	Nitrogen utilization efficiency (kg kg ⁻¹)		
	2014	2015	Mean
Common vetch			
Pigasos	20.3 ± 0.27 a	18.1 ± 0.78 b	19.2 ± 0.19 A
Tembi	19.9 ± 0.25 a	17.9 ± 0.27 b	18.9 ± 0.38 A
Red pea			
Rodos	20.5 ± 0.22 a	18.9 ± 0.94 a	19.7 ± 0.31 A
Argos	19.1 ± 0.24 a	20.7 ± 0.30 a	19.9 ± 0.56 A
Lentil			
Thessalia	19.5 ± 0.18 a	15.0 ± 0.57 b	17.3 ± 0.27 B
Samos	20.3 ± 0.48 a	18.6 ± 1.00 a	19.4 ± 0.85 A
Chickpea			
Kassos	18.8 ± 0.20 b	20.5 ± 0.44 ab	19.7 ± 0.42 A
Andros	19.3 ± 0.86 b	21.6 ± 0.35 a	20.5 ± 0.34 A
Field pea			
Vermio	18.2 ± 0.27 ab	19.0 ± 0.34 a	18.6 ± 0.20 A
Ithomi	18.9 ± 0.20 a	17.5 ± 0.55 b	18.2 ± 0.40 A

Within each species, cultivar means ± standard error in a column followed by different uppercase letter are significantly different at $P = 0.05$

Within each species, means ± standard error followed by different lowercase letter are significantly different at $P = 0.05$

terms of N accumulation, owing to increased seed N accumulation in both years.

There was a noticeable decrease of the N accumulation in aboveground vegetative parts during the seed-filling period in both years, suggesting translocation of N to seeds. It should be noted that in the present study, N translocation to seeds was attributed only to translocation from aboveground vegetative components; nevertheless, some of the seed nitrogen in legumes is derived through N translocation from roots (Pampana et al. 2016). Legumes differed in N translocation, with the differences following those of N accumulation prior to the onset of seed filling, as confirmed by the close correlation between the two measures. This finding means that the higher N accumulation at the GS R5 contributed to improved N translocation to seeds, emphasizing the importance of the pre-podding N pool in determining the extent of N translocation. Previous studies (Fotiadis et al. 2017) have demonstrated the beneficial effect of early N accumulation on the translocation of vegetative N to seeds in grain legumes, particularly those grown under Mediterranean conditions, where terminal heat or water stress are major abiotic constraints limiting rainfed crop production (Yang et al. 2020). Given that most climate change scenarios indicate drier conditions in most geographical areas worldwide

Table 6 Nitrogen harvest index (NHI), nitrogen translocation (NT), nitrogen translocation efficiency (NTE), and nitrogen utilization efficiency (NUtE) as affected by legume species and year of the experimentation

Year/Species		NHI	NT (kg ha ⁻¹)	NTE (%)	NUtE (kg kg ⁻¹)
2014					
Common vetch		0.820 ± 0.01 ab	102 ± 6.48 bc	83 ± 0.80 bc	20.1 ± 0.22 ab
Red pea		0.820 ± 0.01 ab	89 ± 8.24 cd	80 ± 1.44 bcd	19.8 ± 0.05 b
Lentil		0.845 ± 0.01 a	167 ± 17.08 a	90 ± 1.20 a	19.9 ± 0.16 b
Chickpea		0.773 ± 0.02 cd	168 ± 20.65 a	83 ± 1.69 bc	19.1 ± 0.39 bc
Field pea		0.797 ± 0.01 bc	126 ± 8.15 b	84 ± 0.34 ab	18.6 ± 0.19 cd
Mean		0.811 ± 0.01 A	130 ± 9.15 A	84 ± 0.84 A	19.5 ± 0.16 A
2015					
Common vetch		0.737 ± 0.03 de	51 ± 2.22 e	77 ± 2.30 de	18.0 ± 0.44 d
Red pea		0.822 ± 0.01 ab	70 ± 1.23 de	84 ± 0.76 ab	19.8 ± 0.51 b
Lentil		0.715 ± 0.02 e	43 ± 2.20 e	72 ± 3.10 e	16.8 ± 0.69 e
Chickpea		0.860 ± 0.01 a	68 ± 4.98 de	82 ± 2.73 bcd	21.1 ± 0.35 a
Field pea		0.752 ± 0.01 cde	73 ± 1.66 cde	78 ± 1.20 cd	18.2 ± 0.28 cd
Mean		0.777 ± 0.01 B	61 ± 2.91 B	78 ± 1.31 B	18.8 ± 0.39 A
Source of variation	df	Mean squares			
Year (Y)	1	0.011**	48,372**	286.2**	4.984
Species (S)	4	0.004**	2,428**	9.1	4.942**
Y × S	4	0.014**	3,530**	134.6**	7.768**

Within each column, means ± standard error followed by the same lowercase or uppercase letter are not significantly different at $P=0.05$

** Significant at $P=0.01$

(Masson-Delmotte et al. 2021), the findings of the current study suggest that the projected climate change is likely to cause a considerable reduction of N accumulation in legume seeds by reducing the pre-podding N pool or inhibiting the translocation of N from vegetative organs to seeds. Inter-specific differences in N translocation have also been linked to variations in NTE (Pampana et al. 2016), which is the proportion of pre-podding N that has been translocated to seeds. In the present study, except for lentil that received the highest value of 90%, no differences in NTE among species were recorded in 2014. On the contrary, species differences became pronounced under drought conditions in the 2015, with red pea exhibiting the highest NTE (84%) and lentil exhibiting the lowest NTE (72%) (Table 6). In both years, however, the variation among legumes in N translocation was due to species differences in pre-podding N rather than differences in NTE. Moreover, seed N demands were an additional factor involved in N redistribution within plants, as evidenced by the positive correlation between N translocation and seed N accumulation at maturity. Similar associations have been reported for chickpea (Davies et al. 2000) and field bean (Pampana et al. 2016). These findings demonstrate that the dynamics of seed N supply vary across grain legume species. Despite the ability of plants to fix atmospheric nitrogen, N translocation from storage organs to seeds was a key component regulating seed nutrition, irrespective of growing conditions. Furthermore, N translocation from aboveground vegetative parts was highly dependent on both

the source's ability to provide N that could potentially be translocated to seeds and the sink's N requirements.

The N utilization of legumes varied from year to year, highlighting the importance of the growing environment on the exploitation of accumulated N to produce seeds. Under the more humid environmental conditions of 2014, common vetch ranged first in terms of NUtE, with no significant differences with red pea and lentil. Drought conditions in 2015 reduced NUtE in lentil and common vetch by 16 and 10%, respectively, had no effect on red pea and field pea, and increased NUtE in chickpea by 10%, compared with 2014. Thus, during the 2015 drought, chickpea displayed the highest efficiency in utilizing N, producing almost 21 kg seeds per kg of N accumulated. The superiority of chickpea in using N to produce seeds under drought conditions was most likely due to its high adaptation to water stress. These findings are in line with those of Neugschwandtner et al. (2015), who found that chickpea maintained high N utilization efficiency under drought environments. To further understand the variation in NUtE, several physiological factors were considered. In this respect, legumes' differences in NUtE were associated with differences in the ratio of total plant N allocated to seeds at maturity, as demonstrated by the strong relationship between NUtE and NHI. This association suggests that species with greater N allocation to seeds tend to utilize the accumulated N more efficiently in seed production. Thus, genotypic or environmental variables causing an increase in NHI will likewise increase NUtE. Moreover, the contribution of NHI to the establishment of NUtE was greater

in the drier year 2015 than in 2014 ($R^2=0.42$ in 2014 and $R^2=0.96$ in 2015). These findings are consistent with prior research on spring chickpea under Mediterranean conditions, which revealed that the NHI, rather than seed dry weight generated per unit of seed N, was the limiting factor for increasing NUtE (Fotiadis et al. 2019). Furthermore, the low N concentration of vegetative parts at maturity would also be a sign of high NUtE, given the inverse relationship between the two traits.

Seed N accumulation is determined by seed dry matter and N concentration. In the present study, seed N yield variation was related with the differences in seed dry matter, as N concentration in seeds was less variable across treatments (ranging from 40 to 43 g kg⁻¹, data not shown). Thus, the higher seed N yield achieved by all legumes in 2014 was due to higher seed dry matter, because of more favorable growing conditions compared with 2015. Drought conditions in 2015 impaired seed N yield, on average, by 39% compared with 2014, with lentil suffering the greatest reduction (55%) and chickpea the least (19%). Chickpea had the highest seed N yield in both years. By this point of view, chickpea showed the highest N removal by seed harvesting (up to 112 kg ha⁻¹), a value that is slightly higher than what was reported for wheat (≈ 89 kg N ha⁻¹) in the region (Koutroubas et al. 2016). Species with high total above-ground N accumulation at maturity also had high seed N yield, that is in line with findings of previous studies (Giunta et al. 2009). On the other hand, NHI was an influential factor determining seed N yield only under drought conditions.

Within-species differences were generally less pronounced than those among species in most traits studied. The cultivar differences in NUtE for lentil and field pea during the drier year were noteworthy. By this point of view, the lentil cv. Samos and the field pea cv. Vermio should be preferred to improve nitrogen utilization in dry environments. However, because only two cultivars of each legume were used in the present study, more research with a larger group of cultivars is required to investigate within-species variation and the possibility of improving NUtE through breeding.

5 Conclusions

Legumes are ideal plants for crop rotation, yet studies until now have only considered isolated facets of nutrition and sustainability. In the present study, grain legumes differed in their patterns of nitrogen accumulation and utilization, which were also greatly influenced by environmental factors. Water stress inhibited nodulation and suppressed early nitrogen accumulation in all species studied, with chickpea maintaining a high number and mass of nodules even under drought conditions. Despite the ability of legumes to fix atmospheric nitrogen, pre-podding nitrogen accumulation was a key factor for seed nutrition through the translocation

of nitrogen from vegetative storage tissues. Nitrogen translocation was highly dependent both on the ability of the source to supply nitrogen that could potentially be translocated to seeds and on the nitrogen requirements of the sink. Chickpea demonstrated a consistently high nitrogen utilization efficiency regardless of the growing conditions, highlighting its potential role in enhancing the sustainability of agroecosystems in the semiarid Mediterranean regions. In an era where climate change is causing unstable weather, the environmental stability of chickpea is highly encouraging.

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Data Availability The data presented in this study are available on request from the corresponding author.

Declarations

Conflicts of Interest The authors declare no conflict of interest.

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References

- Bouchet AS, Laperche A, Bissuel-Belaygue C, Snowdon R, Nesi N, Stahl A (2016) Nitrogen use efficiency in rapeseed. A review. *Agron Sustain Dev* 36:1–20. <https://doi.org/10.1007/s13593-016-0371-0>
- Chen C, Miller P, Muehlbauer F, Neill KE, Wichman D, McPhee K (2006) Winter pea and lentil response to seeding date and micro- and macro-environments. *Agron J* 98:1655–1663. <https://doi.org/10.2134/agronj2006.0085>
- Davies SL, Turner LC, Palta JA, Siddique KHM, Plummer JA (2000) Remobilisation of carbon and nitrogen supports seed filling in chickpea subjected to water deficit. *Austr J Agric Res* 51:855–866. <https://doi.org/10.1071/AR00018>
- Del Pozo A, Garnier E, Aronson J (2000) Contrasted nitrogen utilization in annual C3 grass and legume crops: physiological explorations and ecological considerations. *Acta Oecol* 21:79–89. [https://doi.org/10.1016/S1146-609X\(00\)00113-2](https://doi.org/10.1016/S1146-609X(00)00113-2)

- Döring TF (2015) Grain legume cropping systems in temperate climates. In Grain legumes. Springer, New York, pp. 401–434
- Ferguson BJ, Mens C, Hastwell AH, Zhang M, Su H, Jones CH, Chu X, Gresshoff PM (2019) Legume nodulation: the host controls the party. *Plant Cell Environ* 42:41–51. <https://doi.org/10.1111/pce.13348>
- Fotiadis S, Koutroubas SD, Damalas CA (2017) Sowing date and cultivar effects on assimilate translocation in spring Mediterranean chickpea. *Agron J* 109:2011–2024. <https://doi.org/10.2134/agronj2017.01.0048>
- Fotiadis S, Koutroubas SD, Damalas CA (2019) Nitrogen utilization and yield determination of spring Mediterranean chickpea as influenced by planting date and environmental conditions. *Int J Plant Prod* 13:59–72. <https://doi.org/10.1007/s42106-018-0034-y>
- Giunta F, Pruneddu G, Motzo R (2009) Radiation interception and biomass and nitrogen accumulation in different cereal and grain legume species. *Field Crops Res* 110:76–84. <https://doi.org/10.1016/j.fcr.2008.07.003>
- Guinet M, Nicolardot B, Voisin AS (2020) Nitrogen benefits of ten legume pre-crops for wheat assessed by field measurements and modelling. *Eur J Agron* 120:126151. <https://doi.org/10.1016/j.eja.2020.126151>
- Hocking PJ, Pate JS (1977) Mobilization of minerals to developing seeds of legumes. *Ann Bot* 41:1259–1278. <https://doi.org/10.1093/oxfordjournals.aob.a085415>
- Hossain Z, Wang X, Hamel C, Gan Y (2017) Nodulation and nitrogen accumulation in pulses vary with species, cultivars, growth stages, and environments. *Canadian J Plant Sci* 98:527–542. <https://doi.org/10.1139/cjps-2017-0114>
- Hunt R, Causton DR, Shipley B, Askew AP (2002) A modern tool for classical plant growth analysis. *Ann Bot* 90:485–488. <https://doi.org/10.1093/aob/mcf214>
- Koutroubas SD, Antoniadis V, Damalas CA, Fotiadis S (2016) Effect of organic manure on wheat grain yield, nutrient accumulation, and translocation. *Agron J* 108:615–625. <https://doi.org/10.2134/agronj2015.0328>
- Koutroubas SD, Papageorgiou M, Fotiadis S (2009) Growth and nitrogen dynamics of spring chickpea genotypes in a Mediterranean-type climate. *J Agric Sci* 147:445–458. <https://doi.org/10.1017/S0021859609008739>
- Koutroubas SD, Papakosta DK, Gagianas AA (1998) The importance of early dry matter and nitrogen accumulation in soybean yield. *Eur J Agron* 9:1–10. [https://doi.org/10.1016/S1161-0301\(97\)00067-1](https://doi.org/10.1016/S1161-0301(97)00067-1)
- Koutroubas SD, Fotiadis S, Damalas CA (2012) Biomass and nitrogen accumulation and translocation in spelt (*Triticum spelta*) grown in a Mediterranean area. *Field Crops Res* 127:1–8. <https://doi.org/10.1016/j.fcr.2011.10.011>
- Koutroubas SD, Antoniadis V, Damalas CA, Fotiadis S (2023) Municipal sewage sludge effects on maize yield, nitrogen use efficiency, and soil properties. *J Soil Sci Plant Nutr* 23:1209–1221. <https://doi.org/10.1007/s42729-022-01115-0>
- López-Bellido RJ, López-Bellido L, Castillo JE, López-Bellido FJ (2004) Chickpea response to tillage and soil residual nitrogen in a continuous rotation with wheat: II. Soil nitrate, N uptake and influence on wheat yield. *Field Crops Res* 88:201–210. <https://doi.org/10.1016/j.fcr.2004.01.012>
- Marrou H, Ricaurte JJ, Ghanem ME, Clavijo Michelangeli JA, Ghaouti L, Rao IM, Sinclair TR (2018) Is nitrogen accumulation in grain legumes responsive to growth or ontogeny? *Physiol Plant* 162:109–122. <https://doi.org/10.1111/pp1.12617>
- Masson-Delmotte V, Zhai P, Pirani A, Connors S, Péan C, Berger S, Caud N, Chen Y, Goldfarb L, Gomis M, Huang M, Leitzell K, Lonnoy E, Matthews J, Maycock T, Waterfield T, Yelekçi O, Yu R, Zhou B (2021) IPCC, 2021: summary for policymakers. *Climate Change 2021: the physical science basis. contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change*. https://www.ipcc.ch/report/ar6/wg1/downloads/report/IPCC_AR6_WGI_SPM_final.pdf. Accessed 24 May 2023
- McCauley AM, Jones CA, Miller PR, Burgess MH, Zabinski CA (2012) Nitrogen fixation by pea and lentil green manures in a semi-arid agroecoregion: effect of planting and termination timing. *Nut Cycl Agroecosys* 92:305–314. <https://doi.org/10.1007/s10705-012-9491-3>
- MSTAT-C (1989) MSTAT-C Statistical Program Version 2.10. Crop and Soil Science Department, Michigan State University, USA
- Nehe AS, Misra S, Murchie EH, Chinnathambi K, Tyagi BS, Foulkes MJ (2020) Nitrogen partitioning and remobilization in relation to leaf senescence, grain yield and protein concentration in Indian wheat cultivars. *Field Crops Res* 251:107778. <https://doi.org/10.1016/j.fcr.2020.107778>
- Nelson DW, Sommers LE (1973) Determination of total nitrogen in plant material. *Agron J* 65:109–111. <https://doi.org/10.2134/agronj1973.00021962006500010033x>
- Neugschwandtner RW, Wagentrift H, Kaul HP (2015) Nitrogen yield and nitrogen use of chickpea compared to pea, barley and oat in Central Europe. *Int J Plant Prod* 9:291–304. <https://doi.org/10.22069/IJPP.2015.2049>
- Oladzad A, González A, Macchiavelli R, de Jensen CE, Beaver J, Porch T, McClean P (2020) Genetic factors associated with nodulation and nitrogen derived from atmosphere in a Middle American Common bean panel. *Front Plant Sci* 11:576078. <https://doi.org/10.3389/fpls.2020.576078>
- Palmero F, Fernandez JA, Garcia FO, Haro RJ, Prasad PV, Salvaggiotti F, Ciampitti IA (2022) A quantitative review into the contributions of biological nitrogen fixation to agricultural systems by grain legumes. *Eur J Agron* 136:126514. <https://doi.org/10.1016/j.eja.2022.126514>
- Pampana S, Masoni A, Arduini I (2016) Grain legumes differ in nitrogen accumulation and remobilisation during seed filling. *Acta Agric Scandinavica B - Soil Plant Sci* 66:127–132. <https://doi.org/10.1080/09064710.2015.1080854>
- Pampana S, Masoni A, Mariotti M, Ercoli L, Arduini I (2018) Nitrogen fixation of grain legumes differs in response to nitrogen fertilisation. *Exp Agric* 54:66–82. <https://doi.org/10.1017/S0014479716000685>
- Preissel S, Reckling M, Schläpke N, Zander P (2015) Magnitude and farm-economic value of grain legume pre-crop benefits in Europe: a review. *Field Crops Res* 175:64–79. <https://doi.org/10.1016/j.fcr.2015.01.012>
- Salon C, Munier-Jolain NG, Duc G, Voisin AS, Grandgirard D, Larmure A, Emery RJN, Ney B (2001) Grain legume seed filling in relation to nitrogen acquisition: a review and prospects with particular reference to pea. *Agronomie* 21:539–552. <https://doi.org/10.1051/agro:2001143>
- Turner NC, Davies SL, Plummer JA, Siddique KHM (2005) Seed filling in grain legumes under water deficits, with emphasis on chickpeas. *Adv Agron* 87:211–250. [https://doi.org/10.1016/S0065-2113\(05\)87005-1](https://doi.org/10.1016/S0065-2113(05)87005-1)
- Virk AL, Lin BJ, Kan ZR, Qi JY, Dang YP, Lal R, Zhao X, Zhang HL (2022) Simultaneous effects of legume cultivation on carbon and nitrogen accumulation in soil. *Adv Agron*. <https://doi.org/10.1016/bs.agron.2021.08.002>
- Walley FL, Clayton GW, Miller PR et al (2007) Nitrogen economy of pulse crop production in the northern great plains. *Agron J* 99:1710–1718. <https://doi.org/10.2134/agronj2006.0314s>
- Yang C, Fraga H, van Ieperen W, Santos JA (2020) Assessing the impacts of recent-past climatic constraints on potential wheat yield and adaptation options under Mediterranean climate in southern Portugal. *Agric Syst* 182:102844. <https://doi.org/10.1016/j.agry.2020.102844>
- Zdruli P, Jones RJA, Montanarella L (2004) Organic Matter in the Soils of Southern Europe. European Soil Bureau Technical Report, EUR 21083 EN, 16pp. Office for Official Publications of the European Communities, Luxembourg