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Winter barley grown in a long-term field trial with a large variation in N supply: Grain yield, yield components, protein concentration and their trends

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ABSTRACT

Information on the responsiveness of winter barley to nitrogen (N) is scarce. Based on a long-term field trial (1978–2015) with different winter barley varieties in northern Germany combined with 64 N fertilizer treatments differing in amount (0–360 kg N ha^{-1}) and distribution, the effects of N fertilizer amount and variety on the grain yield and its components, grain protein concentration (GPC), N use efficiency (NUE), and apparent fertilizer N recovery (AFR) were determined. In addition, quadratic N response curves and linear trends over the experimental period (parameters derived from annual N response curves) were estimated. N fertilization increased grain yield in all varieties, mainly due to a larger number of grains m^{-2} rather than to an improved thousand grain weight (TGW). The newer varieties released since the late 1990s outyielded the older ones, especially at higher N supply, revealing a clear breeding progress, which in turn resulted in an improved NUE and AFR. Only if exceeding 190 kg N ha^{-1} AFR started to decrease. During the trial, GPC in the unfertilized control significantly decreased, while the yield slightly, but non-significantly increased. In contrast, at economic optimum N supply, a significant increase in grain yield and number of grains m^{-2} occurred while TGW and GPC remained stable over time. From the practical view of barley growing in humid climates, our results suggest to provide sufficient grains per area (sinks) in order to tap the full yield potential, especially against the background of climate change with its predicted temperature increase in spring and summer impairing grain filling.

1. Introduction

Nitrogen (N) fertilization has to be optimized in order to minimize environmental impacts, and, at the same time, to ensure high yields. The high yield potential in Northern and Central Europe requires to apply mineral N fertilizers to winter barley at least at 3 growth stages (Maidl et al., 1996), normally at the beginning of spring growth during tillering (N1), at the beginning of stem elongation (N2), and at ear emergence (N3). The better synchronization of N supply and crop N demand allows to reduce losses and, in consequence, to increase fertilizer N recovery (Kanwar et al., 1988; Maidl et al., 1996; Sieling et al., 1998). While a lot of literature dealing with N fertilization of wheat is available, information of the effects of increasing total N amounts and their N splittings on barley yield and apparent N fertilizer recovery (AFR) is scarce.

In their trial series in south Germany, Maidl et al. (1996) used 10 treatments (0–170 kg N ha^{-1}) which were split applied in autumn, at the beginning of spring growth, at growth stage (GS) 30, GS 32 and/or GS 49. The six-row cultivar achieved highest yields if fertilized with 110 kg N ha^{-1}, while the two-row cultivar required 140 kg N ha^{-1}. The authors reported that the latter one apparently required more N in the early growth period to establish an optimum number of fertile tillers, while the yield of the six-row cultivar mostly depended on the formation of large ears, thus needing less N until stem elongation. In years without lodging, apparent N fertilizer recovery (AFR) was similar in both cultivars with higher values being attained by late applications. Sieling and Kage (2021) observed in winter wheat an increased AFR of N application at GS 30 and at GS 49 compared to that at the beginning of spring growth. In a 3-year trial in Ireland, Hackett (2016) tested 6 N treatments (33% at the beginning of spring growth, 67% at the beginning of stem elongation) ranging between 0 and 260 kg N ha^{-1} in a range of winter barley cultivar types (line and hybrid types, two- and six-row types) and found similar N response curves for all types. Depending on the cultivar type, the number of grains per m^{-2} (mainly due to the ear density) was most closely correlated with yield. In trials from Argentina

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(Arisnabarreta and Miralles, 2006; 2015), changes in number of grains per land area better explained N supply induced variations in grain yield than the grain weight, mainly due to an improved radiation interception. This is in line with results of Kennedy et al. (2017) obtained in 2-row spring barley in Ireland.

In order to explain the barley yield variation in UK, Bingham et al. (2007a, b) quantified the potential supply of photosynthates for grain filling by analyzing radiation interception, radiation-use efficiency (RUE), and carbohydrate storage reserves. Within a trial series at six sites and over three years they observed a non-linear relationship between the cumulative interception of post-anthesis photosynthetically active radiation and the respective biomass accumulation (= RUE) for some environments. The authors attributed a RUE decrease during the latter stages of grain filling to a feedback inhibition from a limited sink capacity when the potential assimilate formation exceeds the storage capacity of the grains. Beside the period of grain filling they highlighted the relevance of the phases before anthesis in order to provide a sufficient sink potential (Bingham et al., 2007b; Kennedy et al., 2017).

Several authors investigated the breeding progress of (mainly spring) barley in terms of yield, yield components and N use efficiency in different environments (e.g., Abeledo et al., 2003; Bertholdsson and Kolodinska Brantestam, 2009; Bingham et al., 2012; Mirosavljević et al., 2016, 2020a, b; Rajala et al., 2017). Most of them attributed the yield gain during the last decades to an increased number of grains m^{-2} (mainly due to more ears m^{-2} (Abeledo et al., 2003)) and only partly to a higher thousand grain weight (e.g., Mirosavljević et al., 2016, 2020a; Rajala et al., 2017). This often went along with an increased DM due to a higher radiation interception (Abeledo et a, 2003) and N accumulation around ear emergence (Bingham et al., 2012; Rajala et al., 2017). Additionally, an improved harvest index occurred (Bertholdsson and Kolodinska Brantestam, 2009; Bingham et al., 2012; Mirosavljević et al., 2020a). The increased yield level in combination with a constant N fertilization led to an improved N uptake efficiency (N offtake per unit N supply; Bingham et al., 2012) or N use efficiency (grain yield per unit N supply; Rajala et al., 2017). Bingham et al. (2012) suggested that the positive correlation between N uptake efficiency with post anthesis, but not pre-anthesis, dry matter accumulation and N uptake resulted from an increased N demand from a large grain sink.

Based on a long-term field trial with up to 64 N treatments varying in time of application and amount in winter barley, we estimated N response curves for grain yield, grain protein concentration and related parameters like N offtake or apparent fertilizer N recovery. We hypothesize that newer winter barley genotypes achieved higher grain (N) yields due to an increase in the number of grains m^{-2} and an improved apparent fertilizer recovery. In addition, we tried to identify linear trends over time of several parameters derived from year and genotype specific N response curves based on the N amounts applied at the different growth stages (e.g. grain yield and grain protein concentration at zero or optimal N supply). We expect over time an increase of the grain yield and N offtake with the grain at optimal N supply but decreasing grain protein concentration in the unfertilized treatment due to the dilution effect.

2. Material and methods

2.1. Site and soil

The experiment was carried out at the Hohenschulen Experimental Farm (9°59' E, 54°18' N) of the Kiel University, located in Northern Germany 15 km west of Kiel (Schleswig-Holstein). The soil at the site was a pseudogleyic sandy loam (Luvisol: 100 g kg^{-1} clay, pH 6.7, 82 mg kg^{-1} P, 200 mg kg^{-1} K, 215 mg kg^{-1} Mg, 13.8 g kg^{-1} organic C, 1.1 g kg^{-1} N_{total}).

At the experimental site, total annual rainfall averages 750 mm, with about 400 mm received during April - September (Tab. S1).

Table 1
Winter barley varieties tested in the field trial.

Harvest years	Variety	Type	Year of release
1978–2002	Dura	6-row	1961
1987–1991	Alraune	2-row	1984
1992–1999	Alpaca	6-row	1987
2000–2004	Carola	6-row	1998
2005–2008	Franziska	6-row	2000
2009–2012	Fridericus	6-row	2006
2013–2015	Souleyka	6-row	2009

2.2. Treatments and measurements

Established in 1976, winter barley (varieties see Table 1) was grown within a winter oilseed rape – winter wheat – spring oats - winter barley rotation. Winter barley was fertilized with N as calcium ammonium nitrate with 27% N at three growth stages (GS): beginning of spring growth (below named as N1; 0, 40, 80 or 120 (the latter one since 1981) kg N ha^{-1}), beginning of stem elongation (GS 30/31) (N2; 0, 40, 80 or 120 kg N ha^{-1}), beginning of ear emergence (GS 50/51) (N3; 0, 40, 80 or 120 (the latter one since 2003) kg N ha^{-1}). All single splitting treatments were combined resulting in 64 (4 × 4 × 4) different N treatments (since 2003) with total N amounts ranging from 0 (0/0/0) to 360 (120/120/120) kg N ha^{-1}. Practical constraints required the field trial design to be split-plot with the 3 applications dates as splitting levels within 2 blocks; thus, the N treatments were not completely randomized. The same treatments were applied to winter wheat, however, in an inverse pattern. Oilseed rape (90 kg N ha^{-1}) and oats (60 kg N ha^{-1}) were fertilized below optimum, allowing them to level out differences in the residual N due to the N supply to and the N offtake by the harvest products of the respective preceding crops in order to ensure uniform conditions to the subsequent test crops wheat and barley.

Straw of all crops remained on the plots. Crop management not involving the treatments (e.g. soil tillage, sowing date, application of pesticides and plant regulators) was the same in all plots and according to standard farm practice. Since all plots received the same N amount within a rotation, we assume that no accumulation effects due to the N treatments occurred over time, although differences in the N offtake by the harvest products occurred within the same N fertilizer amount due to varying distribution patterns (e.g. 12 different combinations for 200 kg N ha^{-1}).

Until 2002, the total plot size was 1.5 m × 12 m, of which an area of 1.5 m × 6 m was used for the combine harvest; afterwards it was 3 m × 12 m (with a core of 1.75 m × 6 m for combine harvest). Grain yield was standardized to t ha^{-1} with 86% dry matter (DM) based on the moisture content of a grain subsample. Unfortunately, the number of ears m^{-2} was not counted each year. The number of grains m^{-2} was calculated by dividing the grain yield by the thousand grain weight (TGW), which was determined by default during subsample processing. Grain protein concentration (given at 100% DM) was calculated by multiplying the respective N concentration of the subsample (determined by NIRS (Near-InfraRed-Spectroscopy)) with the conversion factor 6.25). Due to the long experimental period, different NIR analyzers were used; however, additional control measurements (e.g. chemical analysis) were done to ensure correct readings. N offtake by the grain was obtained by multiplying the grain DM with the N concentration of the combine-harvested grain. The difference between N fertilization and N offtake by the grains gave the N balance.

Nitrogen use efficiency (NUE; %) (Eq. 1) was determined by relating N offtake by the grains of the fertilized plots ($Noff_{fertilized}$, kg N ha^{-1}) to the N amount applied (kg N ha^{-1}).

$$NUE = \frac{Noff_{fertilized}}{N \quad amount \quad applied} 100 \tag{1}$$

Apparent fertilizer recovery (AFR; %) (Eq. 2) was calculated by comparing N offtake by the grains (Noff, kg N ha^{-1}) of the fertilized

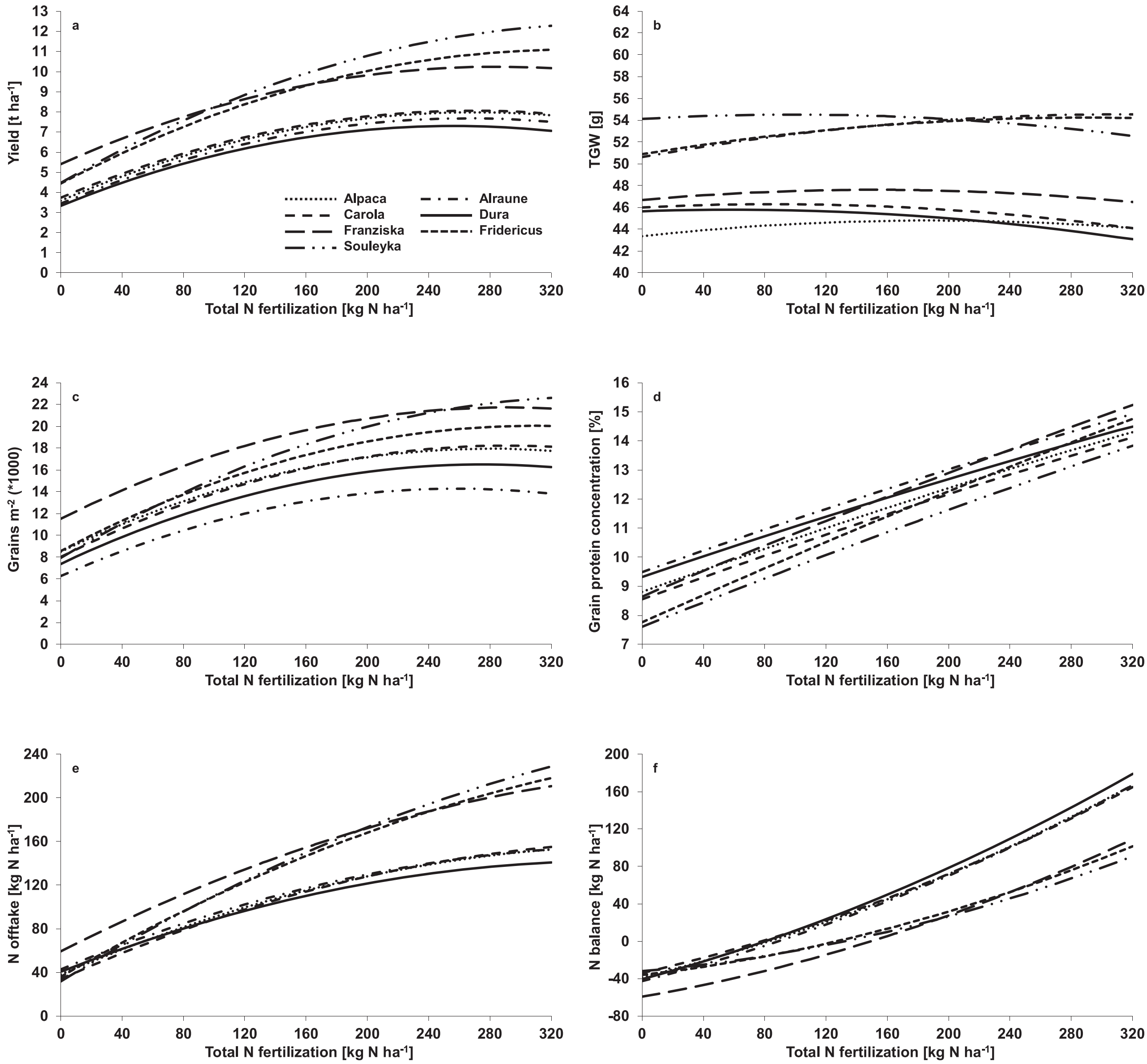

Fig. 1. Mineral N fertilizer effects on grain yield (a), thousand grain weight (TGW) (b), number of grains m^{-2} (c), grain protein concentration (d), N offtake by the grain (e), and N balance (N fertilization minus N offtake by the grain) (f) of winter barley. (N3 = 0, 40 or 80 kg N ha^{-1}, means over the years in which the variety was included in the trial).

plots with Noff of the corresponding unfertilized plot in relation to the N amount applied (kg N ha^{-1}). This approach assumes the same soil N mineralization in both fertilized and unfertilized plots.

$$AFR = \frac{Noff_{fertilized} - Noff_{unfertilized}}{N \quad amount \quad applied} 100 \tag{2}$$

2.3. Statistical analysis

The data from 2011 were considered as outliers and excluded since unfavorable conditions (especially poor field emergence and low temperatures during winter) led to poor crop growth and unusually low yields. In addition, only years where both, grain yield and grain N concentrations, were available, were included in the analyses; missing grain N concentrations caused the exclusion of the harvest years 1976, 1977, 1981 and 1983.

N response curves for different parameters (grain yield (GY, t ha^{-1}), grain protein concentration (GPC, % at 100% DM), N offtake by the grains, yield components) were fitted to the crop data:

$$Y = a + b \bullet N + c \bullet N^2 + d \bullet var + f \bullet var \bullet N \tag{3}$$

where *Y* stands for the different parameters. *N* denotes the amount of fertilizer N (kg N ha^{-1}) applied throughout the growing period, *var* is the variety and *a*, *b*, *c*, *d,* and *f* are constants (Tab. S2). The analysis of covariance was done using the MIXED procedure of SAS statistical package (version 9.4, SAS Institute 2002–2012) with the years as random factor.

For each year x variety combination, the following models for the N response curves were fitted to the crop data:

$$Y = a + b \bullet N1 + c \bullet N2 + d \bullet N3 + e \bullet N1^2 + f \bullet N2^2 + g \bullet N3^2 + h \bullet N1 \bullet N2 + i \bullet N1 \bullet N3 + j \bullet N2 \bullet N3 \tag{4}$$

where *Y* represents GY (t ha^{-1}), GPC (% at 100% DM), and N offtake by the grains (Noff, kg N ha^{-1}), respectively. *N1*, *N2* and *N3* are the amounts of N fertilizer (kg N ha^{-1}) applied at the beginning of spring growth (N1), beginning of stem elongation (N2), and beginning of ear emergence (N3). *a, b, c, d, e, f, g, h, i* and *j* are constants (Tab. S3 - S5) which were estimated using the REG procedure of SAS.

From eq. 4, several parameters (economic N fertilization optimum (Nopt); grain yield (GYopt), grain protein concentration (GPCopt) and N offtake at Nopt; grain yield (GY0), grain protein concentration (GPC0) and N offtake at 0 kg N ha^{-1}) were *ex post* estimated separately for each year and each variety by means of the add-in program 'Solver' of Microsoft Excel™, assuming 1 € (kg N)$^{-1}$ and 185 € t^{-1} barley. The N amount of each splitting was restricted to the range used in the field trial.

These estimated parameters were then analyzed for a linear trend over the duration of the experiment in combination with the variety according to Laidig et al. (2017) and Reckling et al. (2021).

$$Y = a + b\bullet year + c\bullet var \tag{5}$$

where *year* is the duration of the experiment since 1978 and *var* the variety, and *a, b,* and *c*, are constants estimated by the MIXED procedure of SAS.

In order to dissect genetic and non-genetic trends, we used mixed linear models with a linear regression term according to Piepho et al. (2014).

$$Y = a + b\bullet year + c\bullet rlyear \tag{6}$$

where *year* is the duration of the experiment since 1978 and *rlyear* the year of release of the variety (Table 1), and *a, b,* and *c*, are constants estimated by the MIXED procedure of SAS. Statistics for eq. 5 + 6 are given in Tab. S6 in the supplements.

Analyses of variance of NUE and AFR were performed, using the MIXED procedure of SAS, where the years were considered as random factor.

3. Results

The varieties were (mostly) grown in different years (Table 1) and consequently, the interaction year x variety cannot be unraveled. Therefore, the variety effect may include effects resulting from an improved crop management (e.g., crop protection) and from changing environmental conditions (e.g., increasing CO_2 level).

3.1. Grain (N) yield, yield components and N balance

The effects of the total N fertilization were estimated from the respective N response curves (eq. 3, Tab. S2) on average of all years.

Nitrogen fertilization significantly ($P < 0.0001$) increased grain yield of all varieties tested (Fig. 1a). The newer varieties cvs. Franziska, Fridericus and Souleyka outyielded the older ones, especially at higher N levels. In years where 2 varieties were grown together (cvs. Dura and Alpaca, Dura and Alraune, Dura and Carola), cv. Dura which has been released in 1961 yielded significantly less than the newer ones (not shown).

While the thousand grain weight (TGW; Fig. 1b) remained unaffected by the N fertilization, the number of grains m^{-2} showed a similar pattern as the grain yield (Fig. 1c), indicating that the yield increase was mainly the result of an enhanced number of grains m^{-2} rather than of a raised TGW (Fig. S1). As expected, Alraune as a 2-row barley type stood out with its higher TGW in combination with a lower number of grains m^{-2}, while the high TGWs of Fridericus and Souleyka, both 6-row types, were somewhat surprising, although both varieties were classified in their registration as '6' (range of the classification: 0 (very low) - 9 (very high)).

Grain protein concentration increased nearly linearly with an improved total N supply (Fig. 1d); however, the absolute level as well as the slope differed between the varieties with a steeper increase in cvs. Franziska, Fridericus and Souleyka (coefficient in Tab. S2). Although the latter one showed the lowest protein concentration, cv. Souleyka due to its high grain yield, as well as cvs. Franziska and Fridericus, achieved the highest N offtake by the grain, if sufficient N was available (Fig. 1e), indicating an improved ability to utilize available N. Consequently, the older varieties with their smaller N offtake revealed a higher N surplus. Their N balances became positive if N fertilization exceeded 78–90 kg N ha^{-1} compared to 110–150 kg N ha^{-1} in the newer varieties (Fig. 1f).

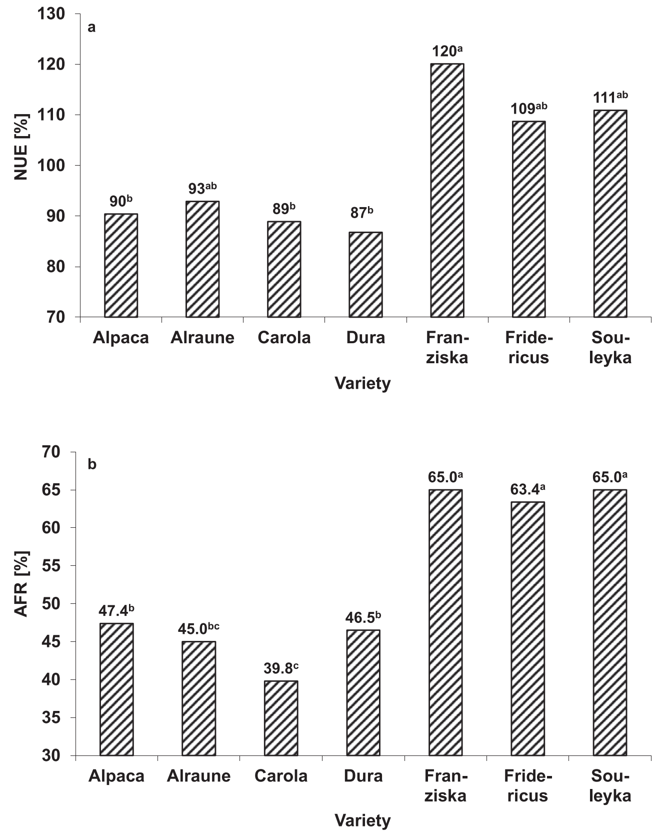

Fig. 2. Nitrogen use efficiency (NUE) (a) and apparent fertilizer N recovery (AFR) (b) of the winter barley varieties tested (1978–2015, 40–240 kg N ha^{-1}). Please note that the varieties were not always grown in the same years.

3.2. Nitrogen use efficiency (NUE) and Apparent N fertilizer recovery (AFR)

In order to ensure orthogonal data sets, the years 2005–2015 were used to analyze the effects of the total N amount (40–360 kg N ha^{-1}) in cvs. Franziska, Fridericus and Souleyka, while those of the other varieties were based on 1978–2015 and N amounts of 0, 40 and 80 kg N ha^{-1} per application date; thus the splitting rate of 120 kg N ha^{-1} at each date was excluded.

On average of the total N amounts varying between 40 and 240 kg N ha^{-1}, the varieties grown in 2005–2015 (cvs. Franziska, Fridericus and Souleyka) used the applied N more efficiently (Fig. 2a) and recovered more ($P < 0.05$) fertilizer N (Fig. 2b) than those released before 2000. Considering only the newer varieties revealed a clear decrease in NUE (relation between N offtake by the grains and total N fertilization) with increasing fertilizer N supply (Fig. 3a). In contrast, AFR (increase in N offtake by the grains due to fertilization related to the total N fertilizer amount) remained constant, but started to decrease linearly if total N fertilization exceeded 189 kg N ha^{-1} (Fig. 3b).

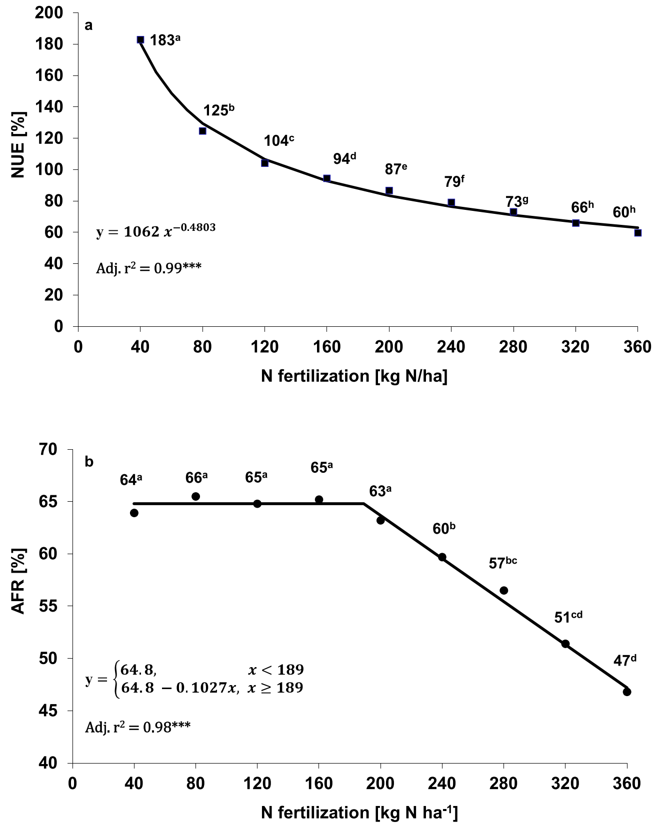

Fig. 3. Nitrogen use efficiency (NUE) (a) and apparent fertilizer N recovery (AFR) (b) of the total N fertilization (2005–2015, cvs. Franziska, Fridericus and Souleyka, 40–360 kg N ha^{-1}). Please note that the varieties were not always grown in the same years.

3.3. Trends over time

The trend of the different parameters over time was estimated from the N response curves using the single splittings (N1, N2, N3) fitted for each year x variety combination separately (eq. 5 + 6; Tabs. S3 - S5).

Without N supply, grain yield seems to increase with the duration of the experiment (Fig. 4a); however, the slope did not significantly differ from 0 ($P = 0.06$; Tab. S6) indicating that the increase was also due to the higher yield potential of the newer varieties ($P = 0.08$). Also, if estimated for each variety separately, no significant yield trend could be observed (except of cv. Dura; $P = 0.048$) (not shown). In contrast, grain protein concentration decreases significantly ($P < 0.05$) with time (Fig. 4b) resulting in quite constant values for the N offtake by the grain in the unfertilized treatment (Fig. 4c).

Total economic N fertilization optimum (Nopt) estimated *ex post* remained constant without any significant trend ($P > 0.05$) (data not shown). However, grain yield at Nopt increased significantly ($P < 0.05$) during the trial (Fig. 5a) apart from the variety effect. Especially the varieties released since the late 1990 s outyielded the older ones. Replacing the dummy variable 'variety' by its 'year of release' (eq. 6) confirmed the breeding progress to be significant ($P < 0.05$). Since the grain protein concentration at Nopt remained constant over time (Fig. 5b), N offtake by the grain showed a similar pattern as the grain yield (Fig. 5c). The (non-significant) increase of the N offtake without N supply and that at Nopt resulted in constant NUE and AFR values over time, but with clear variety effects (Fig. S2; Fig. 2a, b).

While the varieties clearly affected the thousand grain weight at Nopt, no significant trend over time could be observed (Fig. 7a). Consequently, the increase in grains m^{-2} (Fig. 7b) mainly caused the grain yield improvements at Nopt (Figs. 5a, 6).

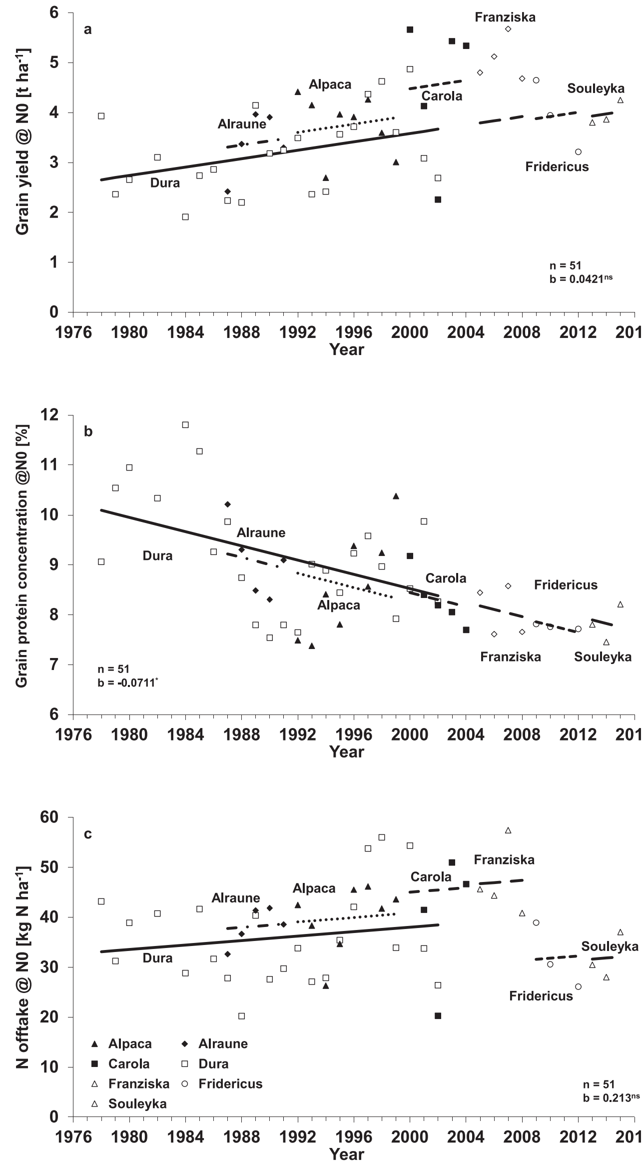

Fig. 4. Trend over time of grain yield (a), grain protein concentration (b), and N offtake by the grain (c), each without N fertilization, of the barley varieties tested.

3.4. Yield performance

In order to quantify the yield performance of the N splittings and their amounts, regressions of grain yields from annual mean depending on the N amounts of each splitting were estimated (Fig. 8, Table 2). At all application dates, the unfertilized treatment showed the lowest ability (slopes ranging between 0.82 and 0.91) to transfer the annual yield potential (as indicated by the average annual yield) to the actual grain yield. In contrast, N splittings of 80 and/or 120 kg N ha^{-1} were required to tap the full potential. Additionally, the lower r^2 values suggest a higher yield variability between the years in the plots without N supply (Table 2).

4. Discussion

The objective of the field trial was to allow the *ex post* identification of the N fertilization required for optimum yield each year (Tab. S3). For this purpose, N fertilization was applied at 3 dates and each splitting was staggered into 0, 40, 80 and 120 kg N ha^{-1} (since 2003). Combining all

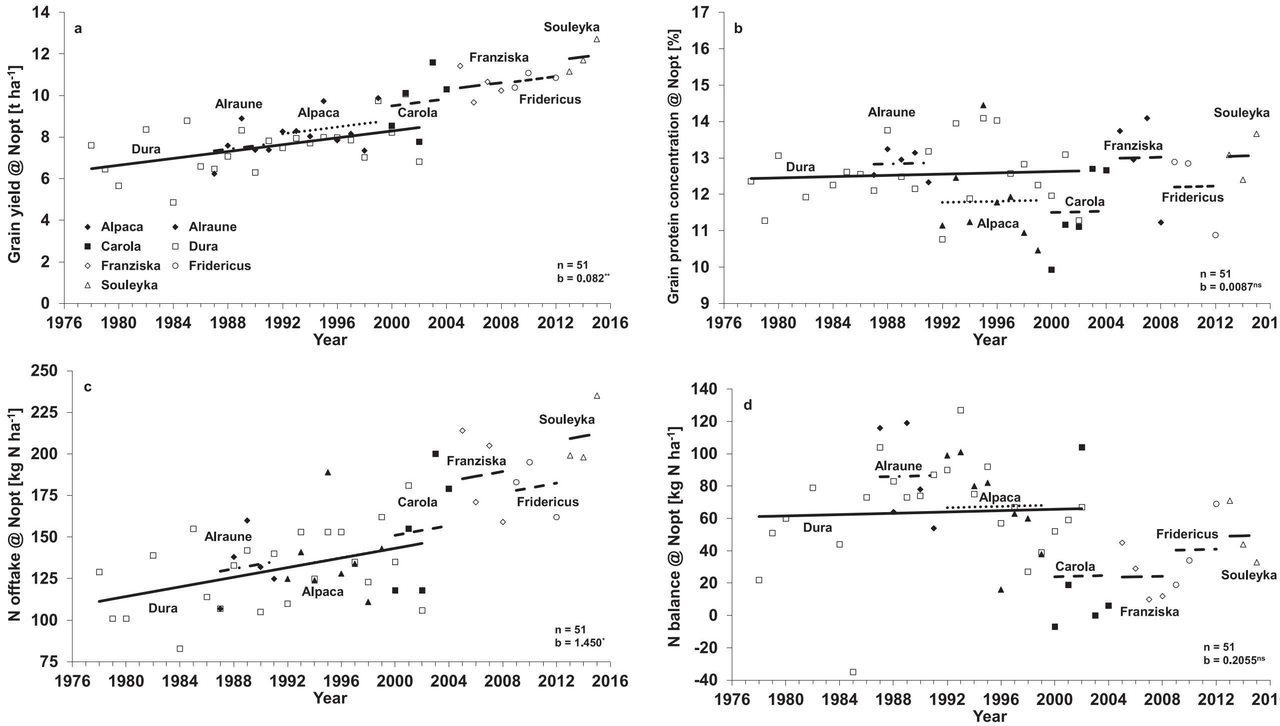

Fig. 5. Trend over time of grain yield (a), grain protein concentration (b), N offtake by the grain (c), and N balance (d), each at economic optimum N fertilization, of the barley varieties tested.

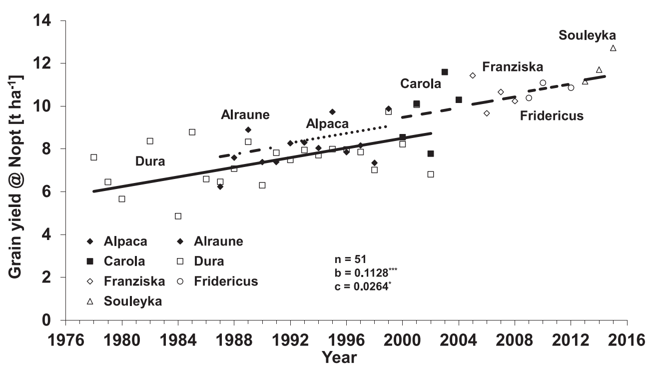

Fig. 6. Trend over time of grain yield at optimum N fertilization of the barley varieties tested. The year of release was used to characterize the varieties according to Eq. 6.

4 N amounts of each splitting resulted in 64 N treatments. Due to the experimental design, only one (2003–2015) or two (1978–2002) varieties were grown within one year. Consequently, changes of environmental conditions (e.g., increasing CO_2 level) or improvements in crop management (e.g., use of pesticides) may interfere with the variety effect. The grain yield increase over time of cv. Dura in the unfertilized treatment suggested such an effect; however, all other parameter tested seemed to remain unaffected from changes of the growing conditions during the duration of the experiment as also shown for the yield of winter wheat grown in the same trial (Sieling et al., 2011).

Located in Northern Germany with its humid climate, precipitation at the experimental site was quite equally distributed throughout the year (Tab. S1). During spring, (mild) drought stress often occurred; however, in most of the years due to sufficient rainfall during grain filling, cereals were able to better utilize late-season N than at sites with a more continental climate (Cruppe et al., 2017).

The quadratic N response curves estimated on average of the years revealed that N fertilization increased grain yield, grain N concentration

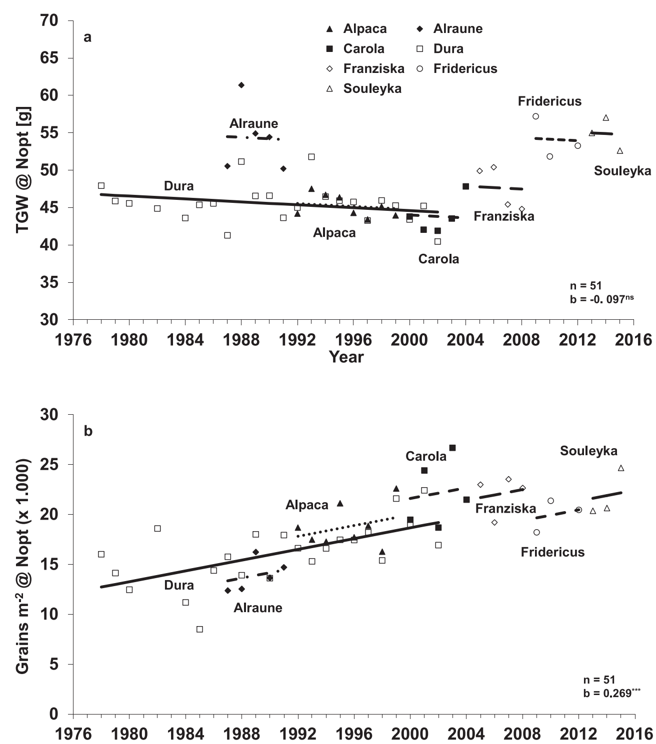

Fig. 7. Trend over time of thousand grain weight (TGW) (a), and grains m^{-2} (b), each at economic optimum N fertilization, of the barley varieties tested.

and N offtake by the grains, while the thousand grain weight (TGW) showed only small changes with higher yields achieved by the newer varieties (Fig. 1). Correlating grain yield with its components clearly demonstrated that the yield increase could mainly be attributed to an

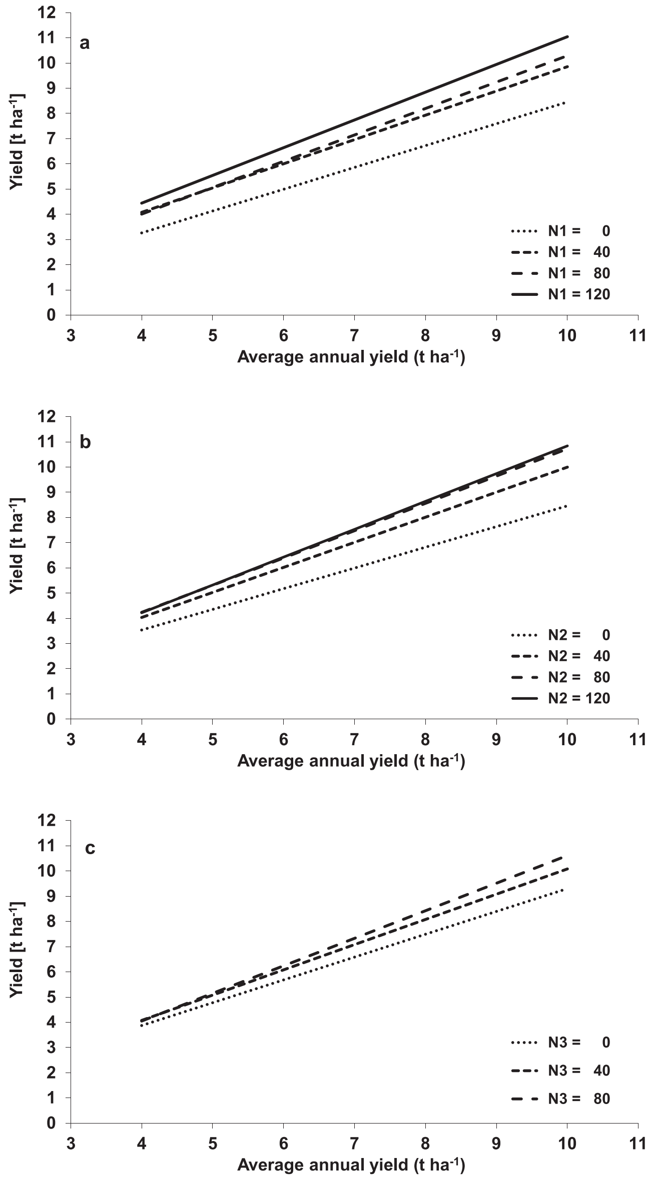

Fig. 8. Regression of winter barley yields from annual mean depending on the different N splitting amounts (kg N ha^{-1}): beginning of spring growth (N1) (a), stem elongation (N2) (b), and ear emergence (N3) (c). Slopes are given in Table 2.

Table 2
Slopes of the regression of winter barley yields from annual mean depending on the different N splittings (kg N ha^{-1}).

N splitting	[kg N ha^{-1}]	Slope[#]	95% confidence interval	Adj. r^2
N1 (beginning of spring growth)	0	0.87^d	0.79–0.94	0.52
	40	0.96^c	0.90–1.02	0.69
	80	1.05^b	1.00–1.09	0.85
	120	1.10^a	1.06–1.14	0.89
N2 (stem elongation)	0	0.82^c	0.73–0.91	0.45
	40	0.99^b	0.93–1.05	0.72
	80	1.08^a	1.04–1.13	0.84
	120	1.10^a	1.06–1.13	0.86
N3 (ear emergence)	0	0.91^c	0.84–0.97	0.54
	40	1.00^b	0.94–1.06	0.67
	80	1.09^a	1.04–1.14	0.76

- different letters indicate significant differences ($P < 0.05$) between slopes within each N splitting

improved number of grains m^{-2} rather than to heavier grains (Fig. S1). Also the breeding progress in grain yield seemed to rely mainly on an increased number of grains m^{-2} (Abeledo et al., 2003; Prystupa et al., 2004; Bingham et al., 2007a; b, 2012; Kennedy et al., 2017). However, several authors reported also of improvements in the grain size and harvest index due to breeding in last decades (Peltonen-Sainio et al., 2007; Bertholdsson and Kolodinska Brantestam, 2009; Arisnabarreta and Miralles, 2006, 2015; Mirosavljević et al., 2016, 2020a; Laidig et al., 2017; Rajala et al., 2017). In their experiments with winter barley in UK, Bingham et al. (2007a) observed a reduction in the radiation use efficiency during the second half of grain filling at some sites and explained it with a 'feedback inhibition from a limited sink capacity'. Since the estimated potential assimilate supply for grain filling exceeded the measured yield, they assumed that 'crops were predominantly sink limited'.

The small or non-existent effect of TGW on grain yield is somewhat surprising. Probably, this may be due to the fact, that TGW is an average value. Apart from the number of ears m^{-2}, N fertilization also increases the number of grains per ear. Results of Schoop (1986) obtained in wheat suggest that grains from positions within the ear which already exist in the unfertilized treatments will become heavier with increasing N supply while those grains additionally produced will remain smaller resulting in more or less unaffected TGWs.

In the unfertilized treatment, GPC decreased during the experimental period, which in combination with the increasing grain yield, resulted in similar N offtakes by the grains, indicating a constant natural N supply (although showing, of course, a considerable inter-annual variation) (Fig. 4). The breeding progress in the last decades led to yield increases if adequate N was available (Figs. 5a, 6), confirming results of other authors (e.g., Bingham et al., 2012; Laidig et al., 2014, 2017; Mirosavljević et al., 2016, 2020a; b; Rajala et al., 2017). Optimum N fertilization enabled the barley crop to maintain GPC constant (Fig. 5b), but not to increase it due to a dilution effect (Laidig et al., 2017; Rajala et al., 2017; Mirosavljević et al., 2020b). Especially the newer varieties were more able to take up higher N amounts and to accumulate them in the grains. Bingham et al. (2012) observed a higher N uptake efficiency which was correlated with post-anthesis N uptake. They suggested that a larger grain sink due to a higher number of grains m^{-2} increased the N demand which could only be met by an adequate N supply like the optimum N fertilization in our trial. The question if and to what extent an increase of CO_2 concentration with the years may have impaired GPC (Erbs et al., 2010; Weigel and Manderscheid, 2012) has to remain open.

Moll et al. (1982) introduced the approach of N use efficiency (NUE) defined as grain production per unit of N available in the soil in order to describe the crop's ability to utilize fertilizer N for its yield formation. However, since NUE according to Moll et al. (1982) neglects the N (or protein) concentration of the grains, we decided to replace grain yield by N offtake by the grains. Raun and Johnson (1999) suggested the term 'Apparent Fertilizer N Recovery' (AFR) (defined as: ((N removed in grain) – (N from soil + deposition))/fertilizer N applied). The amount of the term 'N from soil + rain' is normally represented by the N offtake of an unfertilized control treatment, which, however, assumes that no 'added nitrogen interaction' occurs (Jenkinson et al., 1985). We used a simplified approach (Eq. 2) in our experiment which also indicates directly how well applied fertilizer is removed by the harvest products (Hawkesford and Griffiths, 2019). It should be kept in mind that the consideration of the N offtake in the unfertilized treatment (indicating the natural N supply) causes that the AFR values normally range below 100. As already mentioned by other authors, the breeding progress increased N utilization (e.g., Bingham et al., 2012; Mirosavljević et al., 2020b) (Fig. 2a, b). Increasing N supply normally impairs fertilizer efficiency (Hackett, 2016). However, in our experiment, AFR of the newer varieties did not start to decrease until N fertilization exceeded 180 kg N ha^{-1} (Fig. 3b). As wheat, barley utilized the first application at the beginning of spring growth to a smaller extent than that at stem elongation or ear emergence (data not shown; Sieling and Kage, 2021).

Also Maidl et al. (1996) observed a lower fertilizer N use efficiency in treatments with excessive total N rates and with high N rates applied during early growth stages in either autumn or spring.

In order to get an idea of the yield stability of the different N treatments, we use the regression of yields from annual mean depending on the different N splitting amounts (Fig. 8) according to Macholdt et al. (2020) and Reckling et al. (2021). At all three application dates, the barley crop fertilized with 80 or 120 kg N ha^{-1} was able to realize more of the annual yield potential (as indicated by the average annual yield) (slope > 1) in combination with the smallest variation (higher r^2 values) than the unfertilized control or the 40 kg N ha^{-1} treatment (Table 2). Also Macholdt et al. (2020), who analyzed the yield stability of different cropping systems with winter barley fertilized with N amounts of 0, 70 and 140 kg N ha^{-1}, observed less stable yields and a poorer environmental adaptability in the unfertilized plots.

Our experiment revealed a clear breeding progress in winter barley yields of the last decades mainly due to an increase in the number of grains m^{-2} rather than to heavier grains. In addition, N fertilization seems to act in the same way. One implication for practical barley growing in humid climates is to provide sufficient grains per area (sinks) as proposed by Bingham et al. (2007a, b), especially against the background of climate change with its predicted temperature increase in spring and summer impairing grain filling. Unfortunately, our results do not allow to rate the importance of the number of ears m^{-2} and the number of grains per ear. In contrast to wheat, GPC is less relevant in barley growing. Consequently, the decreasing or at least constant GPC level observed seems to cause no problems.

CRediT authorship contribution statement

Klaus Sieling: Conceptualization, Methodology, Investigation, Statistical analysis, Writing, Reviewing. **Henning Kage:** Editing, Reviewing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2022.126505.

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