Article

Nitrogen Yields and Biological Nitrogen Fixation of Winter Grain Legumes

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Abstract: Grain legumes are valuable sources of protein and contribute to the diversification and sustainability of agricultural systems. Shifting the sowing date from spring to autumn is a strategy to address low yields of spring grain legumes under conditions of climate change. A two-year field experiment was conducted under Pannonian climate conditions in eastern Austria to assess the nitrogen yield and biological N₂ fixation of winter peas and winter faba beans compared to their spring forms. The grain nitrogen yields of winter peas and winter faba beans were 1.83-fold and 1.35-fold higher compared to their spring forms, respectively, with a higher value for winter peas. This was mainly due to higher grain yields of winter legumes, as winter faba beans had a 1.06-fold higher grain nitrogen concentration than spring faba bean. Soil mineral nitrate after harvest was similar for all grain legumes, with by 2.85- and 2.92-fold higher values for peas and faba beans than for cereals, respectively. The N₂ fixation of winter peas and winter faba beans were 3.90-fold and 2.28-fold higher compared to their spring forms, with winter peas having a 1.60-fold higher N₂ fixation than winter faba beans. The negative nitrogen balance of winter peas was smaller than that of winter faba beans as they demonstrated the ability to overcompensate for higher nitrogen removal with grain through higher N₂ fixation. The cultivation of winter grain legumes, especially winter peas, can be recommended under Pannonian climate conditions as they achieve high nitrogen yields and high levels of N₂ fixation.

Keywords: *Pisum sativum*; *Vicia faba*; nitrogen fixation; autumn-sowing; spring-sowing

1. Introduction

Grain legumes, such as pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.), are valuable sources of protein and energy for animals; thus, they have been discussed as an alternative to soybean meal [1]. Currently there is a great deficit of about 70% of high-protein feed for livestock in Europe, which is met by imported soybean and soymeal [2]. To reduce this deficit, grain legume production in Europe has to be increased, e.g., by introducing more drought resistant grain legume species, such as chickpea [3], and by using winter grain legumes [4,5].

Grain legumes contribute to the diversification and long-term productivity of sustainable agricultural systems as they can satisfy the bulk of their nitrogen (N) demand from atmospheric nitrogen through symbiosis with nitrogen fixing soil bacteria (*Rhizobium* spp.), thereby minimizing the demand for N fertilizer inputs within crop rotations [6]. Biological nitrogen fixation (N_{FIX}) plays a critical role in crop production; almost 20% of the worldwide N demand for grain and oilseed crop production is supplied by N_{FIX} [7]. Agronomic management offers opportunities for increased N_{FIX} , e.g., by supporting higher yields through irrigation, phosphate and sulfur fertilization, liming for optimal soil pH, weed control, reducing row spacing and optimizing plant density or sowing dates [8–10].

Shifting from spring to autumn sowing is a strategy for arable farming to address climate change, which in Central Europe is expected to cause higher year-round temperatures and a shift in rainfall patterns towards more rainfall in winter and early spring but less in summer [11]. In Central Europe, grain legumes are commonly sown in the spring. Successful cultivation of both winter peas and winter faba beans has been reported in Austria, Germany and Switzerland [4,5,12–14], while it is a tradition in European regions with less frost, such as France or Spain [15,16].

Winter crops, as compared to spring crops, are usually ready for harvest earlier [4,17,18], therefore, they can escape terminal drought in stressful environments [19]. Their yield stability tends to be higher due to their longer growing period and lower dependence on water availability during spring [20]. In particular, this is an argument for the autumn-sowing of grain legumes, as yields of grain legumes are significantly more variable than non-legume yields in Europe [21].

Diverse results have been reported for winter grain legumes in Austria. No advantage in grain yield or N_{FIX} of winter faba beans compared to spring faba bean has been observed due to limited winter survival and the limited compensation mechanisms of yield components [18]. Whereas in another two-year trial with high winter survival, both winter peas and winter faba beans had higher grain yields than their corresponding spring forms [4].

Knowledge of the nitrogen yields and N_{FIX} of winter pea and winter faba bean as well as of both winter grain legumes compared to their spring forms is scarce for Central European cropping systems. Therefore, this study analyzed (i) nitrogen concentrations, (ii) nitrogen yields, (iii) soil mineral nitrate (SMN) after harvest, and (iv) the N_{FIX} of winter and spring forms of pea and faba bean in eastern Austria in order to gain knowledge of the possible benefits of autumn-sown grain legumes for arable cropping systems in Central Europe. The hypotheses were that winter grain legumes can achieve higher N yields and higher N_2 fixation, whereby more N is also left for the subsequent crop in the field, because they can better use limiting growth factors due to the extended vegetation period. Several varieties were tested to assess if effects are constant over genotypes.

2. Material and Methods

2.1. Environmental Conditions

The experiment was carried out at the experimental farm Gross-Enzersdorf of BOKU University in Raasdorf (48°14' N, 16°35' E; 154 m above sea level) in the years 2013/14 and 2014/15. The soil was classified as a chernozem soil of alluvial origin and rich in calcareous sediments ($\text{pH}_{\text{CaCl}_2} = 7.6$, silty loam, 2.2–2.3% soil organic matter). The conventionally farmed soil has a high fertility. The soil chemical properties and macronutrient concentration in different soil layer of a nearby long-term field experiment are presented in the paper by Neugschwandtner et al. [22]. The temperature during the vegetation period (October to July) was above the long-term average in both years and the precipitation was higher in 2013/14 and lower in 2014/15 than the long-term average (Table 1).

Table 1. Long-term average monthly temperature and precipitation (1983–2012) and deviations during the 2013/14 and 2014/15 growing seasons.

	Temperature (°C)			Precipitation (mm)		
	Mean (1983–2012)	2013/14 (±)	2014/15 (±)	Mean (1983–2012)	2013/14 (±)	2014/15 (±)
October	10.8	0.9	2.0	34.4	−8.2	−3.0
November	5.3	0.8	3.1	37.8	−8.4	−12.3
December	1.1	2.0	2.8	32.4	−22.0	5.9
January	0.4	2.2	2.9	25.3	−20.9	16.5
February	1.6	2.7	1.0	25.5	2.4	−9.7
March	5.9	3.6	0.9	37.8	−28.1	−10.1
April	11.1	1.7	0.2	36.6	89.8	−8.2
May	15.8	−0.6	−0.3	57.9	47.0	−12.5
June	18.7	0.4	1.1	72.7	−20.8	−39.8
July	21.1	0.9	2.7	64.6	44.1	−22.5

2.2. Experimental Setup and Treatments

The experiment setup was a randomized complete block design with four replications, with individual plots of 15 m² (10 m × 1.5 m). Winter varieties and spring varieties of pea and faba bean were compared in terms of their N yields and N₂ fixation levels. The cereals winter wheat (*Triticum aestivum* L.) and winter oat (*Avena sativa* L.) were additionally grown for comparison. The following crops were sown (varieties are given in brackets): winter pea (Aviron, Cherokee, Curling, Enduro, Isard, James), spring pea (Astronaute), winter faba bean (Diva, Hiverna), spring faba bean (Alexia), winter wheat (Xenos), winter oat (Wiland). Breeding company, country of origin and year of release of the different varieties as well as details of seedbed preparation, sowing and plant protection are provided in the paper by Neugschwandtner et al. [4].

The sowing of winter crops was performed on 17 October 2013, and on 13 October 2014, and of spring crops on 4 March 2014, and on 10 March 2015. SMN at 0–0.9 m depth at the time of sowing was as follows: 21.7 (17 October 2013), 13.0 (13 October 2014), 18.4 (4 March 2014), or 11.2 (16 March 2015) g N m^{−2}. Plots were not fertilized. Harvest dates were for winter peas: 23 June 2014 or 22 June 2015, spring pea: 7 July 2014 or 2 July 2015, winter faba beans: 7 July 2014 or 7 July 2015, spring faba bean: 7 July 2014 or 13 July 2015, winter wheat and winter oat: 7 July 2014 or 9 July 2015.

2.3. Plant Sampling, Nitrogen Determination and Calculations

The plant sampling method is described in the paper by Neugschwandtner et al. [4]. Sub-samples of grain, pod walls and shoots (stems plus leaves) were ground to pass through a 1-mm sieve. The N concentrations were determined as the average of duplicate samples of about 50 mg each by the Dumas combustion method [23] using an elemental analyzer (vario MACRO cube; Elementar Analysensysteme GmbH, Langenselbold, Germany). Nitrogen yields of grain, pod wall and shoots were calculated by multiplying dry matter with N concentrations. Subsequently the N harvest index (NHI), which is the percentage of N uptake by the grain relative to the total above-ground dry matter (AGDM), was derived. The N utilization efficiency for the production of grain (NUtE_{GRAIN}) was calculated according to Sinebo et al. [24] as follows:

$$\text{NUtE}_{\text{GRAIN}} \left(\text{g g}^{-1} \right) = \frac{\text{YLD}}{\text{NY}_{\text{AGDM}}} \quad (1)$$

where YLD is the grain yield and NY_{AGDM} is the N yield of the AGDM.

Soil sampling was performed with soil probes (Puerckhauer type, core diameter: 30 mm) after harvest at a depth of 0.9 m. Samples were further divided into three layers: 0–0.3, 0.3–0.6, 0.6–0.9 m. SMN (as nitrate nitrogen: NO₃-N) was determined photometrically

(FIASSTAR 5000, FOSS GmbH, Hamburg, Germany) after extraction with 0.0125 M CaCl₂ in a soil extraction ratio of 1:4 (*w/v*) for 1 h using an overhead shaker [25].

The quantity of biologically fixed N (N_{FIX}) at harvest was estimated by the extended difference method [26], which compares differences in the uptake of N in the above-ground dry matter and in the depletion of SMN (0–0.9 m) between the legume and a non-legume reference crop as follows:

$$N_{\text{FIX}} \text{ (g N m}^{-2}\text{)} = (NY_{\text{AGDM-LEG}} - NY_{\text{AGDM-REF}}) + (SMN_{\text{LEG}} - SMN_{\text{REF}}) \quad (2)$$

where $NY_{\text{AGDM-Leg}}$ and $NY_{\text{AGDM-Ref}}$ are the quantities of N in the above-ground dry matter and SMN_{LEG} and SMN_{REF} are the amounts of SMN under the legume and under the reference crop. Unfertilized winter wheat (cv. Xenos) was used as a reference crop. The second part of the equation gives the differences of SMN removal from the soil among the crops. The lower removal of SMN from the soil by the legume crops compared to the cereal crops during growth is termed as “nitrogen sparing” [27].

The N balance was calculated as the difference of N_{FIX} and N removal with the grain [28] as follows:

$$\text{N balance (g N m}^{-2}\text{)} = N_{\text{FIX}} - \text{grain N yield} \quad (3)$$

2.4. Statistics

A two-factorial analysis of variance with the factors variety and year with subsequent multiple comparisons of means were performed using SAS version 9.2. Means were separated by least significant differences (LSD), when the F-test indicated factorial effects on the significance level of $p < 0.05$. Results are presented for the varieties in both years. The main effects are noted within the figure. Significant factor interactions are shown by LSD.

3. Results

3.1. Biomass Yields

On average over all varieties and years, the grain yield of winter peas was 445 g m^{−2} and 1.93-fold higher than that of the spring pea at 231 g m^{−2}. The grain yield of winter faba beans was 292 g m^{−2} and 1.27-fold higher than that of the spring faba bean at 230 g m^{−2}. Winter cereals had a mean grain yield of 516 g m^{−2} (Figure 1A). The average pod wall yield of winter peas was 83.8 g m^{−2} and 2.60-fold higher than that of the spring pea at 32.2 g m^{−2}. The pod wall yields of winter faba beans were 63.6 g m^{−2} and 1.36-fold higher than that of the spring faba bean at 46.6 g m^{−2} (Figure 1B). The average shoot yield of winter peas was 413 g m^{−2} and 1.49-fold higher than that of the spring pea at 278 g m^{−2}. The shoot yields of winter faba beans were 482 g m^{−2} and 1.93-fold higher compared to that of spring faba bean at 250 g m^{−2}. Winter cereals had a mean shoot yield of 585 g m^{−2} (Figure 1C).

Figure 1. (A–C) Yields, (D–F) N concentrations and (G–I) N yields of grain, pod walls and shoots, (J) total residues N yield, (K) above-ground dry matter (AGDM) N yields and (L) N harvest index of winter peas (WP: Aviron, Cherokee, Curling, Enduro, Isard and James) and spring pea (SP: Astronaute), winter faba beans (WFB: Diva, Hiverna), spring faba bean (SFB: Alexia) and winter cereals (C: winter wheat Xenos, winter oat Wiland) in the years 2014 and 2015. Main effects for variety (V) and year (Y) are significant at $p < 0.001$. Error bars are least significant differences (LSDs) ($p < 0.05$) for the significant interactions of V × Y.

3.2. Nitrogen Concentrations and C/N Ratios

The mean grain N concentration (N %) over all varieties and years was highest for winter faba beans at 4.74%, followed by spring faba bean at 4.46%, spring pea at 4.14%, winter peas at 3.92% and cereals at 1.69%. Thus, the difference for both crops was approximately 6%. The grain N % was higher for winter faba beans than for the spring faba bean (with Diva showing higher values than Hiverna), whereas the spring pea had a higher N % than the winter peas (except for James). Among winter peas, James had a higher N concentration than Enduro, with other varieties showing intermediate values. The grain N % was higher for Curling, James and Hiverna in 2015 and Xenos in 2014 compared to the other years, with no differences between the years for the other varieties (Figure 1D).

The mean pod wall N % over all varieties and years was highest for spring faba bean at 1.55%, followed by winter faba beans at 1.41%, winter peas at 0.93% and the spring pea at 0.84%. The pod wall N % of Cherokee and Isard was higher in 2014 than in 2015, whereas that of Astronaute was higher in 2015 (Figure 1E).

The mean shoots N % over all varieties and years was highest for winter peas at 1.57%, followed by spring pea at 1.18%, spring faba bean at 0.95% and winter faba beans at 0.94%. Shoot N % of cereals was lowest at 0.45%. The shoot N % was higher for winter faba beans than for the spring faba bean, whereas no differences were found between the winter and spring pea varieties. The shoot N % of Hiverna and Alexia was higher in 2014 than in 2015, with no differences between years for other varieties (Figure 1F).

The C/N ratios (means over all varieties and years, without considering sowing dates) were ranked for the crops as follows, for grain: cereals (27.9:1) > peas (11.5:1) > faba beans (9.8:1), pod walls: peas (50.4:1) > faba beans (31.0:1), shoots: cereals (115.6:1) > faba beans (54.7:1) > peas (30.8:1).

3.3. Nitrogen Yields and N Harvest Index

The mean grain N yield over all varieties and years was highest for winter peas at 17.43 g m^{-2} , followed by winter faba beans at 13.84 g m^{-2} , spring faba bean at 10.23 g m^{-2} , spring pea at 9.55 g m^{-2} and cereals at 8.66 g m^{-2} . Therefore, the grain N yields of the winter varieties compared to the corresponding spring varieties were 1.83-fold higher for peas and 1.35-fold higher for faba beans. The grain N yields of winter peas Curling, Cherokee, James and Enduro were higher than those of winter faba beans, whereas Avrion and Isard had similar grain N yields to the winter faba beans in 2014. Both spring grain legumes had similar yields in both years. The grain N yields of spring grain legumes in both years and those of Isard and Hiverna in 2014 were in the same range as those of the cereals (Figure 1G).

The mean pod wall N yield over all varieties and years was as follows: winter peas 0.77 g m^{-2} , spring pea 0.28 g m^{-2} , winter faba beans 0.88 g m^{-2} and spring faba bean 0.73 g m^{-2} . Therefore, the pod wall N yields of winter varieties compared to the corresponding spring varieties were 2.77-fold higher for peas and 1.21-fold higher for faba beans (Figure 1H).

The mean shoot N yield over all varieties and years was as follows: winter peas 6.53 g m^{-2} , spring pea 3.25 g m^{-2} , winter faba beans 4.76 g m^{-2} , spring faba bean 2.43 g m^{-2} and cereals 2.66 g m^{-2} . Therefore, the shoot N yields of winter varieties compared to the corresponding spring varieties were 2.01-fold higher for peas and 1.96-fold higher for faba beans. The shoot N yields of winter peas and Hiverna were higher than those of Diva, spring grain legumes and cereals (Figure 1I).

The mean total residue N yield over all varieties and years was as follows: winter peas 7.30 g m^{-2} , spring pea 3.52 g m^{-2} , winter faba beans 5.64 g m^{-2} , spring faba bean 3.16 g m^{-2} and cereals 2.66 g m^{-2} . Therefore, the total residue N yields of winter varieties compared to corresponding spring varieties were 2.07-fold higher for peas and 1.79-fold higher for faba beans. The total residue N yields of winter peas and Hiverna were higher than those of Diva, spring grain legumes and cereals, with Diva showing a higher value than Wiland. The total residue N yields of all winter grain legumes, but not of the spring grain legumes or the cereals, were higher in 2014 than in 2015 (Figure 1J).

The AGDM N yields over all varieties and years were as follows: winter peas 24.73 g m^{-2} , spring pea 13.07 g m^{-2} , winter faba beans 19.48 g m^{-2} , spring faba bean 13.38 g m^{-2} and cereals 11.31 g m^{-2} . Therefore, the AGDM N yields of winter varieties compared to the corresponding spring varieties were 1.89-fold higher for peas and 1.46-fold higher for faba beans. Winter grain legumes had higher AGDM N yields than spring grain legumes and cereals. The AGDM N yields of winter peas were higher than that of winter faba beans, except for Isard in 2014. Isard had a lower AGDM N yield in 2014 than in 2015, while for all other varieties, there were no differences between years (Figure 1K).

The N harvest index (NHI) over all varieties and years was as follows: winter peas 70.3%, spring pea 72.6%, winter faba beans 69.6%, spring faba bean 76.6% and cereals 76.8%. Therefore, the NHI of spring varieties compared to the corresponding winter varieties was 1.03-fold higher for peas and 1.10-fold higher for faba beans. The NHI was highest for Wiland, Alexia, Diva and Curling and lowest for Avron, Isard and Hiverna, with other varieties showing intermediate values. The NHI was lower in 2014 than in 2015 (Figure 1L).

The mean calculated difference (Δ) of the $NY_{AGDM-Leg}$ and the $NY_{AGDM-Ref}$ was 9.04 g N m^{-2} (mean over all pea and faba bean varieties and both years) (Figure 2A). It was higher for both winter grain legumes than for the spring forms: 17.0-fold for pea (at 12.39 versus 0.73 g N m^{-2}) and 6.87-fold for faba bean (at 7.14 versus 1.04 g N m^{-2}) (means over varieties and years). The Δ was higher in winter pea varieties than in winter faba bean varieties (except for Isard in 2014); there were no differences between spring pea and spring faba bean. Meanwhile, it was slightly negative for Astronaute in 2014 and Alexia in 2015. Differences among years were observed for Avron (with higher values in 2014) and for Isard (with higher values in 2015). Among winter pea varieties, Cherokee, Curling, Enduro and James had higher mean Δ values than Isard (with Avron showing intermediate values).

Figure 2. (A) Δ of $NY_{AGDM-LEG} - NY_{AGDM-REF}$, (B) NUT_{EGRAIN} , (C) total soil mineral nitrate at harvest (SMN, 0–0.9 m), (D–F) soil mineral nitrate (SMN) in the three soil layers of 0–0.3 m, 0.3–0.6 m and 0.6–0.9 m at harvest, (G) SMN sparing in the soil at harvest (0–0.9 m), (H) N_{FIX} and (I) N balance for winter peas (WP: Avron, Cherokee, Curling, Enduro, Isard and James) and spring pea (SP: Astronaute), winter faba beans (WFB: Diva, Hiverna), spring faba bean (SFB: Alexia) and winter cereals (C: winter wheat Xenos, winter oat Wiland) in the years 2014 and 2015. Main effects for variety (V) and year (Y) are significant at $p < 0.05$ (*) or $p < 0.001$ (**). Error bars are LSDs ($p < 0.05$) for the significant interactions of $V \times Y$. NY_{AGDM} = N yields of the AGDM

3.4. Nitrogen Utilization

The $\text{NUE}_{\text{GRAIN}}$ values over all varieties and years were as follows: winter peas 17.9 g g^{-1} , spring pea 17.5 g g^{-1} , winter faba beans 14.7 g g^{-1} , spring faba bean 17.2 g g^{-1} and cereals 46.3 g g^{-1} . Therefore, the $\text{NUE}_{\text{GRAIN}}$ for pea was independent of the sowing date and was 1.17-fold higher for spring faba bean compared to winter faba beans. On average, the varieties ranked across both years as follows: Wiland > Xenos > Curling $\geq$ Enduro, Cherokee $\geq$ James, Astronaute, Alexia $\geq$ Avrion, Isard $\geq$ Diva and Hiverna. Between years, the $\text{NUE}_{\text{GRAIN}}$ was higher in 2015 than in 2014 for Hiverna, Isard, Wiland and Xenos (Figure 2B).

3.5. Mineral N Content in the Soil at Harvest

The SMN contents at harvest in the three soil layer of 0–0.3 m, 0.3–0.6 m and 0.6–0.9 m at harvest are shown in Figure 2D–F. The mean SMN contents over all varieties and years were highest in the upper soil layer and decreased with soil depth; mean values were as follows (in g N m^{-2}): 2.34 (0–0.3 m), 1.65 (0.3–0.6 m) and 1.19 (0.6–0.9 m). The SMN contents in the soil of grain legumes were higher in the first two layers than those of the cereals, whereas no differences occurred between varieties at a depth of 0.6–0.9 m. Mean contents over varieties and years were as follows—at a depth of 0–0.3 m (in g N m^{-2}): 2.62 (peas), 2.72 (faba beans) and 0.78 (cereals); at a depth of 0.3–0.6 m: 1.85 (peas), 1.97 (faba beans) and 0.45 (cereals); at a depth of 0.6–0.9 m: 1.29 (peas), 1.21 (faba beans) and 0.79 (cereals). Therefore, the mean values of pea and faba bean were higher than those of the cereals by 3.36- and 3.49-fold (in 0–0.3 m), 4.11- and 4.38-fold at a depth of 0.3–0.6 m and 1.63- and 1.53-fold at a depth of 0.6–0.9 m, respectively. The SMN contents at harvest were higher in 2014 in all three layers as compared to 2015.

Over the whole soil profile of 0–0.9 m (Figure 2C), the mean SMN contents across varieties and years were as follows (in g N m^{-2}): peas 5.76, faba beans 5.90 and cereals 2.02. The mean values of pea and faba bean were, therefore, higher than those of the cereals by 2.85- and 2.92-fold at a soil depth of 0–0.9 m. All pea varieties had similar SMN contents; among faba beans, the contents after Alexia were higher than those after Hiverna. Between years, the mean SMN contents were 2.02-fold higher in 2014 than in 2015 (with 6.92 or 3.43 g N m^{-2} , means over varieties).

3.6. Nitrogen Sparing and Biological Nitrogen Fixation

The nitrogen sparing in the soil (0–0.9 m) at harvest under pea and faba bean amounted to 3.90 g N m^{-2} (mean across all pea and faba bean varieties and both years). There were no differences between varieties and years (Figure 2G).

The N_{FIX} was 12.94 g N m^{-2} (mean over all pea and faba bean varieties and both years) (Figure 2H). It was higher in the winter forms of both grain legumes than in the spring forms: 3.90-fold for pea (at 16.64 versus 4.26 g N m^{-2}) and 2.28-fold for faba bean (at 10.37 versus 4.54 g N m^{-2}) (means over varieties and years). The N_{FIX} of the winter pea varieties was 1.60-fold higher than those of the winter faba bean varieties; no differences occurred between spring pea and spring faba bean. Among winter pea varieties, Enduro had a higher mean N_{FIX} than Isard (with other varieties showing intermediate values). The percentage of N derived from fixation in the AGDM was higher in 2014 at 60.4% than in 2015 at 51.4% (means over all varieties). It was ranked among crops (over all varieties, with consideration of sowing dates) as follows: winter peas at 67.3% > winter faba beans at 53.2% > spring pea at 32.6% and spring faba bean at 33.9%.

The N balance over all varieties and years was as follows: winter peas— 0.79 g N m^{-2} , spring pea— 5.28 g N m^{-2} , winter faba beans— 3.48 g N m^{-2} , spring faba bean— 5.68 g N m^{-2} and cereals— 8.66 g N m^{-2} . Therefore, the N balance of spring varieties compared to the corresponding winter varieties was 6.66-fold lower for peas and 1.64-fold lower for faba beans. Over both years, it was higher for all peas and winter faba beans than for the cereals, with spring faba bean Alexia showing intermediate values. Winter peas and

Hiverna had positive values in 2014, whereas other varieties in 2014 and all varieties in 2015 had negative N balances (Figure 2I).

4. Discussion

4.1. Winter Grain Legumes Have Higher Biomass and N Yields

The yields of both grain legumes were higher with autumn-sowing than with spring-sowing. Faba beans had a higher grain N% than peas, with a generally higher grain N % for winter faba bean and for spring pea compared to the corresponding other winter or spring forms. Higher grain N concentrations in some winter faba beans compared to a spring faba bean have been reported also by Neugschwandtner et al. [29]. Alternatively, similar to the grain N % of pea, a higher grain N % with spring-sowing compared to autumn-sowing (except for the variety James) was observed for facultative wheat [18] and chickpea [30], where the lower grain yields of spring grain crops clearly resulted in a lower N dilution of N in the grain. The grain N % was higher for some varieties in the drier year of 2015 than in 2014. The lower biomass production in the dry year compared to an average year also resulted in a higher biomass N % of spring-sown crops (chickpea, pea, barley and oat) during the vegetation period [31], and thus, in a higher grain N % of all four crops at harvest [32]. The time for carbon assimilation and partitioning to the grain is limited under warmer temperature, therefore, the grain N % is less diluted and remains high.

Under the conditions present in eastern Austria, the grain N yield, total residue N yield, and therefore, the AGDM N yield, were highest for winter peas followed by winter faba beans. The higher grain N yields of winter grain legumes compared to spring grain legumes were due to the higher grain yield but lower N concentrations for peas, and due to a combination of both higher grain yields and a higher grain N % for winter faba beans. Contrary to this observation, autumn-sowing of facultative wheat resulted—with a higher grain yield but lower grain N concentration—in a slightly, but not significantly, higher grain N yield compared to spring sowing [17]. The grain N yields of spring forms of both grain legumes were just in the range of those of unfertilized cereals. Thus, the aim of obtaining higher protein yields by growing grain legumes compared to winter cereals could not be achieved with spring faba bean and spring pea. Similar to this observation, Reckling et al. [33] conducted several long-term experiments in northern Europe, with most locations demonstrating similar or lower protein yields for spring pea and spring faba bean compared to winter cereals.

The total residue N yields of winter cereals were as high as that of spring sown grain legumes. In any case, the C/N ratio of legume residues is much narrower than that of cereals. Consequently, the N is released more quickly during decomposition and is available for the subsequent crop.

The N harvest index differed between varieties, but no distinctive differences in patterns between crops or sowing dates were observed. Although the grain N % and the pod wall N % of winter faba beans were higher and the shoot N % was lower compared to winter pea varieties, the NHI was within a similar range for both, as winter peas had a considerably higher harvest index than winter faba beans [4]. The NHI can differ between crops and is also affected by fertilization and the intercropping ratio [31,32,34]. A higher harvest index and a higher residue N concentration with pea compared to faba bean was also recorded by Kaul [35].

4.2. Autumn-Sowing Results in Higher Nitrogen Fixation

SMN at harvest did not differ between pea and faba bean or between winter and spring grain legumes, whereas Kaul [36] reported a higher SMN before winter rest and in late winter after spring pea compared to spring faba bean. Grain legumes left an almost three times higher SMN content in the whole soil profile than the cereals, with considerably higher contents in the first two soil layers but very low SMN values in the lowest soil layer. These higher contents in upper layers mean that SMN is already available for subsequent crops during their early growth. Contrary to our observation, smaller

differences between faba bean and winter wheat have been found in the arable layer (0–0.3 m), but higher ones have been found in the subsoil in a previous experiment at the same site [29]. Several reasons were given for the higher mineral nitrogen in the soil after legume growth: their lower need for mineral nitrogen as they are N_2 fixing crops [27]; the lower rooting density, and therefore, lower nitrogen uptake, as shown for faba bean compared to oat [37]; the rapid turnover of roots and nodules of legumes [38]; stimulation of mineralization rates under a legume crop [39]. The high SMN after grain legumes causes the risk of a downward movement of nitrogen in the soil and eventually leads to nitrogen leaching into the groundwater [40]. This risk can be addressed, for example, by relay intercropping of legumes with species of high mineral N demand [41].

Nitrogen sparing in the full soil profile at harvest did not differ between peas and faba bean, sowing dates and years. On average, it was 3.90 g N m^{-2} higher compared to the reference crop, winter wheat, over all legume varieties and years. Contrary to this, the difference in the nitrogen yields in the above-ground dry matter between the legumes and the reference crop were several fold higher in winter than in spring grain legumes. In the case of the winter grain legumes, it surpassed the nitrogen sparing in the soil by 2.91-fold for peas and by 2.21-fold for faba beans. Alternatively, for the spring grain legumes, it amounted to just a small share of the nitrogen sparing in the soil, or was even slightly negative as in the case of Astronaute in 2014 and Alexia in 2015.

To summarize, both the differences in the nitrogen yields in the above-ground dry matter between the legumes and the reference crop, and the nitrogen sparing resulted in the N_{FIX} being considerably higher for winter than for spring grain legumes (almost 3.90-fold for peas and 2.28-fold for faba bean) with higher absolute values for winter peas (16.64 g N m^{-2}) than for winter faba beans (10.37 g N m^{-2}). There were no differences between spring grain legumes (pea: 4.26 g N m^{-2} , faba bean: 4.54 g N m^{-2}). Additionally, McCauley et al. [42] reported a higher N_{FIX} for peas and lentils grown as green manure when planted earlier in the year (in spring compared to summer). In contrast, on the same site in a previous experiment, we observed no differences in the N_{FIX} of winter and spring faba beans in one year, mainly due to the low winter survival of winter faba beans [29]. Additionally, in northern Germany, the N_{FIX} of spring pea and spring faba beans did not differ, but was on a much higher level at 16.6 g N m^{-2} for peas and 20.0 g N m^{-2} for spring faba beans (means over two years, reference crop: spring barley). However, larger differences between years were also noted [28]. In our experiment, the N_{FIX} was higher in the more humid year of 2014 than in the drier year of 2015, through a combination of both a slightly higher Δ of the $NY_{\text{AGDM-Leg}}$ and the $NY_{\text{AGDM-Ref}}$ and a slightly higher level of nitrogen sparing. In our previous study with winter and spring faba beans, very large differences between years were observed as drought resulted in the biomass yields and N_{FIX} in one year being considerably lower. The N_{FIX} in the dry year was 6.3 g N m^{-2} , whereas it was 21.9 g N m^{-2} in the year with optimum conditions (means over winter and spring faba beans) [29].

Furthermore, it has to be mentioned that with the extended difference method [26], N_{FIX} is underestimated as the N contribution of legumes to the soil with N inputs from dying roots, root exudates and nodules was not considered [38]. Additionally, harvestable biomass alone, but not leaf litter shed before harvest—especially from faba bean—was investigated. Values of 6 % [35,43] and 25% [44] of the total above-ground produced biomass have been suggested for the shed leaf litter of spring faba bean before harvest.

The N balance was mostly negative, except in one year for winter peas and for Hiverna. Negative values indicate that the removal of N with grain is higher, while positive values indicate that it is lower than N_{FIX} . The N balance varies greatly and is affected by species, environmental conditions influencing N_{FIX} , and the amount of N removed at harvest [6].

Over all varieties and years, the negative N balance of winter peas was not as high as that of winter faba beans, although the N removal with grain was higher for winter peas. However, their considerably higher N_{FIX} value could overcompensate the removal compared to winter faba beans. Between varieties, no differences in the N balance occurred

between spring pea and spring faba bean, as both had similar grain N yields and N_{FIX} values. Additionally, Wichmann et al. [28] observed no differences in the N_{FIX} and N balance between spring pea and spring faba bean grown in northern Germany. However, the negative N balance of winter peas was smaller compared to that of winter faba beans as they had a considerably higher N_{FIX} with which they could even overcompensate the higher grain N removal of winter peas compared to winter faba beans. Wichmann et al. [28] stated that the N balance decreases with a higher harvest index as more N is removed from the field. As the differences in the N harvest index between winter and spring varieties was not as high in our experiment, the lower negative N balance of winter varieties resulted from their higher N_{FIX} .

Although values of N_{FIX} were high, especially for winter grain legumes, the negative N balance of most grain legumes showed that it was not enough to offset the removed N and that legumes exploited the soil N reserve more than contributing to it [39]. The negative N balance of winter cereals was much higher compared to that of the grain legumes. For assessing the benefit of the grain legumes within a crop rotation, not just the contribution by the legume, but also the N loss through removal of harvest products of an alternative non-legume crop has to be considered. Peoples and Craswell [39] termed this the “apparent benefit”.

Grain legumes are, therefore, important crops within crop rotation. The grain yield benefit of the subsequent crop was estimated to be between 50 and 160 g m^{−2} [45]. In addition to this increase in quantity, legumes increase the quality of the subsequent crop, as shown for the protein yield of winter wheat, especially when unfertilized [36,46]. Positive yield effects of legumes on subsequent non-legume crops result from both N sparing in the soil and the transfer of biologically fixed N via crop residues [27,36]. According to our results, these positive effects might arise predominantly via N sparing for both spring grain legumes and winter grain legumes, but with N transfer through crop residues also playing a significant role in the case of winter grain legumes. The positive effects are consistent over different tillage treatments, as shown for winter wheat following soybean versus sugar beet [47].

5. Conclusions

Winter grain legumes have the potential to become important crops in central European cropping systems due to their higher N yields and higher N_{FIX} compared to their spring forms. For this reason, frost-resistant varieties are necessary and appropriate crop cultivation techniques—such as optimal sowing rates and adapted plant protection procedures—need to be developed.

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