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Originalarbeit

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The level of N_2-fixation of different genotypes of winter pea in comparison to spring pea in pure and mixed stands

Die Höhe der N_2-Fixierung verschiedener Wintererbsengenotypen im Vergleich zu Sommererbsen in Rein- und Gemengesaat

Abstract

In contrast to the common spring pea (SP), little is known about the capacity of winter peas (WP) for symbiotic N_2 fixation in pure and mixed stands. Therefore, seven WP genotypes and one semi-leafless SP in pure stands and two mixtures with cereals (25% (Mix1) or 50% (Mix2) of the pea pure stand sowing density) were examined in field experiments on two sites. The amount of fixed N_2 at flowering and at mature harvest was assessed applying the extended total-N-difference method. At flowering and at grain harvest the N_2 fixation for the five frost-resistant WP genotypes (52 and 85 kg ha^{-1}, respectively) was generally higher than for SP (17 and 42 kg ha^{-1}, respectively). This was traced back to the earlier N_2 fixation of WP and a usually higher above-ground biomass (144 and 75 kg ha^{-1}, respectively) and presumably higher below-ground biomass as compared with SP. Furthermore, average inorganic N in soil at mature harvest in pure stands was higher under WP (69 kg ha^{-1}) than under SP (36 kg ha^{-1}), while for any other treatment similar values on a lower level were estimated. Results show that WP may better contribute to the N supply within crop rotations than SP.

Key words: Difference method, grain legume, mixture, N uptake, inorganic N

Zusammenfassung

Im Vergleich zu Sommererbsen ist wenig über die symbiotische N_2-Fixierleistung von Wintererbsen in Rein- und Gemengesaat bekannt. Daher wurden sieben Wintererbsengenotypen mit einer Sommererbse in Reinsaat und zwei substitutiven Gemengestufen mit Getreide (25% (Mix1) bzw. 50% (Mix2) der Reinsaatstärke der Erbsen) in Feldversuchen auf zwei Standorten untersucht. Die Höhe der N_2-Fixierleistung wurde zur Blüte und zum Korndrusch mit der erweiterten Differenzmethode geschätzt.

Die N_2-Fixierung zur Blüte und zum Korndrusch fiel bei den fünf winterharten Wintererbsen (52 bzw. 85 kg ha^{-1}) allgemein höher als bei der Sommererbse (17 bzw. 42 kg ha^{-1}) aus. Dies wurde auf eine frühere N_2-Fixierung sowie eine gewöhnlich höhere oberirdische Biomasse (144 bzw. 75 kg ha^{-1}) und damit wahrscheinlich einhergehend größere unterirdische Biomasse der Wintererbsen im Vergleich zu Sommererbsen zurückgeführt. Darüber hinaus wurde in Reinsaat unter den Wintererbsen (69 kg ha^{-1}) eine höhere durchschnittliche Menge an mineralischem N als unter der Sommererbse (36 kg ha^{-1}) bestimmt, während in den Gemengevarianten ähnliche Werte auf einem geringeren Niveau vorgefunden wurden. Aus den Ergebnissen kann ein höherer Beitrag von Wintererbsen zur N-Versorgung von Fruchtfolgen im Vergleich zu Sommererbsen geschlussfolgert werden.

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Stichwörter: Differenzmethode, Körnerleguminose, Mischanbau, N-Aufnahme, mineralischer N

1 Introduction

Winter pea is an old crop which – during the last decades – has hardly been cultivated in Germany due to its variable winter-hardiness (e.g. Klapp, 1954; Hertzsch, 1959), an increased import of soybean and a rising use of mineral N fertilizers. Recent research shows that there are frost-resistant genotypes of peas suitable for the climate conditions in Germany (Urbatzka, 2010).

Yet, cropping of grain legumes especially of common spring pea has decreased in most European countries (Sass, 2009) due to specific cropping problems, e.g. weeds, yield instability or low economic competitiveness (Urbatzka et al., 2011). Besides the use of organic fertilisation, the cultivation of leguminous crops is the most important source of nitrogen (N) in organic crop farming and the N supply for the crop rotation is one of the biggest challenges. Hence innovative solutions for such cropping systems must be developed.

The N_2 fixation of spring peas in pure and mixed stands is a well known source of N (e.g. Jensen, 1996; Peoples et al., 2001; Corre-Hellou et al., 2006). In mixture the amount of nitrogen derived from atmosphere (Ndfa) is higher than in spring pea pure stands due to a strong competition of the cereal for soil borne N. Therefore the N use efficiency in intercropping of spring pea and cereals is higher than in pure stands (Hauggaard-Nielsen et al., 2009). The absolute level of N_2 fixation is higher in spring pea pure stands than in mixture due to lower crop density and competition of the cereal to light (Jensen, 1996; Wichmann, 2003; Corre-Hellou et al., 2006).

In contrast to the common spring pea, N_2 fixation of winter peas was examined in very few experiments and above all in pure stands and with winter pea serving as a catch crop, i.e. just until the onset of flowering or pod filling (Stivers and Shennan, 1991; Rochester et al., 1998; Karpenstein-Machan and Stülpnagel, 2000). Accordingly the N_2 fixation of winter peas may be higher than for spring peas, but comparisons of the amounts of fixed N_2 for both winter and spring peas are missing so far. Also there are no published data on the amount of Ndfa as well as the N_2 fixation at mature harvest in pure and mixed stands for winter peas.

Accordingly the aim of this research was to compare symbiotic N_2 fixation and Ndfa (only in pure stands) of different winter and spring pea genotypes in pure and mixed stands both at flowering and at mature harvest.

2 Material and Methods

Field experiments were conducted on the experimental farm of the University of Kassel, Hessian State Domain Frankenhausen (DFH; 51°4' N, 9°4' E) between 2003/04 and 2006/07 and on Waldhof (WH; 52°2' N, 8°8' E), the research farm of the University of Applied Sciences, Osnabrueck during 2005/06 and 2006/07. Both are certified farms according to the standards for organic farming. They are located at 209 to 259 (DFH) and 65 to 72 (WH) m above sea level. At DFH, soil type was a Haplic Luvisol (on loess), soil texture a silty loam (Quintern et al., 2006). At WH, soil texture was a sandy loam and loamy sand, respectively. More details of the locations as well as of experimental and crop management are given in Tab. 1. Weather data of the experimental seasons and a comparison with the long-term means are presented in Tab. 2 and 3.

2.1 Experimental design and crop husbandry

Design of the field experiments in 2003/04 was a Latin Square and in the other seasons a split-plot-strip design, always with four replications. In the mixed design, treatments with spring pea were assigned to strips and the remaining treatments according to a split-plot design. Main plot factor was the crop stand (CS) of peas (three treatments: pure or mixed with cereals) and subplot factor the pea genotype (Pisum sativum L.). Seven winter peas and one spring pea (SP) as a control were compared (Tab. 4). The four winter pea genotypes were derived from the gene bank in Gatersleben, Germany: Griechische, Nischkes Riesengebirgs, Unrra and Württembergische. They had been screened for sufficient winter hardiness and selected from 43 winter pea genotypes (Urbatzka et al., 2005).

The main plot factor consisted of pea pure stands and two replacement designs with either 25% (Mix1) or 50% (Mix2) of the pea pure stand sowing density, complemented by either 75% or 50% of the cereal pure stand sowing density. Sowing density in pure stands (PS) was 80 germinable pea grains m^{-2} and 300 (DFH) or 380 (WH) germinable cereal grains m^{-2}, respectively. As a partner for winter pea in mixture with cereal, winter rye (*Secale cereale* L.), cv. Amilo was selected in 2003/04 and cv. Danko from 2004/05 to 2006/07, while for spring pea oats (*Avena sativa* L.), cv. Aragon was the mixture partner in 2003/04 and barley (*Hordeum vulgare* L.), cv. Ria between 2004/05 and 2006/07.

Experimental plots were ploughed and prepared with a cultivator just before sowing the crop. Distance between rows was 21 cm at DFH and 15 cm at WH. Sowing depth was approx. 4 cm. Prior to sowing the spring crops, soil was prepared twice with a rotary cultivator and – if necessary, depending on weed cover – with a chisel plough beforehand.

2.2 Plant and soil sampling

A biomass harvest was conducted at the onset of flowering (BBCH 61) of peas, harvesting whole above-ground biomass. This resulted in a one up to three weeks earlier harvest for winter peas than for spring peas. Harvested area of pea pure stands was 3 m^2 at DFH and 1.5 m^2 at WH. Mixed stands and cereal pure stands were sampled from 1.2 m^2.

Tab. 1. Soil, experimental and crop management details

	Frankenhausen				Waldhof	
	2003/04	2004/05	2005/06	2006/07	2005/06	2006/07
Soil sampling in autumn	21 Oct 2003	30 Oct 2004	25 Oct 2005	23 Oct 2006	24 Oct 2005	27 Oct 2006
pH ($CaCl_2$)	6.8	6.9	6.6	6.8	5.3	5.8
P (CAL; mg kg^{-1}, 0–30 cm)	65	74	100	100	39	110
K (CAL; mg kg^{-1}, 0–30 cm)	102	108	108	100	66	66
Mg ($CaCl_2$; mg kg^{-1}, 0–30 cm)	42	54	60	54	30	24
NO_3-N, NH_4-N (kg ha^{-1}, 0–90 cm)	66	73	81	77	89	127
Soil sampling	24 Feb 2004	9 Mar 2005	4 Apr 2006	15 Mar 2007	6 Apr 2006	18 Mar 2007
NO_3-N, NH_4-N (kg ha^{-1}, 0–90 cm)	33	31	51	25	11	17
Preceding crop	Spring barley	Winter rye	Potatoes	Carrots	Spelt	Potatoes
Pre-preceding crop	Spring wheat	Winter wheat	Winter wheat	Potatoes	Green lupine	Cereals
Sowing of winter crops	24 Sep 2003	5 Oct 2004	22 Sep 2005	28 Sep 2006	27 Sep 2005	28 Sep 2006
Sowing of spring crops	6 Apr 2004	4 Apr 2005	11 Apr 2006	5 Apr 2007	20 Apr 2006	29 Mar 2007
Grain harvest winter crops	4 Aug 2004	2 Aug 2005	25 Jul 2006	17 Jul 2007	19 Jul 2006	18 Jul 2007
Grain harvest spring crops	18 Aug 2004	2 Aug 2005	30 Jul 2006	24 Jul 2007	26 Jul 2006	24 Jul 2007
Size of sampling plot	6 × 3 m	6 × 3 m	6 × 3 m	8 × 3 m	6 × 1,5 m	6 × 1,5 m
Finger weeding winter crop	–	–	–	–	19 Nov 2005	18 Oct 2006
Manual weeding spring crop	6 May 2004	–	24 May 2006	3 May 2007	11 May 2006	2 May 2007
Manual weeding spring crop, only pure stands	18 Aug 2004	–	8 Jun 2006	23 May 2007	–	13 Jun 2007

Tab. 2. Monthly precipitation and average daily temperature at the experimental site Frankenhausen

	Long-term mean		Difference from long-term mean							
	°C	mm/month	2003/2004		2004/2005		2005/2006		2006/2007	
			°C	mm	°C	mm	°C	mm	°C	mm
September	13.7	56	0.0	−9	1.3	4	1.5	−28	3.4	−38
October	9.5	46	−3.8	−11	0.6	−20	1.9	−10	3.1	−12
November	3.9	55	2.6	−32	0.2	20	1.2	−14	3.6	−8
December	0.8	62	0.7	−27	−0.3	−40	0.9	−18	3.9	−15
January	0.1	57	0.7	−1	2.1	−22	−2.3	−45	5.0	47
February	0.6	44	2.3	−11	−1.4	−13	−0.8	−21	3.8	9
March	4.0	54	0.7	−37	−1.0	−28	−2.3	−11	2.3	11
April	7.9	53	1.3	−12	1.0	−17	0.0	−16	3.5	−51
May	12.4	66	−0.3	−19	0.5	−16	0.9	5	1.6	78
June	15.6	80	−0.5	−32	0.7	−37	0.7	−55	1.7	62
July	17.1	63	−1.1	30	−0.2	−5	4.7	−39	−0.2	24
August	16.9	62	2.1	30	−2.8	4	−1.2	10	−1.3	15
mean	8.5	698	0.4	−131	0.1	−170	0.4	−242	2.5	121

The grain harvest was carried out with a Hege 140 plot combine (Wintersteiger AG, Egging am See, Germany) in Frankenhausen and with a Hege 160 plot combine in Waldhof. Winter crops were usually ready for harvest one week earlier than the spring crops (Tab. 1). In summer 2003/04, harvest of spring crops was delayed by the late ripening of oats, which subsequently was replaced by barley. Yield of above-ground residues without stubble was determined at DFH at grain maturity from 1.5 m^2 that were taken by cutting plants approx. 5 cm above the

Tab. 3. Monthly precipitation and average daily temperature at the experimental site Waldhof

	Long-term mean		Difference from long-term mean			
	°C	mm/month	2005/06		2006/07	
			°C	mm	°C	mm
September	13.9	60	1.3	−25	3.6	30
October	9.7	59	2.5	−12	3.4	−17
November	5.4	66	0.7	3	2.7	−46
December	2.4	70	0.9	−16	3.7	−60
January	1.2	62	−1.2	−40	4.9	81
February	1.4	47	0.1	3	3.8	35
March	4.6	50	−1.8	−6	2.4	16
April	7.9	51	0.9	6	4.7	−50
May	12.5	64	1.8	16	1.6	56
June	15.8	73	2.0	−48	2.1	−2
July	17.0	77	5.4	−8	0.1	35
August	16.7	78	−0.8	81	0.3	−20
mean	9.0	757	1.0	−7	2.8	58

ground and put under a roof until dry enough for threshing. Crops were also threshed with the Hege 140 plot combine. At WH, the above-ground residues were sampled from the whole plot immediately after combine harvest.

Soil samples at biomass and mature harvest were taken at 0–30, 30–60, 60–90 cm soil depth at DFH, and only up to 60 cm soil depth at WH due to the pebbly subsoil being too dry for sampling (except in autumn).

The yield stability of above-ground biomass at flowering and at mature harvest of all treatments was calculated with the coefficient of variation (CV) according to FRANCIS and KANNENBERG (1978):

CV (%) = 100 × standard deviation/overall mean

2.3 Laboratory analysis

Dry matter of biomass at flowering harvest and of above-ground residues and of grains at mature harvest was measured immediately after taking the samples. Samples were dried until mass constancy was reached. A sub-sample of dried above-ground residues was ground (0.5 mm) with a Pulverisette No. 19 laboratory cutting mill (Fritsch, Idar-Oberstein, Germany) and analyzed for total N using a Macro N auto-analyzer (Elementar Analysesysteme, Hanau, Germany). Crude protein concentration of biomass at flowering harvest as well as of pea and cereal grains was determined by Near-Infrared-Spectroscopy. P, K and Mg, pH, and inorganic N (NO_3-N, NH_4-N) of soil were analyzed according to DIN ISO 11464 and DIN ISO 14255 (N_{min}–N), respectively (HOFFMANN, 1991).

2.4 Estimation of N_2 fixation

In order to assess crop N_2 fixation, N uptake until flowering and mature harvest, the N concentration was determined from crude protein concentration according to BUCHHOLZ (1993).

The amount of N_2 fixed (Nfix) was calculated for pure stands (Equation 1) according to STÜLPNAGEL (1982) and for mixed stands (Equation 2) according to KARPENSTEIN-MACHAN and STÜLPNAGEL (2000) at flowering and mature harvest with the extended total-N-difference method. Weeds were harvested with the crops and taken into account.

(1) $N\text{-}Fix_{PS}$ = N-uptake $biomass_{PPS}$ − N-uptake $biomass_{CPS}$ + N_{min}-N-$soil_{PPS}$ − N_{min}-N-$soil_{CPS}$

(2) $N\text{-}Fix_{Mix}$ = N-uptake $biomass_{Mix}$ − N-uptake $biomass_{CPS}$ + N_{min}-N-$soil_{Mix}$ − N_{min}-N-$soil_{CPS}$

With PS = pure stand; PPS = pea pure stand; CPS = cereal pure stand; Mix = mixed stand

The extended total-N-difference method was selected for several reasons: Using this method makes estimation of N_2 fixation rather inexpensive and can be conducted with

Tab. 4. Overview of the genotypes

Genotype	Growing cycle	Kind of genotype	Colour of flower	convariety	Leaftype	Growing periods
Assas	winter pea	cultivar	purple flowered	*speciosum*	regular leaf	2003/04 – 2006/07
EFB 33	winter pea	cultivar	purple flowered	*speciosum*	regular leaf	2003/04 – 2006/07
Spirit and Cheyenne, respectively	winter pea	cultivar	white flowered	*sativum*	semi-leafless	2003/04 and 2004/05 – 2006/07, respectively
Griechische	winter pea	origin	purple flowered	*speciosum*	regular leaf	2003/04 – 2006/07
Nischkes Riesengebirgs	winter pea	origin	purple flowered	*speciosum*	regular leaf	2003/04 – 2006/07
Unrra	winter pea	origin	purple flowered	*speciosum*	regular leaf	2003/04 – 2006/07
Württembergische	winter pea	origin	purple flowered	*speciosum*	regular leaf	2003/04 – 2006/07
Santana	spring pea	cultivar	white flowered	*sativum*	semi-leafless	2003/04 – 2006/07

little technical equipment (Danso, 1995; Unkovich and Pate, 2000). Moreover, at a limited soil N supply as is generally the case in organic farming, there is a good correspondence between the simple total-N-difference method and the isotope-dilution method (Rennie, 1984; Danso, 1995). Taking into account inorganic N in soil increases the accuracy of estimation as compared to the simple total-N-difference method (Stülpnagel, 1982; Loges, 1998), because it allows consideration of the different N uptake from grain legumes and cereal.

The portion (%) of N derived from atmospheric fixation in total N uptake (NdfA) was determined for pure stands (equation 3). In mixed stands, calculation of NdfA had to be omitted because the aboveground residues of rye and winter pea could not be separated due to the pea's high longitudinal growth up to three meters with several branches (Urbatzka, 2010).

(3) $NdfA_{PPS}$ (%) = 100 × $N\text{-}Fix_{PPS}$/N-uptake $biomass_{PPS}$

2.5 Statistical analysis

Data from field experiments were analyzed using the MIXED and GLIMMEX procedure of the software package SAS 9.1 (Piepho et al., 2003). A comparison of means for pea genotypes in different crop stands was conducted applying a Tukey test. Inorganic nitrogen in soil, N_2 fixation of pea genotypes and NdfA were analyzed at both harvest dates (factor *time*) applying the REPEATED statement (Piepho et al., 2004). Residuals were checked for normal (Gaussian) distribution with QQ-plots and the Shapiro-Wilk test using the procedure UNIVARIATE NORMAL (Duffner et al., 2004). Homogeneity of variances was assessed with the modified Levene test (Brown and Forsythe, 1974).

The analysis of variance across years was confined to the growing seasons 2004/05 until 2006/07, due to a change of the winter and spring cereal mixture partner. Due to the mixed experimental designs used from 2004/05 until 2006/07, treatment effects were analyzed with dummies according to the method supplied by Piepho et al. (2006). Factors *year* and *location* were combined, yielding a fixed factor *environment*, to allow a common analysis of data from the two experimental sites. Further fixed factors were crop stands and genotype.

3 Results

Analysis of variance gave highly significant effects of the factors and their interactions in almost all cases except NdfA. Consequently, all parameters were analyzed for every environment individually (except NdfA) and N_2 fixation and inorganic N in soil for every sampling date. Usually, a significant interaction between genotype and crop stands (pure or mixed stands) was found. For reasons of standardization and clearness, the parameters inorganic N in soil and N yield of aboveground biomass in environments with significant main factors are only presented as if significant interactions were the case (Fig. 1a, b and 2b).

The cultivars Assas and Cheyenne were winter killed or greatly damaged over winter in three and four out of six environments, respectively (data not shown). Hence the analysis of variance was conducted with only six genotypes. At DFH, pure stands of winter peas were damaged severely by mice in 2006/07 and as a consequence mature harvest and calculation of N_2 fixation as well as NdfA had to be omitted.

3.1 N uptake until harvest at flowering

Significant interactions for the response of N uptake until flowering harvest were found for genotype and crop stand in five out of six environments. The N uptake of winter pea was higher than for spring pea. This difference was always significant in pure stands (139 kg N ha^{-1} and 49 kg N ha^{-1}, respectively) and for 31 out of 60 treatments in mixture (92 kg N ha^{-1} and 48 kg N ha^{-1}, respectively) (Fig. 1, 2). Higher N uptake was established at DFH than at WH in almost all treatments (111 kg N ha^{-1} and 66 kg N ha^{-1}, respectively).

Spring crops responded more strongly to the environmental conditions than winter crops (Tab. 5). The highest coefficient of variation for winter crops was determined for winter rye. N uptake of winter cereal pure stands varied most markedly (in descending order): DFH in 2005/06 and 2006/07 (94 kg N ha^{-1}) > DFH in 2003/04 and 2004/05 (60 kg N ha^{-1}) > WH (30 kg N ha^{-1}) (Fig. 1, 2).

3.2 N dynamics in soil

At flowering harvest a slightly but significantly higher amount of inorganic N was measured in the growing seasons 2003/04 until 2005/06 under spring peas (24 kg ha^{-1}) compared with winter peas (13 kg ha^{-1}) in eight out of twelve treatments (crop stands × environments), but not in 2006/07 (Fig. 1, 2). During of crop growth inorganic N increased markedly under winter pea pure stands to 62 kg ha^{-1} at mature harvest and were significantly higher than under spring peas in pure stands (30 kg ha^{-1}) (Fig. 1, 2). The values in 2005/06 for both sites were lower for all pea genotypes in pure stands than in the other growing seasons (Fig. 1c, 2b). Moreover, under cereals in both pure and mixed stands, a consistently lower availability of inorganic N was recorded (13 kg ha^{-1}) compared with pea pure stands (56 kg ha^{-1}) (Fig. 1 and 2).

3.3 N uptake until mature harvest

Significant interactions for the response of N uptake until mature harvest were found for genotype and crop stand in four out of six environments. N uptake of winter peas in mixture was significantly higher (185 kg N ha^{-1}) than for spring pea (98 kg N ha^{-1}) in four out of six environments (Fig. 1b, c and 2a, c), whereas no differences was found between winter and spring crops in pea pure stands (Fig. 1, 2). Besides, mixtures obtained higher N yield than pea pure stands in four out of six environments

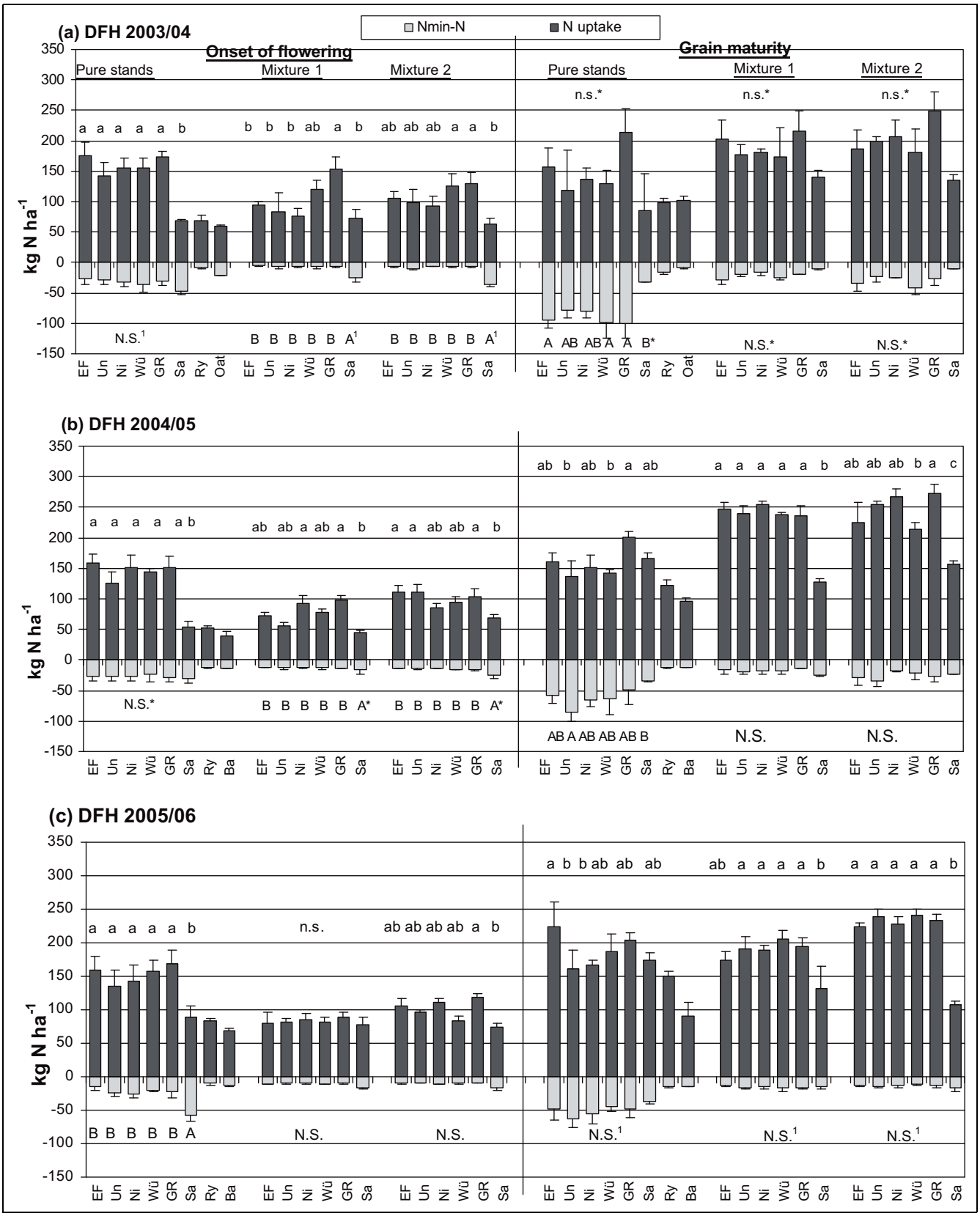

Fig. 1. N-yield of above-ground biomass and inorganic N in soil (0–90 cm) as affected by crop stands and date of sampling (onset of flowering (BBCH 61) or grain maturity (BBCH 89)) (a) DFH 2003/04, (b) DFH 2004/05, (c) DFH 2005/06;
Different letters denote significant differences between pea genotypes (Tukey test, $p < 0.05$): capital letters for N_{min}-N, small letters for N uptake, n.s. = not significant, [1] log-transformation, * no significant interaction of cultivar and crop stand in F-test, results for aboveground residues at grain maturity in DFH 2003/04 were deduced from other years at DFH via N harvest index; EF = EFB 33, Un = Unrra, Ni = Nischkes Riesengebirgs, GR = Griechische, Sa = Santana (spring pea), Ry = rye, Ba = barley.

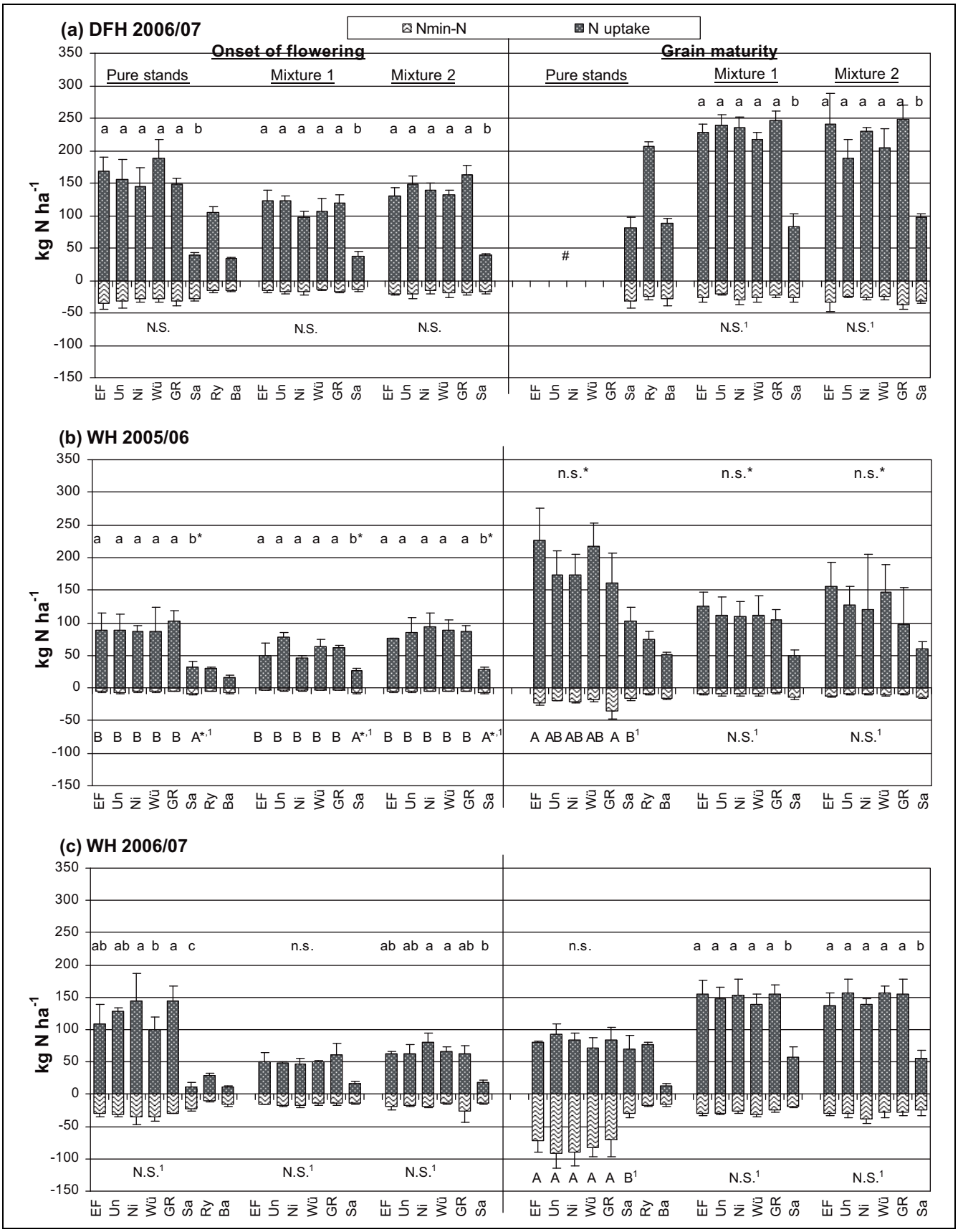

Fig. 2. N-yield of above-ground biomass and inorganic N in soil (0–90 at DFH and 0–60 at WH) as affected by crop stand and date of sampling (onset of flowering (BBCH 61) or grain maturity (BBCH 89)) (a) DFH 2006/07, (b) WH 2005/06, (c) WH 2006/07;
See legend of Fig. 1; [2] Square-root-transformation, # losses due to mice, results for aboveground residues at grain maturity in WH 2005/06 were deduced from other year at WH via N harvest index.

Tab. 5. Coefficient of variation (%) of different pea genotypes in pure stands and mixture and of cereals in pure stands at Frankenhausen 2003/04 – 2006/07 and Waldhof 2005/06 – 2006/07

		EFB 33	Unrra	Nischkes	Wuertt.	GR	Santana	Rye	Spring cereal
Flowering	PS	27.4	23.3	25.0	29.8	19.2	62.1	44.5	55.5
	Mix1	36.2	35.2	30.5	31.8	35.2	52.6		
	Mix2	24.7	31.0	23.9	27.0	31.0	44.7		
Maturity	PS[1]	37.8	34.4	27.0	38.2	33.9	44.0	39.8	47.6
	Mix1	24.8	27.8	27.8	28.6	28.4	43.5		
	Mix2	26.0	25.4	33.5	22.8	34.1	37.1		

PS = pure stands; [1] winter pea except Frankenhausen 2006/07 due to crop losses caused by mice

(Fig. 1a, b and 2a, c). Furthermore, in mixtures and in cereal pure stands the N uptake in DFH was higher than in WH (191 and 112 kg N ha^{-1}, respectively), but in contrast to harvest at flowering, not in pea pure stands.

Usually highest biomass yield stability was measured in mixture of winter peas (Tab. 5). Similar to harvest at flowering the coefficient of variation in winter crops was lower than in spring crops and in winter pea pure stands lower than in rye pure stands. Likewise, rye in pure stands yielded (in descending order, similar to flowering harvest): at DFH in 2005/06 and 2006/07 (178 kg N ha^{-1}) > at DFH in 2003/04 and 2004/05 (110 kg N ha^{-1}) > at WH (76 kg N ha^{-1}) (Fig. 1, 2).

3.4 N_2 fixation at flowering and mature harvest

When harvested at flowering, N_2 fixation for winter peas in 28 out of 30 treatments and for spring peas only in two out of six environments was observed in following order: Pure stands > Mix2 ≥ Mix1 (Tab. 6). When grown in pure stands, winter peas showed a significantly higher N_2 fixation than spring peas (93 kg ha^{-1} and 29 kg ha^{-1}, respectively) in 22 out of 30 treatments. Additionally, mixtures with spring crops were found to have a lower N_2 fixation than mixtures with winter crops, but the difference was only significant in some cases (14 out of 60 treatments). However, slightly negative N_2 fixation was determined in four variants of winter pea-rye mixture at DFH.

During crop growth, not all treatments achieved an increase of N_2 fixation between flowering and mature harvest (Tab. 6, 7), which especially applied for winter pea pure stands in four out of five environments, but also for all spring pea treatments in one environment (Tab. 6d, 7d). At mature harvest N_2 fixation of spring peas in pure stands was in five out of six environments considerably higher than in mixtures (exception shown in Tab. 7d), but only in two out of five for winter peas (Tab. 7b, e). In mixture, winter pea variants showed considerably higher N_2 fixation than spring peas in five out of six environments (Tab. 7a–c, e, f), but only in two out of five for pure stands (Tab. 7a, e). Again at DFH in 2005/06 and 2006/07 in comparison to the earlier growing seasons higher N_2 fixation was usually measured for winter peas, but in contrast to harvest at flowering above all in mixtures with on average 76 kg ha^{-1} lower (Tab. 6a–d, 7a–d).

3.5 Portion of air borne N in total N uptake in pure stands

Analysis of variance over experimental years provided only significant effects for the factors time and year and a significant interaction between these factors.

At flowering harvest, the lowest NdfA of 45 and 55% was measured at DFH in 2005/06 and 2006/07, respectively, which was lower than in the other four environments (64–93%) (Tab. 8). Between flowering and mature harvest, NdfA decreased in three out of five environments and remained on the same level in the other two environments. At WH both at flowering and mature harvest the NdfA was higher than at DFH. Besides, no differences were found between pea genotypes: Ndfa at flowering harvest (66%) was slightly higher than at mature harvest (62%) (Tab. 9).

4 Discussion

4.1 N uptake until flowering harvest

The higher N yields of winter peas in comparison to spring pea may be traced back to their earlier N uptake due to a different course of growth, the different growing pattern (indeterminate vs. determinate) and the consequently different haulm length which considerably affected biomass dry matter (URBATZKA, 2010; Fig. 1, 2). N uptake responded to the quality of the soil at the two experimental sites, being higher at DFH compared with WH in all treatments. Moreover, effects of the individual seasons were apparent and could be traced back to the preceding crop and the weather conditions resulting in different N availability. High N availability as a result of the preceding root crops and prevailing mild weather in autumn and/or winter (Tab. 1, 2) favoured above all the development of rye (Fig. 1c, 2a). In contrast to rye, winter peas as grain legumes seem to have compensated low N availability by N fixation resulting in pronounced biomass yield stability over the two experimental sites (Tab. 5).

Tab. 6. N_2 fixation of different pea genotypes as affected by crop stand and environment at onset of flowering (BBCH 61); mean (kg N ha^{-1}) ± standard deviation

	EFB 33	Unrra	Nischkes	Württemb.	GR	Santana (SP)	all genotypes
(a) DFH 2003/04							
PS	127 ± 26 a A	95 ± 32 a A	113 ± 26 a A	115 ± 9 a A	129 ± 14 a A	35 ± 4 ns B	
Mix1	24 ± 19 b NS	15 ± 32 b	7 ± 17 b	51 ± 18 b	54 ± 13 b	16 ± 14	
Mix2	36 ± 8 b NS	32 ± 19 b	24 ± 7 b	58 ± 14 b	62 ± 14 b	17 ± 9	
(b) DFH 2004/05							
PS	121 ± 13 a A	88 ± 25 a A	114 ± 23 a A	103 ± 7 a A	115 ± 18 a A	32 ± 16 ns B	
Mix1	20 ± 9 b AB	5 ± 5 c B	41 ± 12 b A	26 ± 8 b AB	47 ± 7 b A	10 ± 7 B	
Mix2	60 ± 15 b NS	59 ± 16 b	34 ± 10 b	44 ± 14 b	56 ± 10 b	41 ± 12	
(c) DFH 2005/06*							
PS	81 ± 26	66 ± 15	75 ± 23	84 ± 17	98 ± 22	63 ± 21	78 ± 24 a
Mix1	6 ± 2	−3 ± 8	3 ± 12	−1 ± 6	4 ± 6	10 ± 12	3 ± 5 b
Mix2	22 ± 14	12 ± 8	28 ± 9	−2 ± 10	33 ± 9	7 ± 12	17 ± 12 b
CS	37 ± 23 NS	25 ± 19	35 ± 20	27 ± 21	45 ± 34	27 ± 19	
(d) DFH 2006/07							
PS	85 ± 18 a A	67 ± 33 ns AB	52 ± 39 a AB	97 ± 23 a A	60 ± 17 ns AB	17 ± 5 ns B	
Mix1	19 ± 13 b NS	19 ± 21	−7 ± 22 b	1 ± 18 b	16 ± 21	1 ± 6	
Mix2	31 ± 20 ab AB	50 ± 12 AB	36 ± 10 ab AB	30 ± 5 b AB	61 ± 12 A	6 ± 9 B	
(e) WH 2005/06*							
PS	59 ± 25	60 ± 24	57 ± 12	57 ± 38	72 ± 18	18 ± 6	54 ± 29 a
Mix1	18 ± 15	46 ± 10	15 ± 6	32 ± 13	31 ± 5	10 ± 6	25 ± 26 b
Mix2	47 ± 2	55 ± 23	64 ± 20	58 ± 13	55 ± 10	13 ± 2	49 ± 22 ab
CS	41 ± 25 A	54 ± 21 A	45 ± 26 A	49 ± 29 A	53 ± 21 A	14 ± 6 B	
(f) WH 2006/07							
PS	98 ± 26 a B	120 ± 3 a AB	140 ± 40 a A	95 ± 13 a B	133 ± 23 a A	8 ± 12 ns C	
Mix1	25 ± 13 b NS	25 ± 5 b	23 ± 12 b	23 ± 5 b	35 ± 19 b	4 ± 10	
Mix2	41 ± 9 b AB	39 ± 16 b AB	59 ± 15 b A	39 ± 8 b AB	48 ± 19 b AB	6 ± 6 B	
Mean of all environments							
PS	95	83	92	92	101	29	
Mix1	19	18	14	22	36	8	
Mix2	40	41	41	38	52	15	

Different small or capital letters = significant differences between crop stands or genotypes for every single environment;
ns or NS = no significant differences between crop stands or genotypes, respectively, * = no significant interaction in F-test;
PS = pure stand, Mix1 = Mixture 1, Mix2 = Mixture 2, CS = crop stands, SP = spring pea

4.2 Inorganic N in soil until mature harvest

The elevated concentration of inorganic N in soil at green harvest of spring crops when compared with winter crops in 2003/04 to 2005/06 (Fig. 1a–c, 2b) was probably a consequence of soil tillage prior to sowing in spring and the N uptake by crops in autumn and early winter. In 2006/07, the fact that no significant differences were established for winter and spring crops was presumably due to relatively high N leaching when precipitation and temperature during the preceding winter were unusually high (Fig. 2a, c; Tab. 2, 3).

The strong increase of inorganic N until mature harvest under winter peas in pure stands in comparison to spring pea pure stands was in accordance with the observations by KARPENSTEIN-MACHAN and STÜLPNAGEL (2000) and can not only be explained by N-sparing effects, but also by early mineralization of above ground debris, N-rhizodeposition (URBATZKA et al., 2009) and the subse-

Tab. 7. N_2 fixation of different pea genotypes as affected by crop stand and environment at mature harvest (BBCH 89); mean ± standard deviation

	EFB 33		Unrra		Nischkes		Württemb.		GR		Santana (SP)		all genotypes
(a) DFH 2003/04*													
PS	137 ± 25		134 ± 11		101 ± 9		112 ± 11		198 ± 11		51 ± 10		122 ± 49 a
Mix1	116 ± 20		81 ± 19		81 ± 9		82 ± 46		119 ± 42		39 ± 17		86 ± 43 b
Mix2	105 ± 27		107 ± 8		116 ± 34		106 ± 27		161 ± 35		34 ± 14		105 ± 53 ab
CS	119 ± 13	AB	107 ± 29	B	99 ± 27	B	100 ± 32	B	160 ± 42	A	41 ± 18	C	
(b) DFH 2004/05													
PS	86 ± 26 ns	NS	90 ± 33 ns		84 ± 4 ns		65 ± 25 ns		118 ± 10 ns		94 ± 11 ns		
Mix1	130 ± 25	A	127 ± 3	AB	142 ± 9	A	124 ± 16	AB	116 ± 7	AB	49 ± 22	B	
Mix2	121 ± 47	AB	151 ± 23	A	150 ± 17	A	103 ± 19	AB	163 ± 25	A	72 ± 14	B	
(c) DFH 2005/06													
PS	94 ± 13 a	NS	59 ± 35 ns		57 ± 15 ns		67 ± 25 ns		87 ± 17 ns		106 ± 30 a		
Mix1	23 ± 14 b	NS	42 ± 19		39 ± 12		59 ± 15		46 ± 16		29 ± 10 b		
Mix2	74 ± 12 ab	AB	90 ± 10	A	77 ± 11	AB	87 ± 10	A	83 ± 16	AB	19 ± 25 b	B	
(d) DFH 2006/07*													
PS	#		#		#		#		#		14 12		
Mix1	26 ± 14		30 ± 11		34 ± 13		14 ± 7		37 ± 12		7 ± 13		25 ± 16 ns
Mix2	32 ± 21		1 ± 18		26 ± 12		2 ± 8		52 ± 23		12 ± 11		21 ± 13
CS	29 ± 18	AB	15 ± 11	AB	30 ± 13	AB	8 ± 5	B	45 ± 20	A	10[1] ± 12	B	
(e) WH 2005/06*													
PS	166 ± 39		110 ± 25		112 ± 20		154 ± 25		114 ± 45		53 ± 22		118 ± 48 a
Mix1	51 ± 19		38 ± 26		36 ± 17		38 ± 20		29 ± 3		4 ± 10		32 ± 24 b
Mix2	86 ± 28		54 ± 23		83 ± 46		74 ± 31		53 ± 20		8 ± 11		60 ± 39 b
CS	101 ± 56	A	68 ± 40	B	77 ± 43	AB	89 ± 55	AB	65 ± 47	B	19 ± 17	C	
(f) WH 2006/07													
PS	59 ± 20 ns	NS	92 ± 24 ns		82 ± 13 ns		62 ± 24 ns		60 ± 27 ns		71 ± 24 ns		
Mix1	91 ± 19	NS	83 ± 14		87 ± 27		76 ± 17		86 ± 15		47 ± 21		
Mix2	74 ± 18	NS	93 ± 21		84 ± 4		92 ± 5		90 ± 25		52 ± 20		
Mean of all environments													
PS	108		97		87		92		116		75[2]		
Mix1	73		67		70		65		72		29		
Mix2	82		83		89		77		100		33		

See legend of Tab. 5; # = data missing because of crop losses due to mice, [1] mean of Mix1 and Mix2, [2] mean without DFH 2006/07

quent N priming effects (JENKINSON et al., 1985). In contrast, the higher inorganic N under spring peas in pure stands – in comparison to mixed stands – was probably mainly caused by N-sparing effects (URBATZKA et al., 2009). Inorganic N was markedly lower in 2005/06 (Fig. 1c, 2b), probably as a result of the relatively dry weather conditions during June and July (Tab. 2, 3).

Moreover, the consistently lower availability of inorganic N under cereals in both pure and mixed stands compared with pea pure stands may be a consequence of the different root systems of the partners in mixture (Fig. 1, 2). It is widely-known that cereal plants have a higher root mass and length and display a deeper root soil penetration (e.g. HAMBLIN and TENNANT, 1987; SCHMIDTKE and RAUBER, 2000).

4.3 N uptake until mature harvest

At both harvest dates, N uptake of above-ground biomass in mixture treatments responded to the quality of the soil at the two experimental sites, being higher at DFH com-

Tab. 8. Portion of N derived from atmospheric fixation in total N uptake (%) in the treatments with pure stands, Frankenhausen 2003/04 – 2006/07 and Waldhof 2005/06 – 2006/07; means of environments for both harvest dates over six genotypes, ± = standard deviation

	DFH 2003/04	DFH 2004/05	DFH 2005/06	DFH 2006/07	WH 2005/06	WH 2006/07
Flowering	70 ± 12 ns B	71 ± 12 a B	55 ± 10 a C	45 ± 9 – D	64 ± 10 ns BC	93 ± 5 a A
Mature	73 ± 18 AB	52 ± 12 b B	38 ± 13 b C	#	66 ± 12 B	82 ± 19 b A

Different small or capital letters = significant differences between harvest dates or environments, respectively, $p < 0.05$ (Tukey test); ns or NS = no significant differences between harvest dates or environments, respectively; # data missing because of crop losses due to mice

Tab. 9. Portion of N derived from atmospheric fixation in total N uptake (%) in the treatments with pure stands, Frankenhausen 2003/04 – 2006/07 and Waldhof 2005/06 – 2006/07 (except Frankenhausen 2006/07 at mature harvest due to crop losses because of mice; means of genotypes over both harvests and six environments, ± = standard deviation

	Flowering	Mature
EFB 33	67 ± 16	64 ± 20
Unrra	64 ± 19	64 ± 24
Nischkes	68 ± 18	62 ± 22
Württemb.	69 ± 16	61 ± 23
GR	68 ± 18	61 ± 20
Santana (SP)	60 ± 20	59 ± 22
Mean	66	62

SP = Spring pea

pared with WH. Thereby the same effects of the individual seasons were important for the different levels of N uptakes as discussed in the previous paragraphs. The lower or similar N uptakes from winter pea pure stands in comparison to mixtures were a consequence of the usually higher pea grain yields in mixture due to better growing conditions (URBATZKA et al., 2011) and loss of above ground biomass (leaves, pods) during vegetative growth (URBATZKA et al., 2009), which were not taken into account.

4.4 N_2 fixation and nitrogen derived from atmospheric fixation (NdfA) at flowering harvest

At flowering harvest N_2 fixation of almost all treatments was in accordance with plant density, which usually was a function of crop stand: Pure stands > Mix2 ≥ Mix1 and confirmed by previous research (WATERER et al., 1994; KARPENSTEIN-MACHAN and STÜLPNAGEL, 2000; PEOPLES et al., 2001) (Tab. 6). The higher N_2 fixation of winter peas in comparison to spring pea was most probably a consequence of earlier N_2 fixation and the indeterminate growth pattern resulting in higher above-ground biomass, hence presumably higher below-ground biomass (IWAMA and YAMAGUCHI, 1996) of the winter crop. Accordingly, (still inactive) nodules were found in autumn as early as the four-leaf stage (BBCH 14) (data not shown). For spring peas, JENSEN (1996) also observed a comparatively low N_2 fixation because of lower N_2 fixation rates during vegetative growth and at the beginning of flowering. Besides, the level of soil inorganic N reflected the portion of N derived from atmospheric fixation (Tab. 8) and was in accordance to VOISIN et al. (2002).

The slightly negative values for N_2 fixation (Tab. 6) can be traced back to the fact that the roots of the plants were not taken into account by using the extended total-N-difference method. Recent research showed that below-ground N from roots and N-rhizodeposition represent a substantial part of legume N (MAYER et al., 2003; WICHERN et al., 2007). Hence in accordance to PEOPLES et al. (2002) indirect measurements considerably underestimate N_2 fixation of legumes.

4.5 N_2 fixation and nitrogen derived from atmospheric fixation (NdfA) at mature harvest

JENSEN (1996) also measured constant N_2 fixation from the early pod filling stage onwards for spring pea in mixture with cereals. He concluded that this was due to competition for light in mixture, whereas in our experiments spring pea responded to the unfavourable environmental conditions at DFH in 2006/07 much more than the reference crop, spring barley (six weeks drought in spring resulting in delayed emergence and an unusual high precipitation in May, to which peas react sensitive (GEISLER, 1983; Tab. 2, 7d)).

In contrast to spring pea, the apparent reduction in N_2 fixation of winter peas in pure stands in most environments was a consequence of the loss of biomass during vegetative growth (Tab. 6, 7). This is confirmed by the N_2 fixation of Mix2 of winter peas, which was found to be equally high as or higher than in pure stands in spite of the reduced seeding rates. The debris from above-ground biomass was certainly much more marked than the 2 kg ha^{-1} mentioned by KAUL (2004) for spring peas, because of the winter pea's great haulm length of up to 3 m and the relatively low number of pods at mature harvest (URBATZKA, 2010). Presumably, some of this N was mineralized until mature harvest due to low C/N-ratio, as it was shown for N-rhizodeposition of pea (WICHERN et al., 2007). As a consequence, this fraction was only partially

taken into account including inorganic N, when estimating N_2 fixation by the extended difference method. Hence N_2 fixation of winter peas in pure stands was most probably underestimated. In terms of the rate of its transformation and the amount of N in the debris of above-ground biomass, further research is certainly required.

In contrast to winter peas, a lower N_2 fixation was observed in spring crops mixture compared with pure stands, which was caused by the lower crop density in accordance with other studies (Jensen, 1996; Wichmann, 2003; Corre-Hellou et al., 2006). The calculated values in our study were comparatively low, since in other studies N_2 fixation of spring peas in pure stands ranged between 40 and 240 kg ha^{-1} (e.g. Jensen, 1996; Wichmann, 2003; Corre-Hellou et al., 2006).

The higher N_2 fixation of winter peas in mixture as compared with spring peas was a consequence of earlier N_2 fixation and an indeterminate growth pattern resulting in higher above-ground biomass and probably higher below-ground biomass (Iwama and Yamaguchi, 1996). In pure stands, however, no consistent pattern could be observed, even though, based on the results on N_2 fixation at flowering harvest and at mature harvest (mixture), a higher N_2 fixation of winter peas would be expected. This, again, can probably be traced back to the loss of N (above all debris of above-ground biomass) not accounted for in our experiments.

The great variation of symbiotic N_2 fixation was a result of legume and cereal establishment and growth affected by the prevalent environmental conditions. For example the relative low N_2 fixation for spring pea pure stand at DFH in 2003/04 (Tab. 7a) was probably a consequence of severe weed infestation in the experimental plots (Urbatzka et al., 2011), which suppressed pea development. Also the lower N_2 fixation of winter peas in mixture at DFH in 2005/06 and 2006/07 (Tab. 7c, d) in comparison to the two earlier growing seasons can be explained by a relatively high portion of cereal grain yield (Urbatzka et al., 2011), which again was a result of the relatively high N availability resulting in a suppression of peas (Jensen, 1996). This was, analogous to harvest at flowering, also reflected by the relatively low portion of N derived from the atmosphere (NdfA) (Tab. 8) which was also observed by Voisin et al. (2002).

5 Conclusion

In the presented study the N_2 fixation of different winter and spring pea genotypes was measured at beginning of flowering and at maturity with the extended total N difference method. Certainly the N_2 fixation was underestimated, because N either associated with or derived from nodulated roots was taken into account insufficiently. Hence, the present study shows that further methodological research is required if statements on the course of N fixation of winter grain legumes and rates of N transformation in soil shall be made.

Results show that the earlier crop development of winter peas advances nodulation, and the generally higher above ground biomass and presumably root biomass leads to usually higher N_2 fixation of regular leaf winter peas at flowering and mature harvest (BBCH 61 and 89) as compared with spring peas. Moreover N_2 fixation mostly responds positively to increasing pea crop density. Another striking finding from the work was a similar N_2 fixation at mature harvest when grown in mixture compared with pure stands that could only be established for winter peas. The effect can most certainly be attributed to the better growth conditions in mixture, resulting in higher pea grain yields and N losses (debris) of pure stands that could at least partially be taken into account. Furthermore, N derived from atmospheric fixation of pure stands does not seem to respond to genotype, yet depends on the time of assessment, and is higher the lower soil fertility and/or N availability are.

N yield of aboveground biomass is usually higher for regular leaf winter peas than for spring pea because of earlier N uptake and an indeterminate growth pattern. Data on N uptake at mature harvest clearly show that the higher environmental stress resistance of mixtures is a major argument for cultivation of pea-cereal mixtures. In contrast this is not valid for spring peas probably due to their lower competitiveness concerning crop development and establishment in pure stands and mixture.

Overall, it can be concluded that cultivation of winter peas may be a valuable alternative to spring peas because of their higher level of N_2 fixation. Additionally, when grown in mixture, N availability after mature harvest may be rather low reducing the risk of N leaching.

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