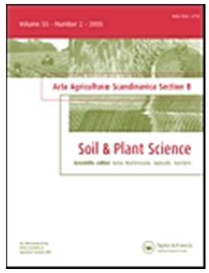

Grain legumes differ in nitrogen accumulation and remobilisation during seed filling

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ORIGINAL ARTICLE

Grain legumes differ in nitrogen accumulation and remobilisation during seed filling

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ORIGINAL ARTICLE

Grain legumes differ in nitrogen accumulation and remobilisation during seed filling

Abstract

Grain legumes are important crops, which are grown worldwide primarily for their high seed protein content, they also release nitrogen into the soil because of their N_2-fixing capacity and enhance yields of subsequent crops. In grain legumes, the N requirements of growing seeds are generally greater than BNF and soil N uptake during seed filling, so that the N previously accumulated in the vegetative tissues needs to be redistributed in order to provide N to the seeds. The differences in N remobilisation among grain legume crops can also be attributed to differences in their N_2 fixation efficiency after flowering.

Four grain legumes [chickpea (*Cicer arietinum* L.), field bean (*Vicia faba* L. var. *minor*), pea (*Pisum sativum* L.), and white lupin (*Lupinus albus* L.)] were grown in soil inside growth boxes for two cropping seasons and harvested twice (full flowering and physiological maturity). The aim was to compare the relative contribution of BNF, soil N uptake and N remobilisation to seed N. The seed N content at maturity was higher than total N accumulation during grain filling in all the four crops and endogenous N previously accumulated in vegetative parts was remobilised to fulfil this demand. N remobilisation from vegetative parts occurred in all four crops, but was crucial in providing N to the seeds of chickpea, pea and white lupin (half of seed N content) although it was less important in field bean (only one third). All the vegetative organs of the legume plants underwent N remobilisation: in all crops shoots were the major contributors to the N supply of seeds but in field bean roots were also important.

Keywords: biological nitrogen fixation; chickpea, field bean, pea, remobilisation, white lupin

Introduction

Grain legumes are important crops which are grown worldwide primarily for their high seed protein content and are used as human food, feed for animals, and industrial demands (Gulmezoglu & Kayan 2011). In addition to being important food crops, grain legumes also release nitrogen into the soil because of their N_2-fixing capacity and enhance yields of subsequent crops, playing an important role in cereal cropping systems. The accumulation of nitrogen in legume plants depends on the N fixed by biological nitrogen fixation (BNF) and on the N assimilated from the soil. However in grain legumes, the N requirements of growing seeds are generally greater than BNF and soil N uptake during seed filling, so that the N previously accumulated in the vegetative tissues needs to be redistributed in order to provide N to the seeds (Salon et al. 2001; Schiltz et al. 2005). Remobilisation of nutrients such as N and P from vegetative tissues to reproductive organs plays an important role for legume grain yield. The extent of the contribution of N remobilisation to seed N yield varies markedly among legumes, ranging from 0% to 90% because of genotype and environmental factors (Kurdali 1996; Davies 2000; Schiltz et al. 2005; Soltani et al. 2006). Kumarasinghe et al. (1992) hypothesised that in bean plants, N translocation and BNF during grain filling are complementary. They suggested that when N_2 fixation was high enough to satisfy virtually all the N demand of the rapidly filling seeds, the remobilisation of N from the vegetative tissues was not necessary. Thus the differences in N remobilisation among grain legume crops can also be attributed to differences in their N_2 fixation efficiency after flowering.

According to Salon et al. (2001), all vegetative organs undergo N remobilisation, however the efficiency with which it can be transferred to growing seeds and the rate of

N remobilisation are higher in leaves and stems than in roots, although little research has been carried out on the N remobilisation from roots (Van Kessel 1994).

The aim of this study was to assess the complementarity between N remobilisation and BNF during seed filling in four grain legumes by comparing the relative contribution to seed N by BNF, soil N uptake, and N remobilisation from vegetative aerial part and roots. The crops used were chickpea (*Cicer arietinum* L.), field bean (*Vicia faba* L. var. *minor*), pea (*Pisum sativum* L.), and white lupin (*Lupinus albus* L.), which are some of the most commonly cultivated grain legumes.

Materials and methods

The research was carried out in 2012 and in 2013, at the Research Centre of the Department of Agriculture, Food and Environment of the University of Pisa, Italy, which is located approximately 4 km from the sea (43° 40′ N, 10° 19′ E) and 1 m above sea level. The climate of the area is hot-summer Mediterranean (Csa) with a mean air temperature of 14.9 °C and a mean rainfall of 971 mm.

In both years, experimental treatments consisted of four legume crops and two harvesting stages. The legumes were chickpea (cv. "Pascia"), field bean (cv. "Chiaro di Torrelama"), pea (cv. "Iceberg"), and white lupin (cv "Multitalia"). The harvesting stages were full flowering and physiological maturity. Flowering was defined when plants had more than one node with flowers open and one node with one pod set (Knott 1987-1990). Durum wheat (*Triticum durum* L. "Claudio") was used as a non-fixing reference crop (RC) to determine plant-available soil N and to estimate biological nitrogen fixation. Harvest times of durum wheat were the same as legume crops.

In both years the research was carried out in an open-air facility consisting of 48 growth boxes (24 for legume crops and 24 for durum wheat) of 300-L volume (0.50 m^2 and 0.6 m depth), spaced 20 cm apart, and embedded in expanded clay to prevent daily fluctuations in soil temperature. In both years, approximately six months before seeding, growth boxes were filled with soil collected from a field previously cultivated with rapeseed.

Differences in soil properties for the two years were negligible, and the averaged soil properties were: 74.4% sand (2>Ø>0.05 mm), 20.2% silt (0.05>Ø>0.002 mm), 5.4% clay (Ø<0.002 mm), 8.0 pH, 1.3% organic matter (Walkley and Black method), 0.5 g/kg total nitrogen (Kjeldahl method), 8.8 mg/kg available P (Olsen method), 72.4 mg/kg available K ($BaCl_2$-TEA method), and 0.4 mg/kg NO_3-N.

All the legume crops and durum wheat were sown on 14 February 2012 and on 4 February 2013. Just prior to sowing, field bean and pea seeds were inoculated with *Rhizobium leguminosarum* biovar. *viciae*, chickpea seeds with *Bradyrhizobium* sp. (cicer) and white lupin seeds with *Bradyrhizobium lupinus*. The seeding rate was 32 seeds m^{-2} for chickpea, 56 seeds m^{-2} for field bean and pea and 40 seeds m^{-2} for white lupin. For all legume crops a 30-cm row spacing was used. The seeding rate of durum wheat was 400 seeds m^{-2} with a 16-cm row spacing. All legume crops and durum wheat were fertilised pre-planting with urea, triple mineral phosphate and potassium sulphate, at rates of 30 kg ha^{-1} of N, 65 kg ha^{-1} of P and 125 kg ha^{-1} of K. In both years all the crops were irrigated from flowering to maturity to prevent water stress. A total of 100 mm of water was applied in May and June. The crops were kept free of weeds by hand hoeing when necessary.

Flowering occurred between 7-14 May 2012 and 9-17 May 2013, and physiological maturity occurred between 22 June - 9 July 2012 and between 1 - 22 July 2013. At both stages, plants from each box were cut at ground level and were partitioned into leaf + stem, pod wall and seed. All dead leaves were collected. Roots from each box were separated from the soil by gently washing with a low flow from sprinklers. They were then immersed in a 10% sodium hexametaphosphate and sodium bicarbonate dispersant solution for 18 h. Nodules were separated from roots; total root fresh weight was recorded and taproots and rootlets were separated and weighed. The dry weight of all plant parts was determined by oven-drying at 60 °C to constant weight, and samples were analysed for N concentration by the micro Kjeldahl method. Nitrogen content was obtained by multiplying N concentration by dry weight.

The two most commonly used methods for estimating N_2 fixation during the growth period are ^{15}N-isotope dilution and N difference. With N difference method, the total N content of the non-fixing crop is subtracted from the total N content of the N_2-fixing legume. The two methods deliver the same results when comparing BNF in soil with a low N concentration (Herridge et al. 2008). Thus, the N_2 fixation was estimated using the N difference method as improved by Evans and Taylor (1987): [N yield (legume) - N yield (RC)] + [N soil (legume at harvest) - N soil (RC at harvest)].

We assumed that all N lost from vegetative parts between flowering and maturity was translocated to the seeds, thus N apparent remobilisation was calculated as the difference between total plant N content at flowering and total N content of vegetative plant parts (leaf + stem + pod wall + root + nodule) at maturity (Koutroubas et al. 2009).

In both years, the experiment was arranged in a split plot design with four main plot treatments (four legume crops) and two subplot treatments (two stages) with three replicates. Significantly different means were separated at the 0.05 probability level by the least significant difference test (Steel et al. 1997).

Results and discussion

There were no significant differences between years in all growth variables or interaction "Year x Crop x Stage", "Year x Crop", and "Year x Stage". This is because differences in temperature between the two years were negligible and crops were irrigated when necessary. Accordingly, only interaction "Crop x Stage" is reported.

From flowering to maturity, shoot dry matter (stems + leaves + pod walls) statistically increased in all crops by approximately 50%, root dry matter did not change appreciably, and nodule dry matter decreased by approximately 18% (Figure 1). Field bean was the most productive in seeds (577 g m^{-2}) followed by pea (487 g m^{-2}), chickpea (397 g m^{-2}), and white lupin (379 g m^{-2}).

During seed filling, the amount of plant N increased in all four crops, thus confirming that they are able to maintain N acquisition during reproductive development. However, the extent of the increase differed markedly among crops and in field bean (17 g m^{-2}) was about twice the amount than in chickpea, pea, and white lupin (approximately 8 g m^{-2}). In all crops the increase was entirely due to seeds, whose N content at maturity was 26 g m^{-2} in field bean and approximately 16 g m^{-2} in chickpea, pea, and white lupin. At maturity, seeds contained between 55% and 70% of total plant N, although they made up only from 25% to 44% of the total dry matter.

Nitrogen concentration and content of vegetative plant parts drastically decreased during seed filling in all legume crops. The decrease in N concentration was higher in nodules (from 5.6 to 3.1 g kg^{-1}) and shoots (from 3.0 to 1.2 g kg^{-1}) than in roots (from 1.8 to 1.0 g kg^{-1}) without statistical differences among crops. In all species the decrease in N content was higher in shoots than in roots and in nodules (Figure 2). In white lupin, the decreases in both shoot and root N content were statistically higher than in the other three crops. The decrease in nodule N content did not differ among species.

From flowering to maturity, BNF represented between 83% and 90% of the exogenous N in all legume crops. However, the amount of N fixed during seed filling differed markedly among legumes and in field bean was more than twice the amount than in pea and white lupin, and three times the amount than in chickpea (Figure 3). Competition for carbon by developing pods and seeds is likely to result in decreased C supply to the Rhizobia bacteria, resulting in the decrease in N fixation which occurs during early flowering (Hooda et al. 1989; Kurdali 1996). Herridge and Pate (1977) reported that at the time of seed filling, the ability of the nodules of grain legumes to fix N decreases, because the plant feeds the developing seed rather than the nodule, and BNF decreases. We found that during seed filling, BNF was very important for N acquisition by the plants, representing 26% of total BNF in chickpea, 35% in pea and white lupin, and 42% in field bean.

The demand for N by developing seeds is known to be high, and in our study, the N content of the seeds at maturity was higher than total N accumulation during seed filling in all four crops. Accordingly, soil N uptake and BNF were not able to sustain the demand for N of filling seeds, and endogenous N previously accumulated in vegetative parts was remobilised to fulfil this demand. Despite the great differences among legumes in grain yield, grain N content and total N content at flowering and maturity, nitrogen remobilisation during seed filling differed little among species ranging from 7.1 g m^{-2} in chickpea to 8.9 g m^{-2} in field bean. However, the importance of N remobilisation for N economy in the plants differed markedly among legumes. Indeed, N remobilised was equal to BNF in white lupin, was about 23% higher in chickpea and pea, and was 50% lower in field bean.

Koutroubas et al. (2009) found that most of the variation in N remobilisation in chickpea could be accounted for by the differences in total N content at the beginning of seed growth. This finding is likely for varieties within the same species, but not among different crops. In our research the total N content of field bean at flowering was higher than that of pea, while N remobilisation was similar. This is because legume crops differed in their N remobilisation efficiency. Field bean had the highest N content at flowering, but was also the species with the lowest N remobilisation efficiency (35%) during seed filling, while pea had the lowest N content at flowering but the highest N remobilisation efficiency (54%).

Davies et al. (2000) hypothesised that a higher pod number and seed yield could be indicative of a higher requirement for seed N, and that the ability to remobilise high pre-podding N may be related to this N sink demand. In our research this hypothesis was confirmed in field bean, which was the most productive plant both in terms of seed and N yield, and had the highest N remobilisation, but not for pea which had a higher seed yield than white lupin but the same N remobilisation.

Our results confirm that the vegetative parts of plants are important sources of N for seed development during periods when N requirements exceed those provided by soil and N_2 fixation (Zapata et al. 1987; Kurdali et al. 1997). Remobilised N was crucial to provide N to the seeds of chickpea, pea and white lupin for which N remobilisation supplied half of the seed N content at maturity but was less important in field bean where it contributed only a third (Figure 4). All the vegetative organs of the legume plants underwent N remobilisation (Figure 2), however the N remobilisation efficiency varied among the organs. In chickpea and pea, shoots and roots remobilised the same percentage of the N allocated at flowering, while in field bean, the N allocated in shoots remobilised less than that in roots (30% and 47%), and in white lupin, the remobilised N was higher in shoots than in roots (46% and 28%). Finally, nodule N content at flowering was remobilised by 51-57% in all crops. However, owing to the different N content at flowering, shoots were the major contributors to the N supply of seeds for all four legume crops (Figure 5) although their relative importance ranged from 85% in white lupin to 70% in chickpea and pea, to only 58% in field bean. The N contribution of roots to N seeds was only 11% in white lupin and 23% in chickpea and pea but up to 37% in field bean. Finally, nodules overall contributed less than 8%.

Conclusions

Our results highlight that the vegetative parts of grain legume crops are important sources of N for seed development during periods when N requirements exceed those provided by soil and N_2 fixation. In addition, the results show that remobilised N and endogenous N had the same impact in terms of N supply to seeds in chickpea, pea and white lupin, while in field bean the exogenous N was more important than endogenous. Field bean maintained the highest BNF during seed filling and had the lowest N remobilisation. This thus suggests that BNF is the primary source of N supply to seeds, and that N remobilisation is used to integrate BNF in order to fulfil the N demand of seeds. All vegetative organs underwent N remobilisation, however owing to the different N contents at flowering; shoots were the largest contributors to the N supply of seeds in all crops. Roots were also involved while the contribution of nodules was almost negligible.

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Figure captions

Figure 1. Shoot, root and nodule dry matter at flowering and maturity. Interaction "crop x stage". Vertical bars denote LSD at $P \leq 0.05$.

Figure 2. Shoot, root and nodule N content at flowering and maturity. Interaction "crop x stage". Vertical bars denote LSD at $P \leq 0.05$.

Figure 3. Biological nitrogen fixation, N remobilisation, and N soil uptake during seed filling. Vertical bars denote LSD at $P \leq 0.05$.

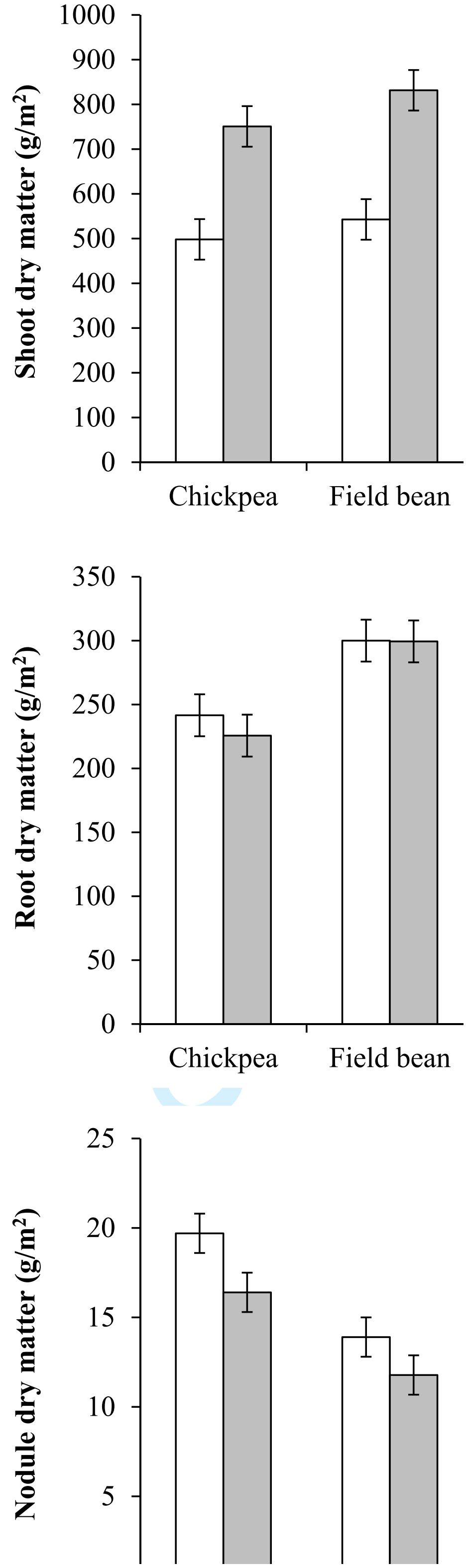
Figure 4. Relative contribution of BNF, N remobilisation, and N soil uptake to seed N content. Vertical bars denote LSD at $P \leq 0.05$.

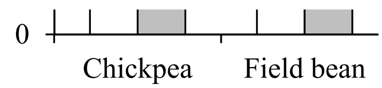
Figure 5. Relative contribution of shoot, root, and nodule to N remobilised to seeds. Vertical bars denote LSD at $P \leq 0.05$.

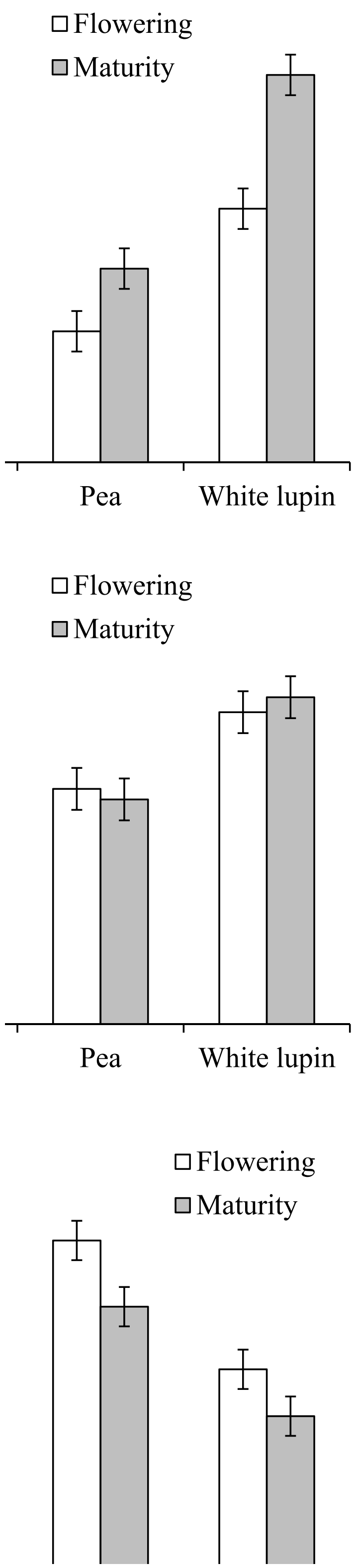
Dry matter

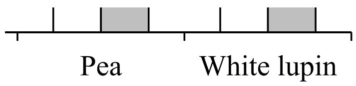
	Shoot		Root	
	Flowering	Maturity	Flowering	Maturity
	g/m2	g/m2	g/m2	g/m2
Chickpea	498	751	242	226
Field bean	543	832	300	299
Pea	293	433	184	176
White lupin	567	866	244	256

Nodule	
Flowering	Maturity
g/m2	g/m2
20	16
14	12
19	16
12	9




0
Chickpea
Field bean

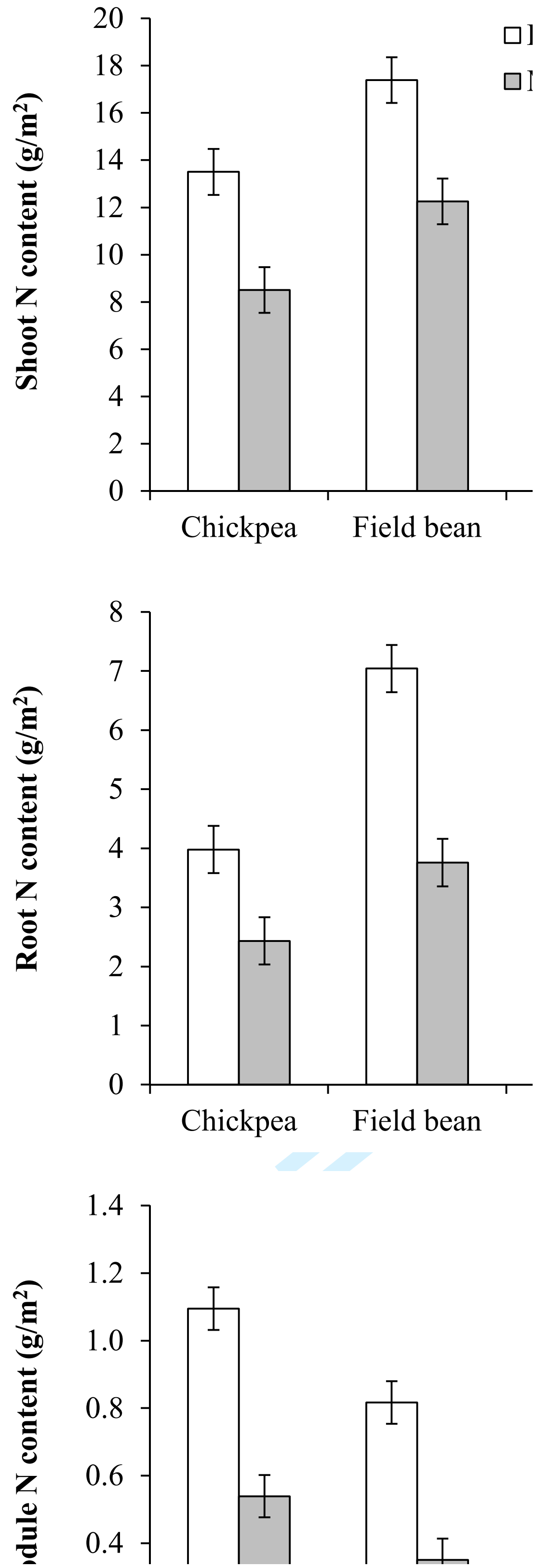

Flowering
Maturity
Pea
White lupin
Flowering
Maturity
Pea
White lupin
Flowering
Maturity

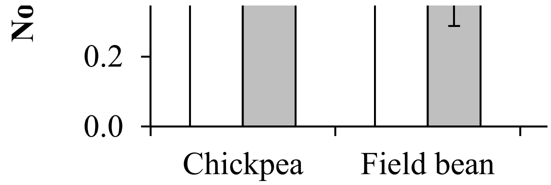

Pea
White lupin

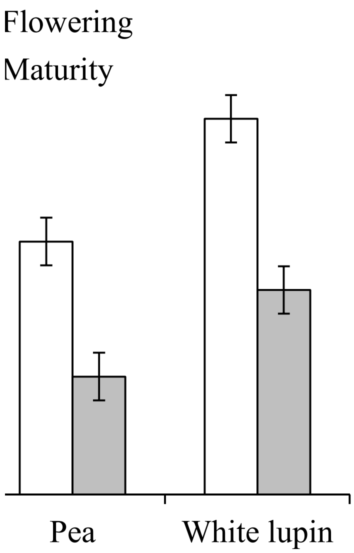
Nitrogen content

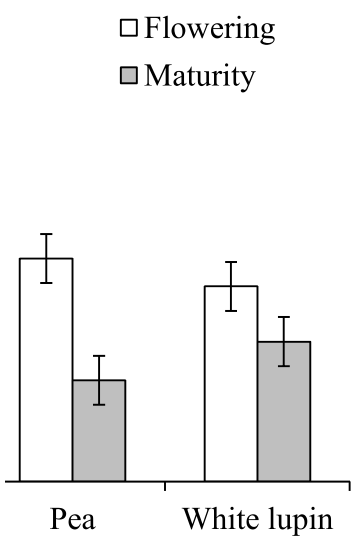
	Shoot		Root		Noc
	Flowering	Maturity	Flowering	Maturity	Flowering
	g/m2	g/m2	g/m2	g/m2	g/m2
Chickpea	14	9	4	2	1.1
Field bean	17	12	7	4	0.8
Pea	10	5	4	2	1.0
White lupin	15	8	3	2	0.7

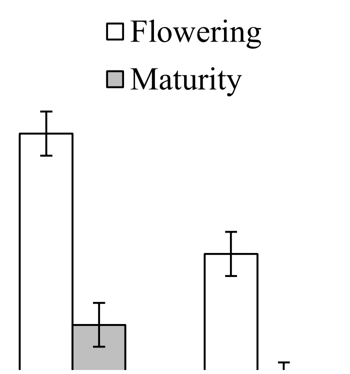
dule
Maturity
g/m2
0.5
0.4
0.5
0.3

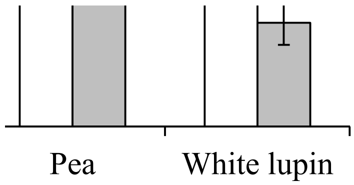



No
0.2
0.0
Chickpea
Field bean


Flowering
Maturity
Pea
White lupin

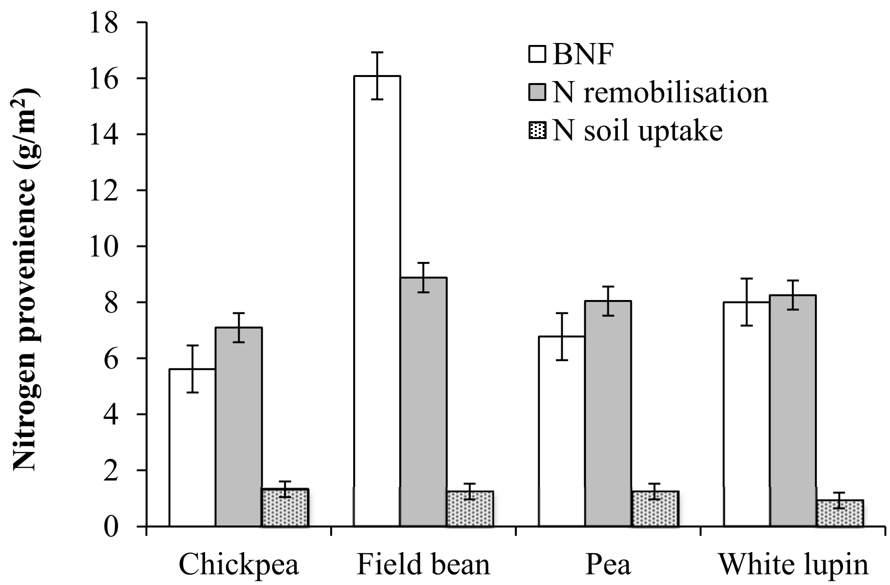

Flowering
Maturity
Pea
White lupin


Flowering
Maturity


Pea
White lupin

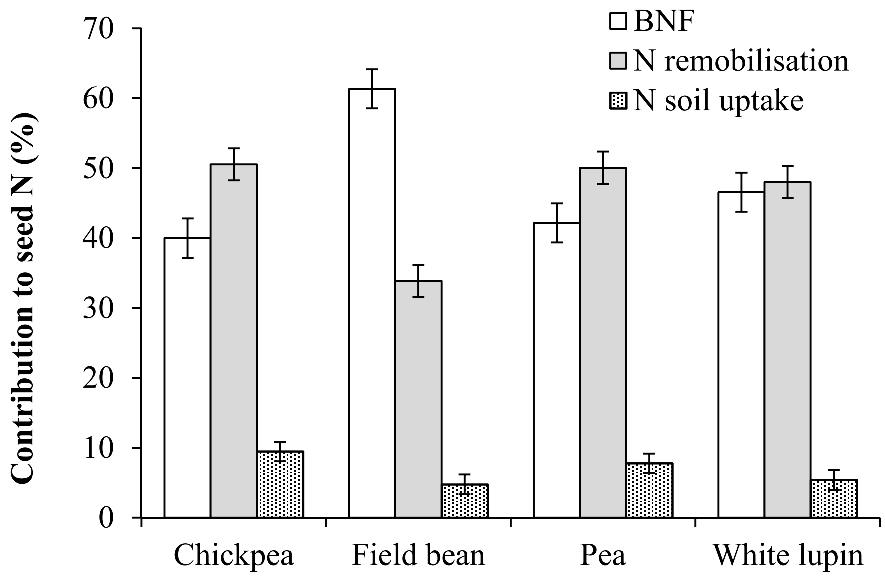
N during seed filling

	BNF	N remobilisation	N soil uptake
	g/m2	g/m2	g/m2
Chickpea	6	7	1
Field bean	16	9	1
Pea	7	8	1
White lupin	8	8	1


Nitrogen provenience (g/m²)
18
16
14
12
10
8
6
4
2
0
BNF
N remobilisation
N soil uptake
Chickpea
Field bean
Pea
White lupin

N in seeds

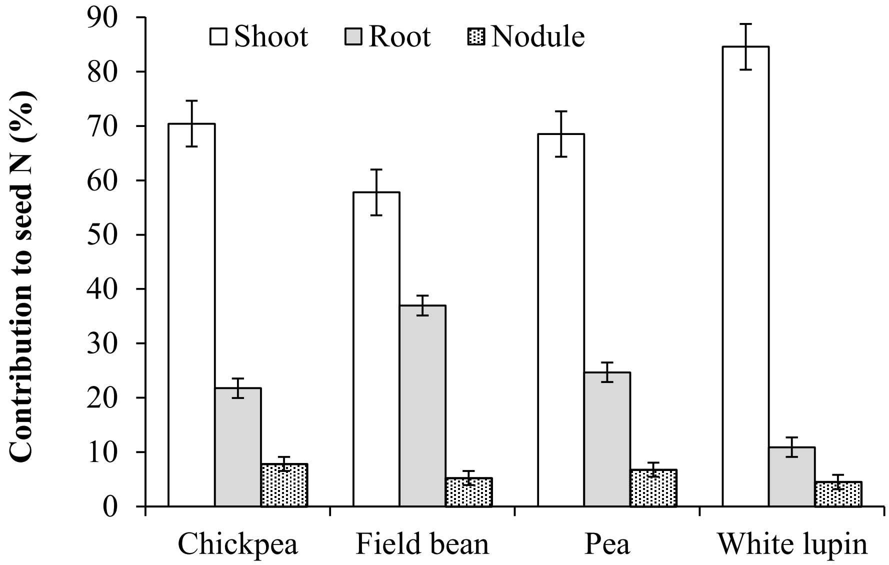
	BNF	N remobilisation	N soil uptake
	%	%	%
Chickpea	40	51	9
Field bean	61	34	5
Pea	42	50	8
White lupin	47	48	5

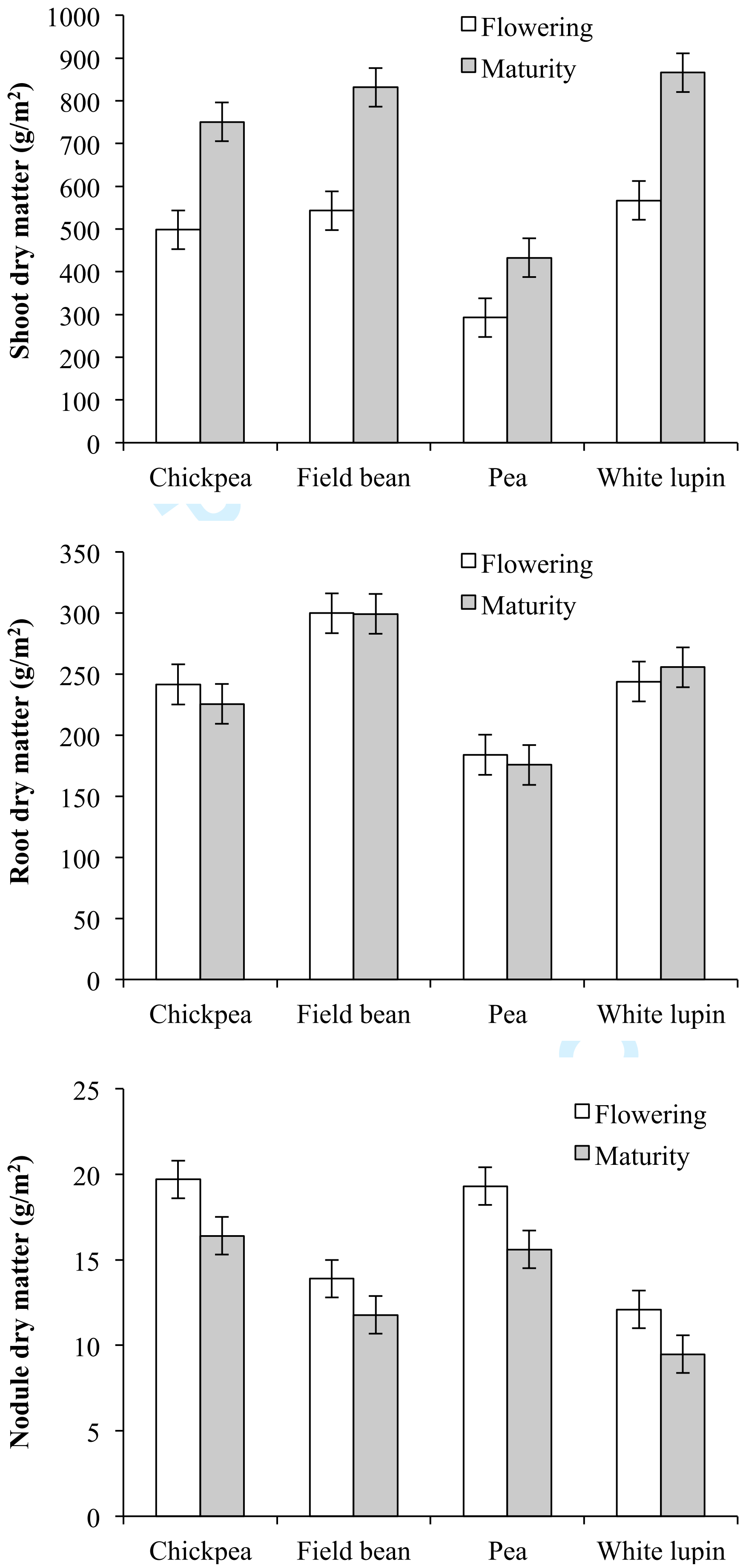


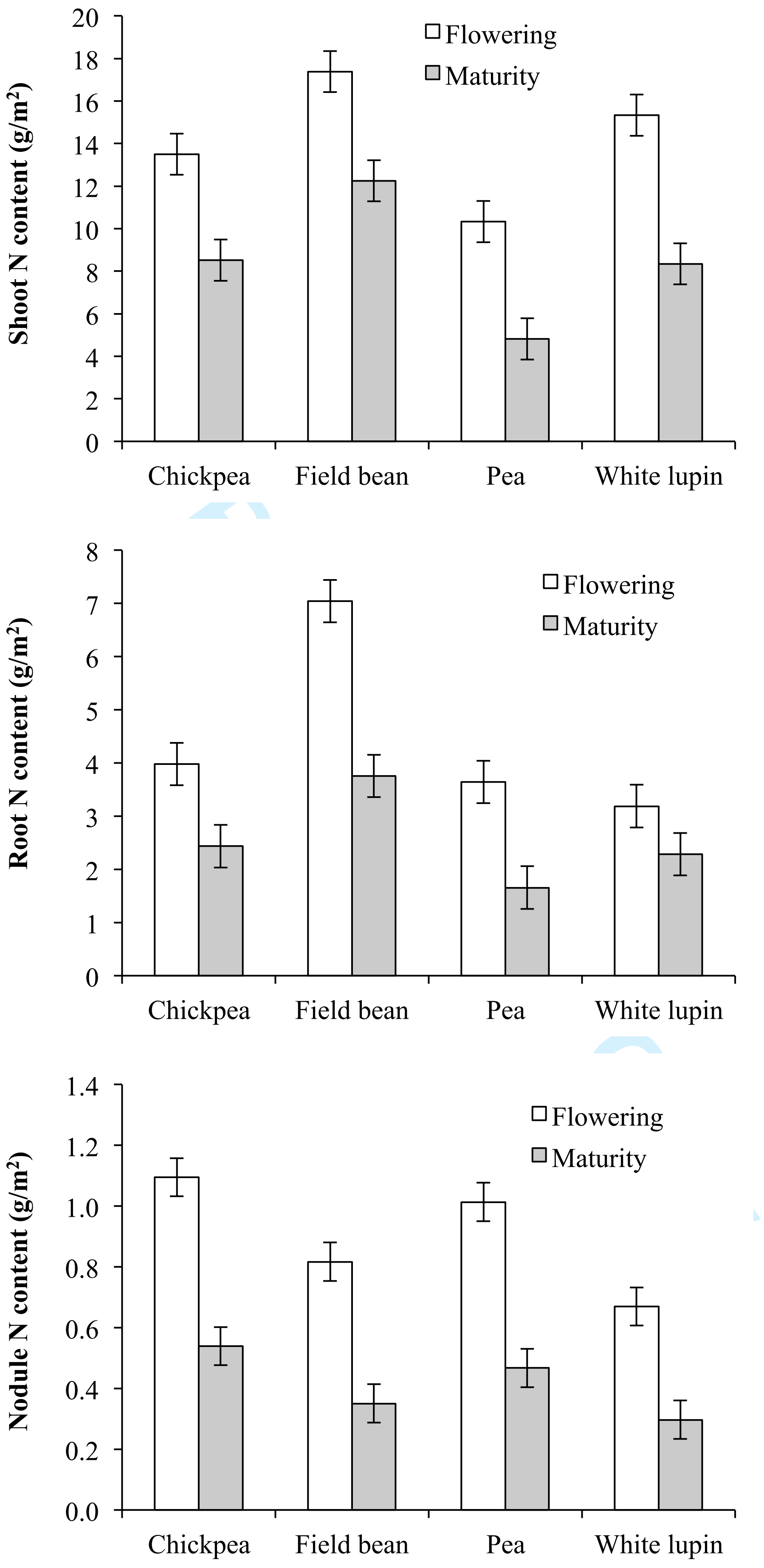
BNF
N remobilisation
N soil uptake
Contribution to seed N (%)
0
10
20
30
40
50
60
70
Chickpea
Field bean
Pea
White lupin

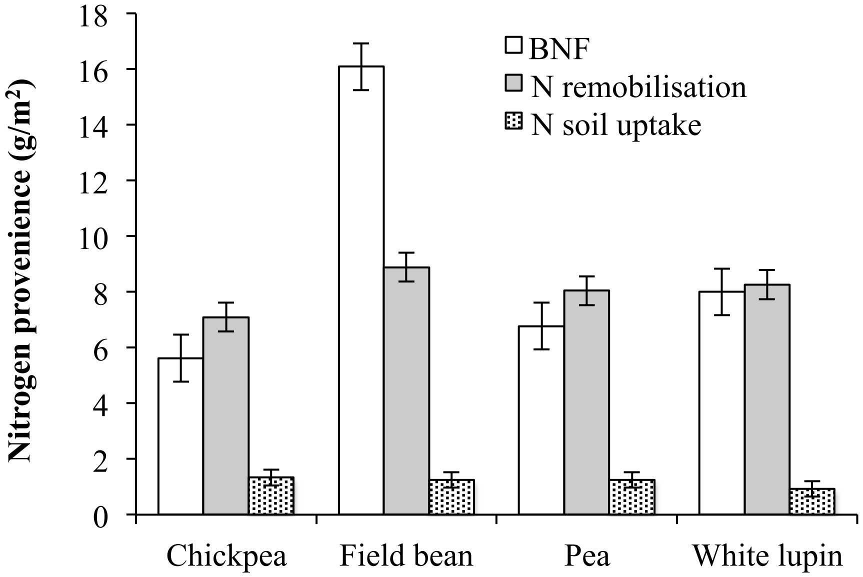
Contribution to seed N

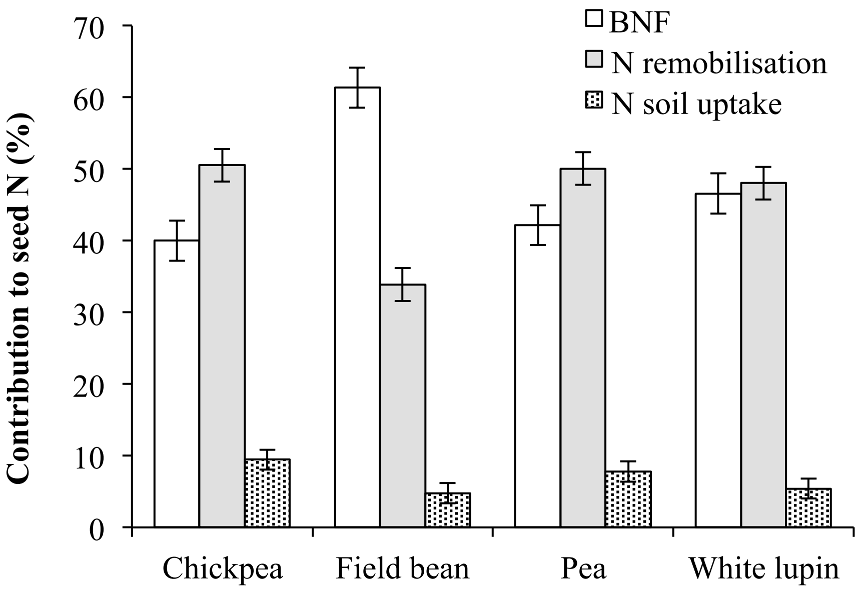
	Shoot %	Root %	Nodule %
Chickpea	70	22	8
Field bean	58	37	5
Pea	69	25	7
White lupin	85	11	5

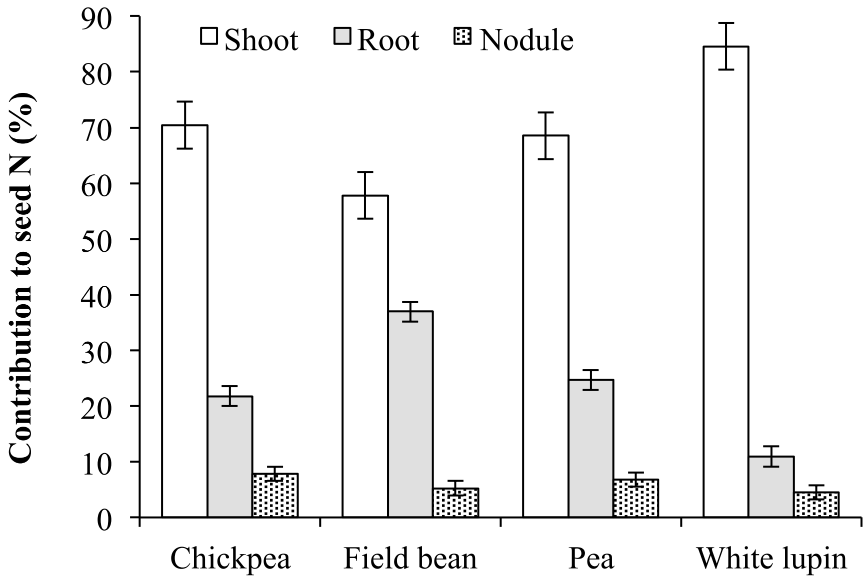

Shoot
Root
Nodule
Contribution to seed N (%)
90
80
70
60
50
40
30
20
10
0
Chickpea
Field bean
Pea
White lupin


Shoot dry matter (g/m^2)
1000
900
800
700
600
500
400
300
200
100
0
Flowering
Maturity
Chickpea
Field bean
Pea
White lupin
Root dry matter (g/m^2)
350
300
250
200
150
100
50
0
Flowering
Maturity
Chickpea
Field bean
Pea
White lupin
Nodule dry matter (g/m^2)
25
20
15
10
5
0
Flowering
Maturity
Chickpea
Field bean
Pea
White lupin


Shoot N content (g/m²)
20
18
16
14
12
10
8
6
4
2
0
Flowering
Maturity
Chickpea
Field bean
Pea
White lupin
Root N content (g/m²)
8
7
6
5
4
3
2
1
0
Flowering
Maturity
Chickpea
Field bean
Pea
White lupin
Nodule N content (g/m²)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Flowering
Maturity
Chickpea
Field bean
Pea
White lupin


Nitrogen provenience (g/m²)
0
2
4
6
8
10
12
14
16
18
BNF
N remobilisation
N soil uptake
Chickpea
Field bean
Pea
White lupin


BNF
N remobilisation
N soil uptake
Contribution to seed N (%)
70
60
50
40
30
20
10
0
Chickpea
Field bean
Pea
White lupin


Shoot
Root
Nodule
Contribution to seed N (%)
0
10
20
30
40
50
60
70
80
90
Chickpea
Field bean
Pea
White lupin