Title: Performance of eleven winter wheat varieties in a long term experiment on mineral nitrogen and organic fertilisation

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Abstract

There is an increasing awareness about the need for improving nitrogen use efficiency in crop production in order to meet economic and ecological standards. The present work provides insight into the varietal factors of wheat that determine nitrogen use in the plant. The performance of eleven winter wheat varieties in terms of grain yield and nitrogen uptake and use efficiency was tested within the framework of a 40-year long term field experiment of organic enrichment and mineral nitrogen fertilisation treatments. Globally, organic enrichment had a beneficial effect on the yield and grain nitrogen concentration and showed a strong interaction with the amount of applied mineral nitrogen fertiliser. Manure application generally had positive effects on varietal performances mainly due to indirect long term effects on the soil properties. Varieties showed a broad range of responses to the treatments of the long term experiment, revealing significant genotype x environment interactions. Nevertheless, the varieties which performed well at high input levels were also the best at low input levels, suggesting that the genotype x environment interactions were not strong enough to inverse the performance ranking. Similarly, the varietal traits associated with high yielding or grain nitrogen concentration in high input conditions were the same as those identified under low input conditions. To conclude, these results suggest that the selection of wheat for nitrogen efficiency is possible under any nitrogen fertilisation regime. However, to be adapted to low input or organic agriculture, varieties also need traits other than nutrient use efficiency, for example, disease resistance, resilience to abiotic stresses and competitiveness against weeds.

Keywords: nitrogen use efficiency, low input production, genotype x environment interactions, grain protein concentration, nitrogen depleted environment

1. Introduction

Nitrogen is one of the most important factors controlling crop development (Frink et al., 1999; Marschner, 1995). The increasing use of mineral nitrogen fertilisers has thus driven the explosion of crop production observed worldwide during the last century (Hirel et al., 2007; Ladha et al., 2005; Tilman, 1999). Yet, the overuse of fertilisers is a major source of environmental pollution, due to nitrate leaching and run-off (Huggins and Pan, 1993; Raun and Johnson, 1999). In addition, the fabrication of mineral nitrogen is costly and highly energy consuming. These drawbacks have motivated important changes in recent fertilisation practices, such as reduced nitrogen fertiliser use and the increasing interest in wheat varieties with elevated nitrogen use efficiency.

As crop development and performance are closely linked to nitrogen availability, the challenge resides in reducing the nitrogen input without affecting yield and quality. One possible approach to solving this problem is breeding varieties that use nitrogen more efficiently (Foulkes et al., 2009; Hirel et al., 2007; Sylvester-Bradley and Kindred, 2009; Tilman, 1999). Nitrogen efficiency of varieties can be described using the nitrogen use efficiency (NUE) approach (Van Sanford and Mackown, 1986; Sadras and Lemaire, 2014). Several studies have shown that the traits linked to the absorption and use of nitrogen, as well as to the formation of yield, are genetically determined and vary between genotypes and species (Austin et al., 1977; Barraclough et al., 2010; Barraclough et al., 2014; Bogard et al., 2010; Flood and Martin, 2001; Gaju et al., 2011; Le Gouis et al., 2000; Lemaire et al., 2008; Ye et al., 2011). Components of NUE are therefore potential targets for breeding of wheat varieties (Foulkes et al., 2009; Gaju et al., 2011). Yet, these traits are also subject to significant genotype x environment interactions. Therefore, breeding for nutrient use efficiency requires a good knowledge of the underlying genetic and environmental factors (Annichiarico, 2002; Ceccarelli, 1996). When decreasing the nitrogen input, or replacing mineral nitrogen with nitrogen from organic sources, the yield, quality and environmental performances of the varieties may change as a function of their genetic background (Ceccarelli, 1996; Charles et al., 2006; El Bassam, 1998; Ruiz et al., 2008; Vlachostergios and Roupakias, 2008). Today, varieties are usually selected under high or medium nitrogen conditions. Several studies suggest that traits selected under nitrogen rich conditions may not be the same as those required for high performance under nitrogen limiting conditions (Ceccarelli, 1996; El Bassam, 1998; Ruiz et al., 2008; Vlachostergios and Roupakias, 2008). Consequently, in order to improve nutrient use efficiency in wheat, the performance of genotypes under contrasting soil fertility conditions needs to be understood.

A few studies have investigated varietal responses when only very low nitrogen is available thus identifying useful genetic traits for low input systems (Dawson et al., 2008). Under most low input conditions, the seasonal course of nitrogen availability in soil is the determining factor of the progression of nitrogen accumulation in plants, followed by their capacity of N remobilisation from vegetative biomass and post-anthesis accumulation (Berry et al., 2002; Dawson et al., 2008; Masclaux et al., 2001). These processes may even be more important in production systems using organic amendments, because the release of organic nitrogen depends on the dynamics of mineralisation, which in turn depends on climatic and other environmental conditions. Chemical fertilisers, on the other hand, release nitrogen rather quickly. The release of organic nitrogen is therefore slow and spread over a longer period of time (Berry et al., 2002). In low input systems in stockless farms, soil organic matter decreases gradually on a long term perspective (Maltas et al., 2012b; Riley et al., 2008). Yet, the decrease in soil organic matter affects the dynamic of nitrogen availability in the soil. It is thus important to identify wheat genotypes able to cope with these conditions.

Consequently, there is a need to better select plants adapted to limited nitrogen conditions, to the form of its supply (mineral, organic), to its nature in soil (high, low organic matter content) and to the dynamics of its availability to the crop. For instance, it has been suggested that varieties absorbing nitrogen early in the season perform better in conditions with low nitrogen availability (Baresel et al., 2008). Breeding for nitrogen efficient varieties that perform well under nitrogen limiting or organic conditions has to account for the genetic basis of the crop but also all cultivation and environmental factors that influence the absorption and the allocation of nitrogen in the plant. This raises the question of the need for a specific breeding scheme for organic production methods as varieties have to cope with stronger stresses and higher environmental variability in contrast to high input systems (Lammerts van Bueren et al., 2002; Löschenberger et al., 2008; Müllner et al., 2014; Reid et al., 2011; Wolfe et al., 2008).

To address the question of varietal responses to low input and organic fertilisation, a study was made within a long-term field experiment of nitrogen fertilisation and organic enrichment, established in 1976 in Switzerland. This experiment offered the unique opportunity to study eleven European winter wheat varieties (*Triticum aestivum* L.) in unusually contrasting environments of different soil fertility within a single field and over two years. The objectives of this study were 1. to investigate the varietal responses to various sources and amounts of nitrogen fertilisation and various soil organic matter content in the same soil, 2. to better understand the agronomic traits that characterise the behaviour of modern varieties under these different conditions, their capacity to exploit soil nitrogen mineralisation, and the dynamics of resource acquisition, 3. to evaluate the need to undertake specific selection for low input systems and 4. to identify the crucial traits necessary for the selection of best adapted varieties. For this, varietal responses in terms of yield, nitrogen content, nitrogen accumulation and nitrogen related traits were systematically explored.

2. Material and methods

2.1. Experimental site

The experiment was carried out at the Agroscope Changins (46° 24’ N, 06° 14’ E, 430 m above sea level) in Nyon, Switzerland, during the years 2005-2006 and 2006-2007. The soil is a well-drained brown soil (Calcaric Cambisol) with 14% clay and 39% silt, and a depth of 100 cm. For this site, the average total annual precipitation is 999 mm and the mean temperature 10.2°C (30-year averages, 1981-2010). While 2005 was a rather dry year, 2006 and 2007 were in line with the mean long term values (2005: 707 mm, 10.3°C; 2006: 930 mm, 11.0°C; 2007: 992 mm, 11.1°C). The total precipitation and mean temperature during the two growing periods (February to June) were similar in 2006 and 2007 (2006: 472 mm, 9.8°C; 2007: 450 mm, 11.9°C), though the distribution of precipitation differed (Table 1).

The long term experiment was established in 1976 in Changins. It follows a split plot design with four replicates, six main treatments of organic enrichment, and four sub treatments of mineral nitrogen fertilisation (Maltas et al., 2012a).

This study focused exclusively on two levels of organic enrichment and three levels of mineral nitrogen fertilisation. The two main treatments were 1. no organic enrichment (Mineral) and 2. 70 t/ha manure every three years (Manure). The sub treatments represented three levels of mineral nitrogen fertilisation: 1. no nitrogen supply (noN); 2. suboptimal fertilisation, nitrogen supply 40 kg/ha below the optimal dose (lowN); 3. over fertilisation, nitrogen supply 40 kg/ha above the optimal dose (highN). The long term treatments and sub treatments modified soil characteristics substantially (Maltas et al., 2012b; Vullioud et al., 2006). In particular, soil organic matter content, its C/N ratio and total soil nitrogen increased significantly in the Manure treatment compared to the Mineral one (Maltas et al., 2012b).

The crop rotation of the long term experiment alternates winter and spring crops, with 67% of cereals (winter wheat, spring barley and oat), complemented with winter rapeseed and maize. At harvest, cereal straw is removed while rapeseed and maize residues are incorporated into the soil. This standard rotation was interrupted in 2005 after a maize crop to allow the settlement of the present study.

2.2. Wheat varieties

The long term experiment was used to test eleven recently released winter wheat varieties. The varieties originated from different European countries and breeders and bore different genetic backgrounds (Table 2). They are all registered in the European variety catalogue. Pireneo and Aszita are organic varieties, and all other Swiss varieties are recommended for low input and integrated systems. Varieties were chosen according to a relative similarity in precocity and maturation to avoid impact on development, growth and yield performance. The varieties covered a large diversity in terms of physiological traits and baking quality.

2.3. Experimental setup

The experimental setting consisted of the eleven winter wheat varieties sown in strips across the nitrogen fertilisation subplots of the long term experiment. The plots (3 m x 1.5 m for each single plot) were machine sown in mid-October (12 October 2005 and 27 October 2006) at a rate of 350 seeds/m^2. The optimal nitrogen dose for the mineral treatment was computed according to the Swiss fertilisation guidelines (Ryser et al., 2001). It reached 160 kgN/ha and 140 kgN/ha respectively for the first and the second year. The dose was higher in the first year to compensate for the nitrogen immobilisation induced by the incorporation of maize straw. N fertiliser was machine applied as NH_4NO_3 in a two- or three-way split during the growing season (Zadoks growth stages 25, 30, 40 in 2006, and 25, 30, 37 in 2007) (Table 1). The last farmyard manure was applied before the 2005 maize in the treatment Manure. Phosphorus and potassium were supplied in order to warrant non limiting conditions on all main treatments, according to the Swiss fertilisation guidelines (Ryser et al., 2001), and taking into account phosphorus and potassium from organic origins. Herbicides were applied depending on weed pressure, and standard phytosanitary protection was applied according to integrated crop protection principles (Häni et al., 1990). Crops received no growth regulator to avoid hormonal effect on biomass growth.

The plots were machine harvested at the end of July (25 July 2006 and 20 July 2007).

2.4. Dry matter and nitrogen related traits

At harvest (Zadoks growth stage 89), grain yield (DMgrain), adjusted at 0% humidity, grain N concentration (%N), and thousand kernel weight (TKW), adjusted at 0% humidity, were assessed for each plot. At anthesis (Zadoks growth stage 61-65), plants were removed from the third row in each plot, over a length of 0.5 m. These samples were used to determine the total aboveground dry matter and the corresponding N concentration. At harvest, additional data on total aboveground dry matter (DMmat) and N concentration in straw were collected in a similar way. Nitrogen concentrations in biomass and in kernels were determined by near infrared spectrometry using a NIRS6500 (FOSS NIRSystems, Inc., Laurel, Md, USA). Kernel number per square meter (KN) was obtained by dividing yield per square meter by thousand kernel weight. Nitrogen uptake of grain (Ngrain), and of total aboveground biomass at anthesis (Nanth) and maturity (Nmat), were calculated based on their respective N concentration and dry weight. Similarly, harvest index (HI) and nitrogen harvest index (NHI) were calculated as the ratio of DMgrain to DMmat and Ngrain to Nmat, respectively. In addition, six traits linked to nitrogen accumulation and use were derived:

- N post-anthesis accumulation (NpA): Nmat - Nanth
- fraction of total N uptake accumulated after anthesis (fNpA): (Nmat - Nanth) / Nmat
- N use efficiency (NUE): DMgrain / N supply
- N utilisation efficiency (NutE): DMgrain / Nmat
- N uptake efficiency (NupE): Nmat / N supply
- N use efficiency for protein (NUEP): Ngrain / N supply

Several methods for estimating N supply (Nsup) exist (Limon-Ortega et al., 2000). Here, Nsup was defined as the sum of mineral N fertilisation and of the maximal whole plant total N uptake observed in the sub treatment with no nitrogen fertilisation (Nmat at noN), and at the respective main treatment levels (Mineral or Manure) and replicates. However, it has been shown that, though the choice of the method influences the values of NUE and NupE, it has little effect on the relative comparisons of this value between treatments (Bingham et al., 2012).

2.5. Data analysis

An analysis of variance was performed on each trait. The full experimental design corresponded to a split-strip plot with four replications, with organic enrichment (two levels) as the main plots, nitrogen fertilisation (three levels) and varieties (eleven levels) as the orthogonal strips.

A principal component analysis (PCA) was performed to investigate how varieties were characterised by the measured traits. This analysis was performed on the correlation matrix of the mean variety values (mean over all replicates, treatments and years), with the R package vegan (Oksanen et al., 2011).

N supply allowed sorting the treatments relative to the nitrogen made available for the crop. This value was used to situate the individual varietal responses (*i.e.* deviation from the variety mean) for the different traits studied, as a function of nitrogen availability. Independently for each variety, a linear regression of its deviation from the variety mean trait was adjusted, as a variant of the procedure proposed by Finlay and Wilkinson (1963).

Pearson correlations were performed between yield (DMgrain), grain N concentration (%N), and the other traits, to assess the relationship between varietal characteristics and performance. Correlations were computed on the mean values of each variety across the four replicates, for each treatment independently.

The relative contributions of N uptake (NupE) and utilisation (NutE) efficiency to the variation in N use efficiency (NUE) among the varieties, in each treatment, were analysed using the method described in Moll et al. (1982). This method allows determining the contribution of each component of a trait to the mean squares of the compound trait. The analysis is performed on the logarithms of the values in order to linearise the product. The mean values for each variety across the four replicates were used for the analysis. In addition, to better understand how grain nitrogen concentration is built up, four other relationships were analysed with this same method:

1. NUE = NupE x NutE (on logarithms)
2. Ngrain = DMgrain x %N (on logarithms)
3. DMgrain = DMmat x HI (on logarithms)
4. Ngrain = Nmat x NHI (on logarithms)
5. Nmat = Nanth + NpA

All statistical analyses were performed using R 3.1.1 (R Core Team, 2014).

3. Results

Grain yield, nitrogen concentration and uptake were significantly influenced by the factor year (Table 3). As the factor year interacted also with several other factors (Table 3), the results were generally analysed separately for each year and then synthesised.

3.1 Overall response of wheat to the long term field trial treatments

3.1.1 Yield and nitrogen utilisation

Grain yield (DMgrain) and nitrogen content (%N and Ngrain) were significantly influenced by the type of organic enrichment and the amount of nitrogen fertiliser (Table 3). They rose with increasing quantities of nitrogen fertiliser and with the addition of manure (Table 4). Yet, organic enrichment and nitrogen fertilisation strongly interacted, especially for %N (Table 3, Figure 1).

All the traits concerning dry matter as well as nitrogen accumulation and use were significantly influenced by the level of nitrogen fertilisation, except the fraction of total nitrogen accumulated after anthesis (fNpA) which was not influenced by any of the treatments (Supplementary data Table S1 and Figures S1 and S2). Organic enrichment had an effect on the kernel number (KN), dry matter at maturity (DMmat), nitrogen uptake at anthesis (Nanth) and at maturity (Nmat), nitrogen post-anthesis accumulation (NpA) and N utilisation efficiency (NutE). In treatments with organic enrichment in combination with mineral nitrogen fertilisation, positive and significant interactions were observed for the use of nutrients by the plant, in particular NUE, NutE, NupE and NUEP, similar to observations made for %N (Table S1 and Figures S1 and S2).

3.1.2 Total nitrogen uptake at maturity as a characterisation of available nitrogen

Each combination of organic enrichment and nitrogen fertilisation treatments was characterised by its mean nitrogen uptake Nmat, related to the amount of nitrogen available for the crop. Nmat ranged from 38 kgN/ha to 217 kgN/ha in 2006, and from 60 kgN/ha to 198 kgN/ha in 2007. In both years, the treatments were ranked in the same order relative to Nmat: 1. Mineral-noN, 2. Manure-noN, 3. Mineral-lowN, 4. Manure-lowN, 5. Mineral-highN, 6. Manure-highN.

The nitrogen uptake at maturity (Nmat) with Manure-lowN and Mineral-highN was very similar, in both years (non-significant differences: 154 kgN/ha and 165 kgN/ha in 2006 (lsd 5% = 15), and 159 kgN/ha and 179 kgN/ha in 2007 (lsd 5% = 20)). It is conceivable, that these two treatments provided almost the same amount of nitrogen to the plants, yet from different sources (organic + mineral or only mineral). The difference in added nitrogen between these two treatments represented 80 kgN/ha.

These two treatments also resulted in comparable grain yields (DMgrain; respectively 6.13 t/ha and 5.86 t/ha in 2006 (lsd 5% = 0.39), and 6.28 t/ha and 6.26 t/ha in 2007 (lsd 5% = 0.82)) (Figure 1) but not the same grain N concentrations (%N; respectively 1.98% and 2.18% in 2006 (lsd 5% = 0.07), and 2.18% and 2.47% in 2007 (lsd 5% = 0.06)). Only 2006 gave similar Ngrain (respectively 120 kgN/ha and 126 kgN/ha in 2006 (lsd 5% = 9), and 136 kgN/ha and 154 kgN/ha in 2007 (lsd 5% = 17)).

3.2 Individual response of the wheat varieties

3.2.1 Yield and nitrogen performance of individual varieties

Yield (DMgrain) and nitrogen concentration (%N) and uptake (Ngrain) differed significantly between varieties (Table 3). On average (on all treatments and years), the lowest DMgrain was achieved by Aszita (4.21 t/ha) and the highest by Tapidor (5.47 t/ha), whereas the lowest %N was observed for Tapidor (1.90 %N), and the highest for Aszita (2.33 %N) (Table 4 and Supplementary data Table S2). The lowest Ngrain was measured for Toras (99 kgN/ha), and the highest for Farandole (108 kgN/ha). The rankings of varieties in terms of DMgrain and %N was generally similar in both years (Figure 2A). A negative relationship between DMgrain and %N was observed, on average, and within each treatment (Figure 2A). No significant differences were observed between varieties for N accumulation at anthesis (Nanth) nor for post-anthesis accumulation (NpA) and fNpA, while differences were present at maturity (Nmat) (Table S1). A significant effect of the factor variety was observed on all the other traits of dry matter and nitrogen accumulation and use (Table S1).

3.2.2 Grouping of varieties

Three main groups of varieties were delineated according to their performances (Figure 2A). A first group consisted of the varieties Arina, Aszita and Titlis (#2, #3 and #4) with low grain yield (DMgrain) and high grain nitrogen concentration (%N). A second group was composed of Pireneo and Zinal (#1 and #5) with intermediate values. The third group contained the varieties Ephoros, Pegassos, Toras, Caphorn, Farandole and Tapidor (#6, #7, #8, #9, #10 and #11), characterised by high DMgrain and low %N.

The principal component analysis confirmed the classification into three groups and allowed a more precise characterisation of the groups (Figure 2B). The first two axes of the PCA together accounted for 86% (axis 1: 55%; axis 2: 31%) of the total variance. The first group (Arina #2 – Aszita #3 – Titlis #4) was characterised by a high %N and DMmat as well as by a low harvest index (HI), nitrogen harvest index (NHI), NutE and NUE. In contrast, the third group (Ephoros #6 – Pegassos #7 – Toras #8 – Caphorn #9 – Farandole #10 – Tapidor #11) was characterised by an elevated harvest and nitrogen harvest indices, and high nitrogen utilisation and use efficiencies. However, the third group was rather inhomogeneous for Nmat, Ngrain, NupE and NUEP, with low (Ephoros #6 – Toras #8) or high (Farandole #10 and Tapidor #11) values. Looking at the same criteria, these two latter varieties were also comparable to the second group (Pireneo #1 – Zinal #5).

3.2.3 Stability of the performances of the varieties with increasing nitrogen inputs

According to the analysis of variance, the factor variety tended to interact with the factors organic enrichment and nitrogen fertilisation (Table 3). This means that the performance of the single varieties was not consistent along the increasing nitrogen supply but depended also on its origin (mineral or organic).

The varietal response to nitrogen supply Nsup is highlighted in Figure 3 and in supplementary data Table S3. Here, the performance of each varietiy in relation to all other varieties is displayed on the gradient line from low to high nitrogen supplies, for yield, N concentration and N accumulation. For yield (DMgrain), the varieties of the first group showed significantly decreasing performances, with respect to the average of all varieties, with increasing nitrogen supply. This suggests that their yield benefited less from the increase of available nitrogen compared to the other varieties. The other varieties exhibited a steady increase of yield with increasing Nsup, yet were either lower than the average yield (second group Pireneo #1 and Zinal #5) or higher (highest values for Tapidor #11 and Farandole #10). Only the variety Toras (#8) showed a significant increase from a very low yield in the absence of mineral fertilisation to a yield over the mean by intensive fertilisation.

With respect to grain nitrogen concentration (%N), Pireneo (#1) and especially Titlis (#4) increased more than the average with increasing nitrogen supply (Figure 3). In contrast, Ephoros (#6) and Toras (#8) (both from the third group) were unable to follow the average increase in %N with increasing availability of nitrogen. The other varieties exhibited mostly very stable responses along the increase of Nsup, performing either above (Aszita #3, Arina #2, Zinal #5) or below the mean values curve (Pegassos #7, Caphorn #9, Tapidor #11).

For grain N uptake (Ngrain), Zinal (#5) showed higher accumulation of nitrogen with increasing nitrogen availability compared to the mean value (Figure 3) than all other varieties.

Concerning the dynamics of nitrogen accumulation, the varieties exhibited very similar responses at anthesis (Nanth) to the increase of available nitrogen (except Farandole #10 but this effect is due to an outlier value) (Table S3). The accumulation of nitrogen at maturity (Nmat) was also very similar in all varieties, except for Zinal (#5) that increased accumulation with more nitrogen at disposal, and Pegassos (#7) with poor reaction to the available nitrogen (Table S3). For post-anthesis accumulation (NpA), Ephoros (#6) did not react to increased nitrogen availability.

The direct comparison of the performance of the varieties in the two most extreme systems in terms of nitrogen input, Mineral-noN and Manure-highN, suggested however that the ranking of the varieties was in principle consistent at low and at high nitrogen inputs.

Strong correlations between the varieties for %N at low and high nitrogen inputs were observed (Pearson's coefficient of correlation r: 0.87, $p<0.001$ in 2006 and $r=0.95$, $p<0.001$ in 2007). The correlation was also significant for yield in 2006 ($r=0.75$, $p=0.005$), but not in

2007 (r=0.38, p=0.25), though a single outlier (Toras #8) was responsible for this non-significant correlation (without Toras: r=0.79, p=0.006). The more pronounced correlations for N concentration than for yield suggested that genetic determinism is stronger for this trait.

3.2.4 Varietal response to distinct sources of nitrogen

As pointed out previously, the two treatments Mineral-highN and Manure-lowN supplied similar amounts of nitrogen to the plants, which showed similar mean yield and grain N uptake values. When comparing the varieties, it was possible to highlight those varieties reacting more strongly to the source of nitrogen (mineral only or organic + mineral). Figure 4 displays how the performances of the varieties deviate from the general pattern. Concerning yield (DMgrain), Tapidor (#11) in 2006 and Pegassos (#7) in 2007 diverged more than the other varieties, showing a preference to mixed sources of nitrogen (organic + mineral in the Manure-lowN treatment) (Figure 4, upper row). For grain N concentration (%N), Zinal (#5) showed a divergent reaction in both years, towards mixed sources in 2006 and towards mineral nitrogen in 2007 (Figure 4, lower row). Here again, the response of %N was more consistent than that of DMgrain.

3.3 Relationship between varietal traits and performance

3.3.1 Correlations of varietal traits with yield and nitrogen concentration

Among the studied traits, harvest index (HI) and kernel number (KN) were strongly positively correlated with yield (DMgrain) and negatively with %N (Supplementary data Table S4). Nitrogen utilisation efficiency (NutE) also displayed strong positive correlations with DMgrain and negative correlations with %N for all six treatments, whereas N uptake efficiency (NupE) was not correlated with either variable (except with yield at lowN in 2007). Nitrogen use efficiency for protein (NUEP) was generally correlated positively with DMgrain, but not with %N. Interestingly, the three traits associated with the dynamics of nitrogen accumulation (Nanth, NpA, fNpA) were correlated neither with DMgrain nor with %N (except in a few situations) (Table S4).

3.3.2 Relative contributions of varietal traits

The amount of nitrogen accumulated in the grain (Ngrain) is the product of N concentration (%N) and yield (DMgrain). In all treatments (except Mineral-noN in 2006 and Mineral-highN in 2007), variations in %N contributed less to the Ngrain than did DMgrain (Table 5). Grain dry matter (DMgrain) is itself a function of the total dry matter at harvest in the whole plant (DMmat) and of the harvest index (HI). Variations in DMgrain among varieties were better explained by variations in HI than in DMmat. Since the nitrogen harvest index (NHI) was relatively stable, the differences in grain nitrogen uptake (Ngrain) depended on the quantity of nitrogen accumulated at maturity (Nmat). Depending on the nitrogen treatment, the variation of nitrogen at maturity (Nmat) was alternatively more influenced by the quantity of nitrogen at anthesis (Nanth) or by the post-anthesis nitrogen accumulation (NpA) (Table 5). In 2006, Nanth contributed more to Nmat in the Mineral treatments and NpA more in the Manure treatments, thus showing not only a clear influence of the presence of organic enrichment to the dynamics of nitrogen absorption, but also an effect of the year (climate). In 2007 the pattern was less clear, but with a greater influence of Nanth on Nmat in all cases except Mineral-lowN and Manure-highN (Table 5).

The variation in nitrogen use efficiency (NUE) was more influenced by nitrogen utilisation efficiency (NutE) than by nitrogen uptake efficiency (NupE), in all treatments (Table 5). This effect was stronger at high than at low nitrogen fertilisation levels.

4. Discussion

The availability of nitrogen is fundamental for the expression of the yield and the baking quality potential in wheat. Under nitrogen limiting conditions, each wheat genotype must choose to attribute the available nitrogen either for yield or for quality. In the present work, the interplay between the form of added nitrogen and the wheat genotype has been investigated in a long term experimental scheme. The principal aim was to understand which proportion of the available nitrogen is taken up and allocated in the different plant metabolic compartments. Before dissecting genotype effects of nitrogen metabolism, it was necessary to characterise the gross availability of nitrogen in the single long term treatments.

4.1 Response of wheat to organic enrichment, nitrogen fertilisation and their interaction

Most of the traits, in particular grain yield and nitrogen, were strongly influenced by both the organic enrichment and the nitrogen fertilisation treatments. As expected, the more nitrogen is readily available, the higher is the yield and the grain N concentration. The long term experiment made the study of different types of readily and delayed nitrogen availability possible, especially in treatments with organic and mineral nitrogen allowances. The dynamics of the organic matter and the availability of nitrogen in these different treatments have been characterised in previous studies (Maltas et al., 2012ab). The addition of mineral nitrogen tended to increase the soil organic matter (values ranging, in 2004, from 1.45% in Mineral-noN to 1.75% in Mineral-highN, and from 1.98% in Manure-lowN to 2.23% in Manure-highN), which is likely to be due to the greater amount of residues and root biomass produced in fertilised plots, which then has a positive influence on crop growth (Maltas et al., 2012b).

The addition of manure also significantly affected plant performance, even though manure was incorporated before the preceding crop in spring 2005. Here, the positive effect relies essentially on the indirect and long lasting effects of manure addition and not on the direct input of easily available nitrogen. Indeed, the availability of nitrogen for crops after manure application lasts for several years, and is still consequent in the second year after application (Eghball and Power, 1999; Maltas et al., 2012b; Sinaj et al., 2009). Such long term effects of manure can be explained by the increase of soil organic matter content (Zhang et al., 2009), as has been observed in the present long term experiment (Maltas et al., 2012b). Manure application, and other organic enrichments such as compost, can also improve the soil structure and the soil physical properties. Moreover, manure generally enriches the soil in macro- and micronutrients that promote crop growth (Dick, 1992; Lal, 2009; Meng et al., 2005; Nemecek et al., 2008; Singer et al., 2004; Watson et al., 2002; Zhang et al., 2009). Organic enrichment also modifies the characteristics of organic matter, by increasing its carbon/nitrogen (C/N) ratio (Maltas et al., 2012b; Yang et al., 2007).

In addition, the beneficial effect of manure on grain yield and on nitrogen uptake was enhanced by the concomitant presence of mineral nitrogen. Several studies have shown that nitrogen fertilisation promotes manure mineralisation through the stimulation of microbial activity (Khan et al., 2007; Sakala et al., 2000). This process increases in turn the amount of nitrogen available for the crop, and is the likely explanation for the results presented here.

4.2 Genotype x environment interactions

Overall, the presentresults indicate that combined treatments of organic enrichment and nitrogen fertilisation built contrasting growth environments in which the specific response of each variety could be tested. The eleven varieties included in this study showed large variations in grain yield and N concentration, in response to the fertility levels offered by the long term experiment. In the nitrogen depleted plots, the varietal responses were challenged to highlight the respective nitrogen allocation strategies under low input conditions. For yield and for grain nitrogen concentration, the factor variety generally interacted with nitrogen fertilisation, but also, to a lesser extent, with organic enrichment.

To understand how nitrogen was used, three groups of varieties were identified, based on their yield and N concentration. Within each group, the response to the increase of nitrogen was consistent. The groups corresponded largely to the baking quality classes shown in Table 2, further validating this cataloguing. The group composed of Arina, Aszita and Titlis showed a decreasing yield response, with the increasing nitrogen supply. However, in comparison with high yielding varieties, they remained low yielding varieties at low input too. Obviously, the interaction was not strong enough to inverse the ranking of the varieties at low input. In contrast, this group of varieties was characterised by high N concentration, which is a sign of high bread making quality. Yet, Titlis exhibited a strongly positive response of grain N concentration with the increase of N supply. This could be explained by its really stable grain nitrogen uptake, which allowed it to concentrate nitrogen as its yield response slowly decreased. Thus, Titlis is a more responsive variety, in terms of quality, which benefits from highly fertile conditions.

In the second group (intermediate yield and N concentration), Zinal (quality class 1) showed a capacity of post-anthesis nitrogen assimilation superior to the mean and increasing with N supply, leading to high total and grain N uptake at high N supply. This is thus typically an interesting variety, responding when nitrogen is available at high doses, and able to profit from nitrogen available late in the season.

In the third group (high yield and low N concentration, quality classes 3-5), Ephoros had a decreasing response of N concentration when nitrogen supply increased. This was linked to a weak response of nitrogen post anthesis accumulation and total nitrogen uptake at maturity, leading to low nitrogen use efficiency with high nitrogen supply. This variety responded weakly to an increase of nitrogen supply. A more responsive variety was Tapidor, which exhibited a strong increase in its number of grains leading to high yields with high nitrogen supply. The choice of the best variety will thus depend on the level of soil fertility available for its growth, the timing of its availability and on the importance of quality stability.

4.3 Efficiency of breeding for low input systems

The results showed a strong interaction between the genotype and the environment for both yield and nitrogen concentration potentials. Despite this interaction, the ranking of the varieties in the different treatments hardly changed. High yielding varieties under high input conditions also exhibited high yields under low input conditions. The same was true for the N concentration and several other traits, revealing thereby non-crossover interactions (cf. Ceccarelli, 1996). Interestingly, the varieties Aszita and Pireneo were selected under low input conditions while all other varieties were selected under medium to high input levels. The present results do not support the hypothesis that varieties specifically selected for low input are better adapted to low input conditions. Many similar findings have been reported (Barraclough et al., 2010; Guarda et al., 2004; Hasegawa, 2003; Le Gouis et al., 2000; Wang et al., 2011), and advocate the fact that specific selection programs for low input systems may not be necessary. In contrast, other studies show opposite results postulating that direct selection in low input environments helps to breed better adapted varieties (Brancourt-Hulmel et al., 2005; Ceccarelli, 1996; Dawson et al., 2008; El Bassam, 1998; Müllner et al., 2014; Presterl et al., 2002; Ruiz et al., 2008). Results from this study suggest that breeding may be carried out under any fertility conditions. Yet, testing advanced breeding lines and varieties systematically under a wide range of contrasting environments is still recommended, including high as well as low input conditions. This procedure, combined with baking quality assessments, will allow characterization of the nitrogen allocation of the variety and its aptitude for low input agriculture.

The treatments Mineral-highN and Manure-lowN provided comparable levels of plant total nitrogen uptake, thus differing basically by the source of nitrogen but not by the amount. The results showed only slight differences in terms of grain yield and N concentration that, moreover, were not consistent through the years. Nevertheless, high yielding varieties tended to better use mixed sources of nitrogen. It is however likely that potential effects are masked by environmental factors in particular by variability between the years. In contrast, other studies have given evidence to differential responses of varieties to organic fertilisation conditions (Baresel et al., 2008; Dawson et al., 2008; Löschenberger et al., 2008; Murphy et al., 2007; Reid et al., 2009, 2011; Vlachostergios and Roupakias, 2008; Wolfe et al., 2008). In addition, when breeding for low input conditions or for organic agriculture, other traits have also to be included in the selection scheme such as disease resistance, resilience to abiotic stress and competitiveness against weeds.

4.4 Development of selection criteria

In order to improve nutrient use efficiency of crops and thus yield and N concentration, it is necessary to identify appropriate targets of selection (Foulkes et al., 2009). Many studies have found genetic variability among traits involved in nitrogen processes, and genetic associations between these traits and the focal properties (Austin et al., 1977; Barraclough et al., 2010, 2014; Bogard et al., 2010; Foulkes et al., 2009; Gaju et al., 2011; Le Gouis et al., 2000). In the present study, almost all traits responded to a variety effect, suggesting genetic determinism. Nitrogen use efficiency, nitrogen utilisation efficiency and harvest index were strongly correlated with yield and N concentration in grain. However, these correlations were observed within all treatments, whatever the level of nitrogen fertilisation and organic enrichment. These traits can therefore not be used to target specifically high performance (yield or %N) in low input or organic environments.

The contributions of nitrogen uptake efficiency and nitrogen utilisation efficiency to nitrogen use efficiency were also explored in detail (following Moll et al., 1982). It was found that nitrogen utilisation efficiency plays a more important role than nitrogen uptake efficiency in nitrogen use efficiency, and this in all environments. This stresses the paramount importance of grain biomass production. Several other authors support this finding (Barraclough et al., 2010; Bingham et al., 2012; Gaju et al., 2011). However, contradictory results about the importance of uptake and use efficiency have been reported in different experiments and for different species. Indeed, other studies attribute a more important role to nitrogen uptake efficiency for the determination of nitrogen use efficiency, especially at low nitrogen levels (Dhugga and Waines, 1989; Le Gouis et al., 2000; Moll et al., 1982; Ortiz-Monasterio et al., 1997; Wang et al., 2011).

Present results showed that for grain nitrogen uptake, dry matter accumulation was more important than nitrogen concentration. Furthermore, these results postulated a major role of nitrogen accumulated in the whole plant at maturity and of the harvest index for the determination of grain nitrogen concentration at harvest. In these experiments, organic enrichment has been observed to change the respective contribution of nitrogen accumulated at anthesis and post-anthesis in total N uptake at maturity. In 2006, late accumulated nitrogen had a major role in the presence of manure, compared to the mineral treatments. This is probably explained by the increased rate of organic nitrogen mineralisation when temperatures rise in spring, which makes more nitrogen available for the crops after anthesis. This effect might have been more noticeable in 2006 than in 2007 because the last manure application was in spring 2005.

4.5 Conclusions

In the present experiment, the allocation of nitrogen for yield or for grain nitrogen (i.e. quality) has been investigated on an array of eleven modern wheat varieties in a 40-year long term field experiment. The experiment offered the conditions to compare the effect of mineral and organic nitrogen fertilisation in an experimentally robust scheme. Overall, the varietal responses depended strongly on the organic enrichment and mineral nitrogen fertilisation. Transition from high input production to low input or organic production would potentially alter the performance of varieties, mostly selected at high input levels, since the availability of nitrogen is delayed, compared to production systems exclusively fertilised with mineral nitrogen. Results presented here indicate that even if an important variability between varieties was observed, their ranking in terms of yield and nitrogen accumulation in grain was the same in nitrogen depleted and in nitrogen fertilised plots. Thus, varieties efficient in high input environments tended also to outcompete the other varieties in low input or organic fertilised environments. In conclusion, these results suggest that selection of wheat for nitrogen efficiency can be carried out under any nitrogen fertilisation regime. Yet, selection for low input agriculture must also integrate other traits, such as disease resistance, resilience to abiotic stresses and competitiveness against weeds.

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Supplementary Data

Table S1: Analysis of variance for the studied traits.

Table S2: Mean values of grain yield, grain nitrogen concentration (%N) and uptake (Ngrain) for each variety in each treatment.

Table S3: For each variety, linear regression coefficients of its difference from the mean treatment values on the nitrogen supply Nsup.

Table S4: Correlation coefficients between dry matter and nitrogen related traits.

Figure S1: Distribution of the studied traits as a function of organic enrichment and nitrogen fertilization treatments in 2006.

Figure S2: Distribution of the studied traits as a function of organic enrichment and nitrogen fertilization treatments in 2007.

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Table and figure legends

Table 1: Cumulated rainfall [mm] and growing degree days from sowing (T_{base} = 10°C) [°C] at different growth stages, and nitrogen fertilisation splits and amounts [kgN/ha] for each fertilisation treatments, for the two experimental years. Zadoks stage 89 corresponds to harvest.

Table 2: Wheat varieties tested in this study. Origin: A = Austria, CH = Switzerland, D = Germany, F = France. Year: year of release. Swiss class: estimation of the corresponding swiss class, based on variety quality. Use: principal use of the variety.

Table 3: Analysis of variance (p-values) for grain yield (DMgrain), grain nitrogen concentration (%N) and uptake (Ngrain), total dry matter at maturity (DMmat), nitrogen uptake at anthesis (Nanth) and total nitrogen uptake at maturity (Nmat). Factors: experimental year (Year), organic enrichment (Organic), mineral nitrogen fertilisation (Nitrogen) and Variety.

Table 4: Overall mean values of grain yield, grain nitrogen concentration (%N) and uptake (Ngrain), total dry matter at maturity (DMmat), nitrogen uptake at anthesis (Nanth) and total nitrogen uptake at maturity (Nmat), for each factor (experimental year, organic enrichment, mineral nitrogen fertilisation and variety).

Table 5: Decomposition analysis of varietal traits. %N: nitrogen concentration, Ngrain: grain nitrogen uptake, DMgrain: grain yield, DMmat: dry matter at maturity, HI: harvest index, Nmat: nitrogen uptake at maturity, NHI: nitrogen harvest index, Nanth: nitrogen accumulated

at anthesis, NpA: post-anthesis nitrogen accumulation, NUE: nitrogen use efficiency, NupE: nitrogen uptake efficiency, NutE: nitrogen utilisation efficiency.

Figure 1: Nitrogen concentration (%N) as a function of yield for the organic enrichment and nitrogen fertilisation treatments. Mean values over all the varieties and replicates are presented. The shape of the symbols stands for the mineral fertilisation treatments, circle: no nitrogen supply (noN), square: sub fertilisation (lowN), triangle: over fertilisation (highN). Text labels specify the organic enrichment treatments, Min: Mineral and Man: Manure. Year 2006 values are in darkgrey, and 2007 values in white. The dashed lines represent isolines of grain nitrogen uptake. The small grey symbols represent the individual variety values.

Figure 2: Characterisation of varieties. A. Nitrogen concentration (%N) as a function of yield (mean values over all treatments and replicates). B. Principal components analysis of the mean values of varieties (over all factors). Each number stands for a variety (see Table 2). Descriptors: yield = grain yield, %N = grain nitrogen concentration, Ngrain = grain nitrogen uptake, DMmat = total dry matter at maturity, Nmat = total nitrogen uptake at maturity, HI = harvest index, NHI = nitrogen harvest index, NUE = nitrogen use efficiency, NutE = nitrogen utilisation efficiency, NupE = nitrogen uptake efficiency, NUEP = nitrogen use efficiency for protein.

Figure 3: A. Grain yield (DMgrain), B. grain nitrogen concentration (%N) and C. grain nitrogen uptake (Ngrain), as a function of mean total nitrogen supply Nsup, for the two years. Left side: raw values, Right side: differences from the treatment mean. Variety specific linear regressions of the differences from the mean on the total nitrogen uptake are shown. Significant regressions are indicated with bold lines.

Figure 4: Pairwise comparisons of treatments Mineral-highN and Manure-lowN for A. grain yield in 2006, B. grain yield in 2007, C. grain nitrogen concentration (%N) in 2006 and D. grain nitrogen concentration (%N) in 2007. Each number stands for a variety (see Table 2). The grey lines have a slope of one, and pass through the overall mean of all the varieties.

Table 1

	2006				2007			
Zadoks stage	**25**	**30**	**40**	**89**	**25**	**30**	**37**	**89**
GDD [°C]	46	65	153	863	20	21	166	735
rainfall [mm]	324	496	576	681	337	349	359	701
noN [kgN/ha]	0	0	0		0	0	0	
lowN [kgN/ha]	40	80	0		40	60	0	
highN [kgN/ha]	70	80	50		70	70	40	

Table 2

#	Variety	Origin	Year	Swiss class*	Use
1	Pireneo	A	2005	1-TOP	Bread
2	Arina	CH	1981	1	Bread
3	Aszita	CH	2005	2	Bread
4	Titlis	CH	1996	TOP	Bread
5	Zinal	CH	2003	1	Bread
6	Ephoros	D	2004	3-5	Bread
7	Pegassos	D	1998	3	Bread
8	Toras	D	2004	3	Bread
9	Caphorn	F	2000	2-3	Bread
10	Farandole	F	1999	3	Bread
11	Tapidor	F	2002	5	Forage

*estimation of corresponding swiss classes based on Swissgranum classification requirement (http://www.swissgranum.ch/files/agrar_6_2015_getreideliste_f.pdf)

Table 3

	df	**Yield**	**%N**	**Ngrain**	**DMmat**	**Nanth**	**Nmat**
Year	1	**0.038**	**0.001**	**0.004**	0.198	**0.005**	0.125
Error	3						
Organic	1	**<0.001**	**0.001**	**<0.001**	**<0.001**	**0.003**	**<0.001**
Year:Organic	1	0.084	**0.046**	**0.040**	0.055	**0.029**	**0.035**
Error	6						
Nitrogen	2	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Year:Nitrogen	2	**0.015**	**<0.001**	0.749	**<0.001**	**<0.001**	**0.026**
Organic:Nitrogen	2	**0.018**	**<0.001**	**0.001**	0.123	0.124	**0.002**
Year:Organic:Nitrogen	2	0.504	**<0.001**	**0.039**	0.695	0.209	0.127
Error	24						
Variety	10	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.137	**0.001**
Year:Variety	10	**<0.001**	**<0.001**	**0.024**	**<0.001**	0.282	**0.026**
Organic:Variety	10	**0.040**	0.485	0.537	0.389	0.123	0.562
Year:Organic:Variety	10	**0.029**	**0.017**	0.205	**0.035**	0.053	0.156
Error	120						
Nitrogen:Variety	20	**<0.001**	**<0.001**	**0.001**	**0.002**	0.134	**<0.001**
Year:Nitrogen:Variety	20	**<0.001**	**0.001**	**0.010**	0.516	0.119	0.069
Organic:Nitrogen:Variety	20	0.434	0.090	0.361	0.077	0.095	0.076
Year:Organic:Nitrogen:Variety	20	0.984	0.696	0.852	0.993	0.053	0.680
Error	240						

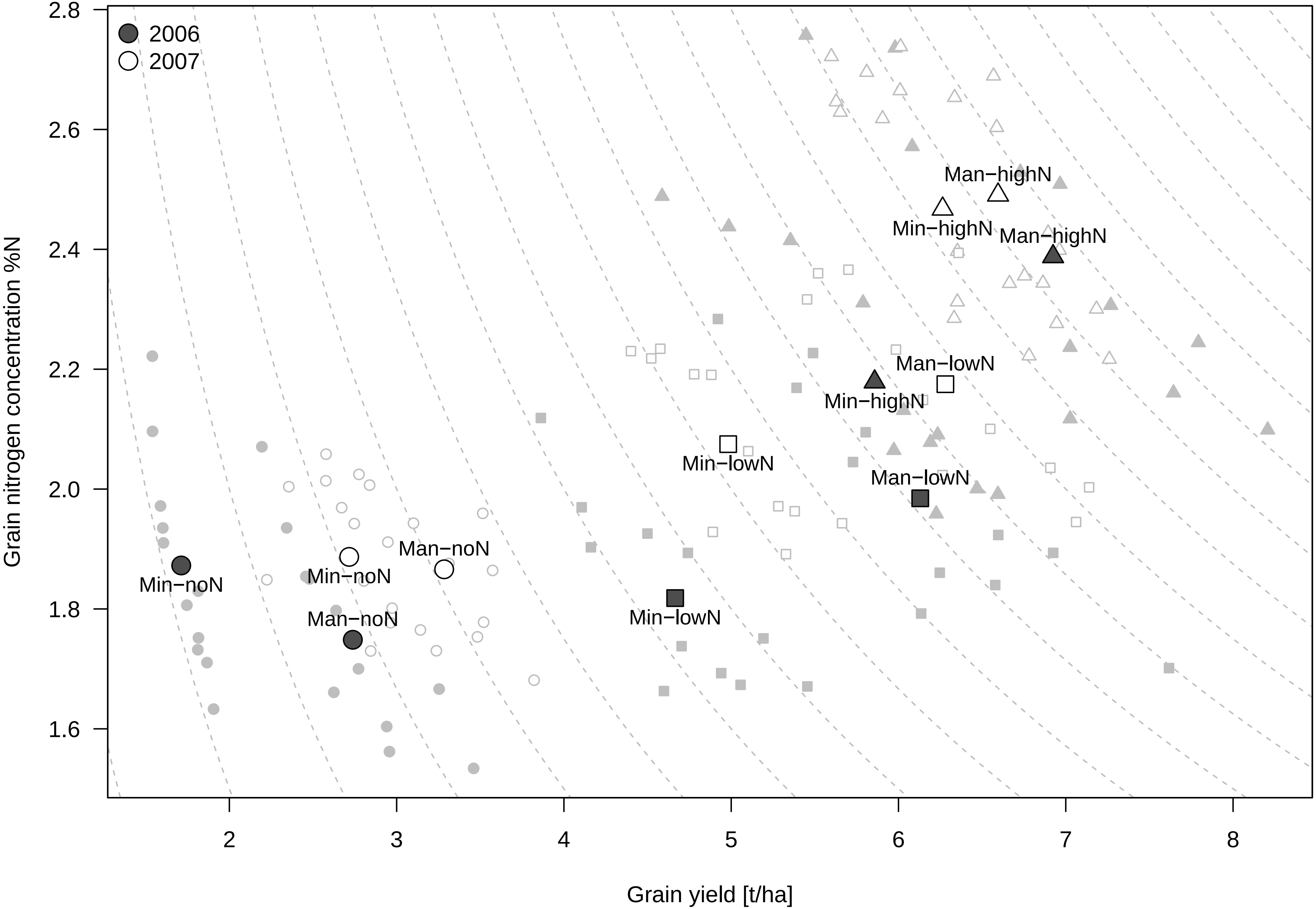
Table 4

	Yield [t/ha]	% N	Ngrain [kg/ha]	DMmat [t/ha]	Nanth [kg/ha]	Nmat [kg/ha]
Overall	4.85	2.08	104	11.60	69	127
2006	4.67	2.00	96	11.94	80	123
2007	5.02	2.16	111	11.26	58	131
lsd 5%	*0.31*	*0.04*	*6*	*1.31*	*9*	*12*
Mineral	4.37	2.05	92	10.30	61	111
Manure	5.33	2.11	115	12.90	77	143
lsd 5%	*0.27*	*0.02*	*6*	*0.77*	*8*	*7*
noN	2.61	1.84	48	6.45	29	57
lowN	5.51	2.01	111	13.13	74	135
highN	6.41	2.38	152	15.22	104	190
lsd 5%	*0.25*	*0.03*	*5*	*0.68*	*6*	*7*
1. Pireneo	4.77	2.20	109	11.99	66	125
2. Arina	4.30	2.25	100	12.20	69	126
3. Aszita	4.21	2.33	101	10.61	75	127
4. Titlis	4.28	2.25	100	12.16	65	120
5. Zinal	4.69	2.16	105	11.14	74	130
6. Ephoros	5.16	1.87	100	11.83	65	124
7. Pegassos	5.14	1.96	104	11.79	70	132
8. Toras	4.89	1.97	99	11.88	69	131
9. Caphorn	5.13	1.99	105	11.75	71	128
10. Farandole	5.26	1.99	108	11.30	69	125
11. Tapidor	5.47	1.90	107	10.95	66	129
lsd 5%	*0.20*	*0.04*	*4*	*0.53*	*8*	*6*

Table 5

			Mineral			Manure		
	Resultant trait	Component traits	noN	lowN	highN	noN	lowN	highN
2006	log Ngrain	log DMgrain	-0.46	1.17	1.71	1.65	1.53	1.26
		log %N	1.46	-0.17	-0.71	-0.65	-0.53	-0.26
	log DMgrain	log DMmat	0.43	0.02	-0.13	0.36	0.08	-0.09
		log HI	0.57	0.98	1.13	0.64	0.92	1.09
	log Ngrain	log Nmat	0.87	1.02	0.79	0.93	0.99	0.74
		log NHI	0.13	-0.02	0.21	0.07	0.01	0.26
	Nmat	Nanth	1.66	1.07	0.94	0.46	0.39	0.45
		NpA	-0.66	-0.07	0.06	0.54	0.61	0.55
	log NUE	log NupE	-0.07	0.27	0.14	0.25	0.21	0.00
		log NutE	1.07	0.73	0.86	0.75	0.79	1.00
2007	log Ngrain	log DMgrain	1.00	1.04	0.23	1.17	1.03	0.90
		log %N	0.00	-0.04	0.77	-0.17	-0.03	0.10
	log DMgrain	log DMmat	0.34	0.11	-0.25	0.59	0.19	-0.12
		log HI	0.66	0.89	1.25	0.41	0.81	1.12
	log Ngrain	log Nmat	0.84	0.89	0.85	1.01	0.84	0.92
		log NHI	0.16	0.11	0.15	-0.01	0.16	0.08
	Nmat	Nanth	0.92	0.18	1.51	0.93	0.76	-0.27
		NpA	0.08	0.82	-0.51	0.07	0.24	1.27
	log NUE	log NupE	0.48	0.19	-0.06	0.53	0.24	0.21
		log NutE	0.52	0.81	1.06	0.47	0.76	0.79

Figure 1



2006
2007
Man−highN
Min−highN
Man−highN
Man−lowN
Min−highN
Min−lowN
Man−lowN
Man−noN
Min−noN
Min−noN
Man−noN
Min−lowN
2.8
2.6
2.4
2.2
2.0
1.8
1.6
2
3
4
5
6
7
8
Grain nitrogen concentration %N
Grain yield [t/ha]

Figure 2

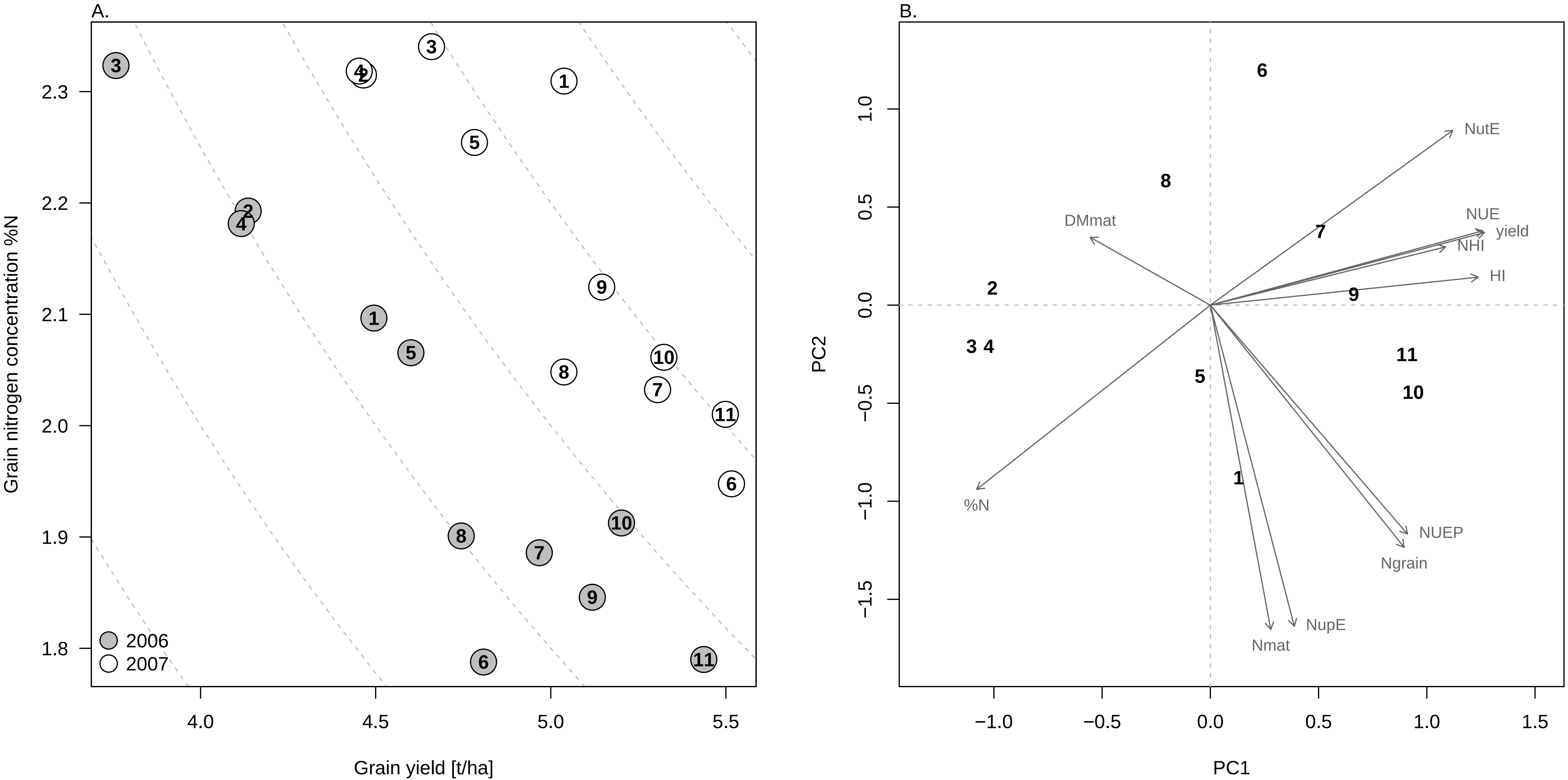

A.
Grain nitrogen concentration %N
Grain yield [t/ha]
1.8
1.9
2.0
2.1
2.2
2.3
4.0
4.5
5.0
5.5
3
4
2
3
1
5
2
4
9
1
5
10
8
7
11
6
8
7
10
9
6
11
● 2006
○ 2007
B.
PC2
PC1
1.0
0.5
0.0
−0.5
−1.0
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
6
NutE
8
NUE
DMmat
7
yield
NHI
2
HI
9
3 4
11
5
10
1
%N
NUEP
Ngrain
NupE
Nmat

Figure 3

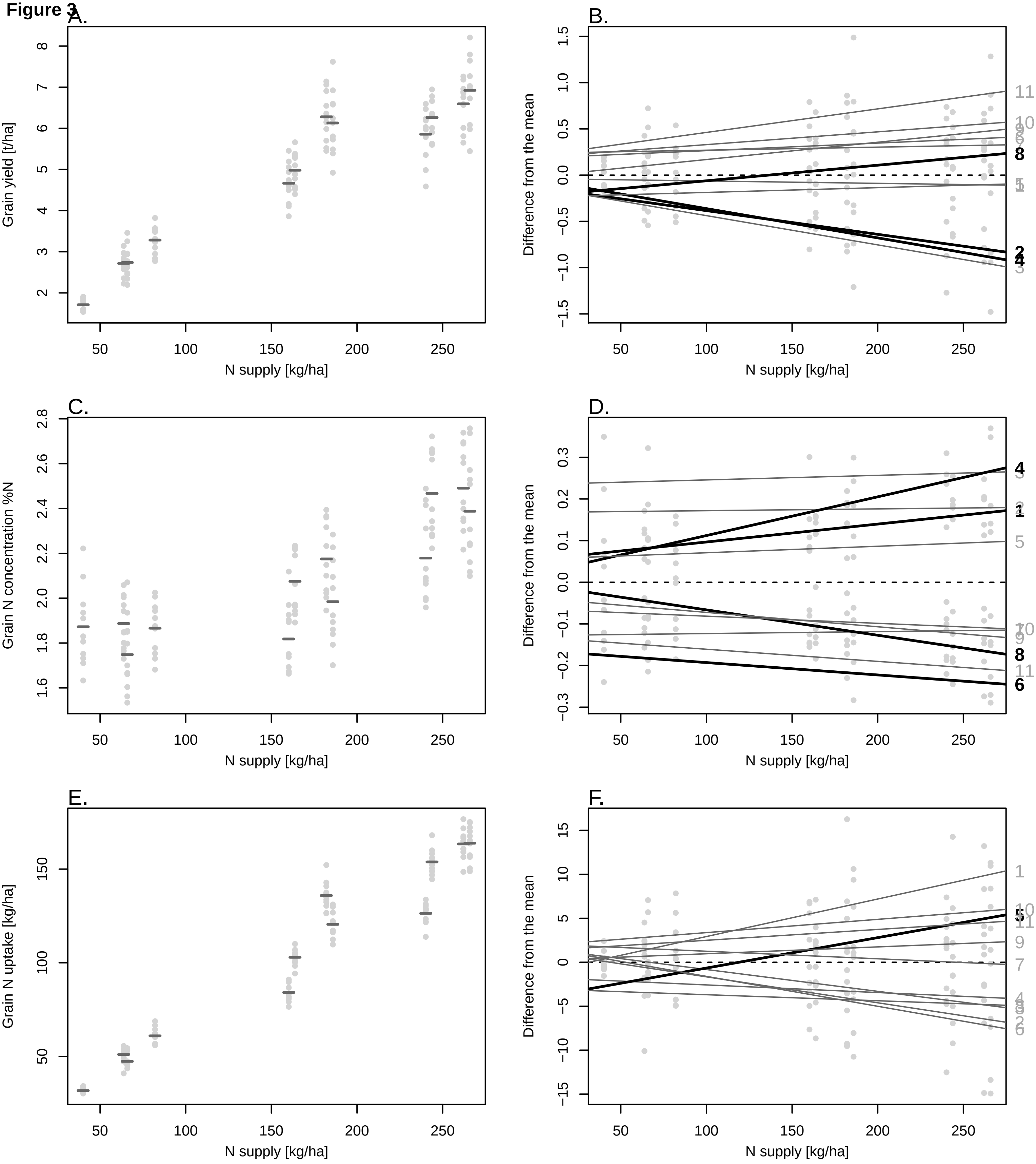
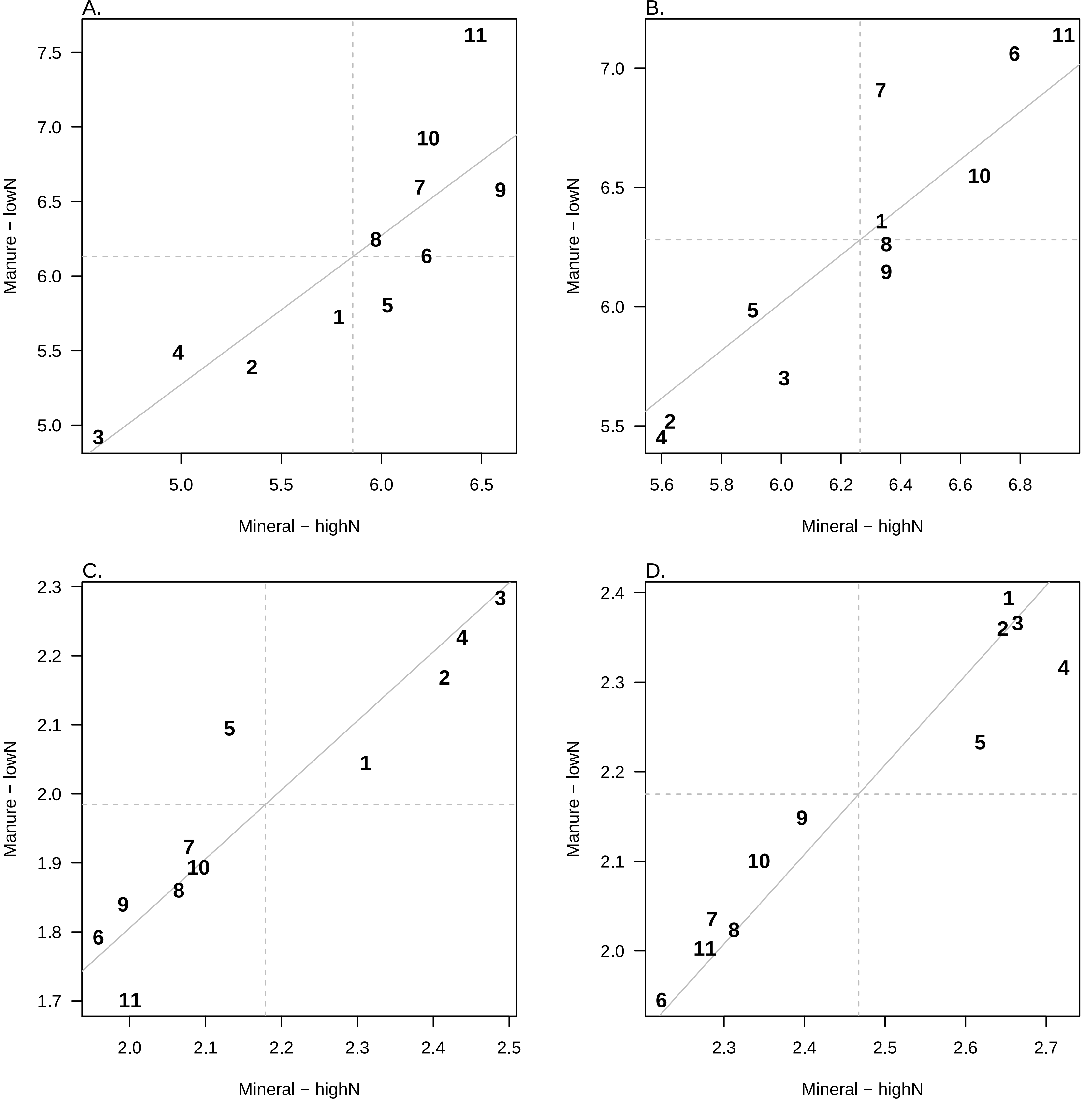

A.
Grain yield [t/ha]
2
3
4
5
6
7
8
N supply [kg/ha]
50
100
150
200
250
B.
Difference from the mean
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
11
10
9
6
7
8
5
1
2
4
3
C.
Grain N concentration %N
1.6
1.8
2.0
2.2
2.4
2.6
2.8
D.
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
4
3
2
1
5
10
7
9
8
11
6
E.
Grain N uptake [kg/ha]
50
100
150
F.
15
10
5
0
−5
−10
−15
1
10
8
11
9
7
4
5
3
2
6

Figure 4


A.
Manure − lowN
Mineral − highN
5.0
5.5
6.0
6.5
7.0
7.5
11
10
7
9
8
6
1
5
4
2
3
B.
Manure − lowN
Mineral − highN
5.5
6.0
6.5
7.0
5.6
5.8
6.2
6.4
6.6
6.8
11
6
7
10
1
8
9
5
3
2
4
C.
Manure − lowN
Mineral − highN
1.7
1.8
1.9
2.0
2.1
2.2
2.3
2.4
2.5
3
4
2
5
1
7
10
8
9
6
11
D.
Manure − lowN
Mineral − highN
2.0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
1
2
3
4
5
9
10
7
8
11
6

Supplementary data