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Abstract

Improving arable crops Nitrogen Use Efficiency (NUE) is a major target of plant breeding. In wheat, a large part of the Nitrogen (N) harvested in the grain originates from N remobilization from vegetative organs during the post-anthesis period. While N remobilization from above-ground organs such as leaves and stems has been extensively studied, studies about N remobilization from roots are scarce. The existence of genetic variability for biomass allocation to the root pool as well as for root N concentration and remobilization may strongly affect the N economy of the crop. By studying the root system of 16 elite wheat genotypes under two contrasted N treatments, we showed that the biomass allocation to roots was strongly related to crop tillering dynamics and N nutritional status. Therefore, the apparent genetic variability for biomass allocation to roots is not intrinsic, but rather a consequence of genetic variability for crop growth and N utilization taken as a whole. In addition, we showed here that the N amount potentially remobilized from roots to the grain is extremely small. Existing genetic variability for root N content and remobilization efficiency cannot explain observed differences in genotypes grain N concentration or N yield. These results indicate that there is little prospect for breeding strategies specifically aiming at optimizing wheat root biomass allocation and N remobilization for improving NUE and GPC for elite genotypes at least in highly productive conditions. These results nevertheless do not imply that the root compartment should be totally discarded in all breeding programs since architectural traits such as root length or distribution may impact strongly crop performance.

Wheat; gentic variability; root; NUE

1. Introduction

Until very recently, productivity has been the major target of agronomical sciences. The growing demand for low input agriculture, driven both by economical and environmental considerations emphasizes now the improvement of resource use efficiency. Nitrogen (N) fertilizers play a major role in crop productivity, but are also one of the main sources of agricultural pollution either through nitrous oxide (N_2O) volatilization or nitrate (NO_3-) leaching (IPCC, 2001; DECC, 2010). N fertilization represents also a major cost for farmers. Increasing N use efficiency (NUE) received therefore much attention in the recent years whether through improved agronomical practices (Cui et al, 2011) or plant breeding (eg Hirel et al. 2007; Foulkes et al., 2009; Gaju et al., 2011). This quest for highly efficient wheat cultivars and agronomical practices is made more complex by the fact that not only grain yield but also grain protein concentration (GPC), the major determinant of wheat end-use quality (eg Shewry 2007; Oury et al., 2010) should be improved. The involvement of N in both biomass accumulation and grain protein concentration determination creates a need for studying the complex interactions between carbon and N metabolisms that determine crop productivity and quality (Triboi and Triboi-Blondel, 2002).

In wheat, the major part of N uptake occurs before anthesis. After anthesis, N remobilization from the vegetative organs toward the growing grains is a strong determinant of both GPC and grain yield through the senescence process that alters the crop photosynthetic capacity (Borell et al., 2001; Triboi et al 2006; Bogard et al. 2011). Understanding finely N remobilization process is therefore a key question for the optimization of the N economy of wheat crops. N distribution and remobilization patterns in aboveground organs have received much attention in past years (e.g. Gregersen et al., 2008; Taylor et al., 2010). In particular, it was shown that the remobilization pattern of these vegetative organs follow a robust first-

order kinetic that is independent of organ age, genotype and N nutrition (Bertheloot et al., 2008). Genetic variability for N remobilization efficiency has also been characterized (Barbotin et al 2005, Uauy et al., 2006; Kichey et al 2007) showing relatively large genetic variation for this trait and strong interaction with the environment and N fertilizer regimes.

Oppositely little is known about the N allocation and remobilization pattern in the wheat root system. In controlled conditions, wheat roots have the ability to remobilize N to the grain (Andersson et al., 2005) and are the last organs to senesce (Peoples and Dalling, 1988). In these conditions, N partitioning to the roots and redistribution of N from the roots to the grain may play an important role for the nitrogen budget of the whole plant (Andersson et al., 2005) since a large part (10 to 20%) of the total plant N at maturity appears to remain in the roots (Andersson and Johansson, 2006). This may have several consequences since most studies occult the root compartment. First, accounting for the root compartment may affect to some extent whole plant NUE if it is associated to different shoot/root allocations (Andersson et al., 2005). Substantial genetic variation has already been observed for this trait in wheat (Siddique et al., 1990; Hoad 2001). Second, if variations in shoot/root allocation were associated to large differences in root N content and remobilization patterns, it may impact other agronomical traits such as grain protein concentration. The aim of this study was to analyze the N allocation to roots in wheat and the net N remobilization from roots during the post-anthesis period under field conditions. A particular emphasis was put on the evaluation of the genetic variability associated with these processes. Root biomass, relative allocation and mass loss were studied on 16 bread wheat cultivars grown in the field at two N levels. Root N content and remobilization were quantified in order to evaluate the potential role of N remobilization from roots in the N economy of the crop.

2. Materials and Methods

2.1. Plant material and growing conditions

Sixteen genotypes of winter bread wheat (Table 2) were studied in a field experiment carried out at Clermont-Ferrand, France (45°47' N, 3°10' E, 329 m elevation) during the 2007-2008 growing season. This panel of genotypes represents a large part of the genetic variability for nitrogen use efficiency and its components (Le Gouis *et al.*, 2000; Gaju *et al*., 2011) and for the deviation from the negative correlation between grain yield and protein concentration (Oury et *al.*, 2003) reported in UK and France elite germplasms. Soil characteristics f are presented in Table 2. Crops were sown at a density of 250 seeds m^{-2} on 06 November 2007. A high (HN) and a low (LN) N treatments were applied. For the HN treatments, the rates of N fertilisation were determined using the balance sheet method (Rémy & Hébert, 1977) to optimize grain yield. N was applied in four splits as ammonium nitrate granules (33.5% N) with 4, 8, 8, and 4 g N m^{-2} being applied when Rialto, which has a rate of development in the middle of the range of the sixteen cultivars used in this study (Table 2), reached growth stages (GS; Zadocks *et al*., 1974) GS21 (beginning tillering), GS31 (ear at 1cm), GS39 (male meiosis), and GS61 (anthesis) respectively. In the LN treatment, 4 g N m^{-2} were applied in one split when Rialto reached GS31. The experimental field was not irrigated. All other crop inputs including weed, disease and pest control, and potassium, phosphate and sulfur fertilizers, were applied at levels to prevent nutrients or weeds, diseases and pests from limiting yield. The experimental design was a split-plot in which N treatment was randomized on main plots, cultivars were randomized on the sub-plots and each treatment was replicated three times. Sub-plot size was 7 × 1.5 m with an inter-row spacing of 0.17 m.

2.2. Plant sampling

Plants were sampled at anthesis (GS61) and full grain maturity (GS92). When each genotype reached the appropriate stage, 0.5 m² per sub-plot was cut at ground level. The total fresh mass of the samples was determined and a 25% sub-sample was randomly selected. The main and secondary shoots were separated and counted. Shoots were classified as main when the top of their ear was in the top 0.15 m layer of the canopy. Thirty main shoots were randomly selected and dissected into individual leaf laminae, stems (including leaf sheaths) and ears. The fresh mass of the secondary shoots of the sub-samples was determined, and sub-samples of secondary shoot (20%) were randomly selected. Their fresh mass was determined before separating them into leaf laminae, stems (including leaf sheath) and ears. For both main and secondary shoots, green and dead photosynthetic (identified by their brownish colour) tissues were analysed separately.

2.3. Root sampling

Root sampling was performed with a square-shaped soil corer (height = 40 cm, section = 18 × 18 cm). The corer was positioned on the soil, centered on a plant row and drove into the soil to a depth of 30 cm with a sledge hammer. The soil core was then retrieved, soaked into 10 L of water to facilitate soil disaggregation and washed abundantly with water above a 1 mm mesh-size sieve until roots were totally free of soil.

2.4. Plant tissues dry mass and nitrogen concentration,

Lamina, stem, chaff, grains and roots dry mass (DM) of the main shoots were determined after oven drying at 80°C for 48h. Samples were then ground and their total N concentration (N mass per unit dry mass) was determined with the Dumas combustion method (AOAC

140 method n° 7.024) using a FlashEA 1112 N/Protein analyser (Thermo Electron Corp.,
141 Waltham, MA, USA).
142
143 *2.5. Calculations*
144 Several composite traits were calculated. Biomass allocation was investigated by calculating
145 the root/rhoot ratio (R/S) as follows:
146 $$R/S = SDM / RDM \quad (1)$$
147 where SDM is the total aboveground dry mass including leaves, stems, chaff and grain and
148 RDM the total root dry mass. The N root/shoot ratio (RN/SN) was calculated similarly as:
149 $$RN/SN = RN / SN \quad (2)$$
150 where SN and RN are the N amount in the aboveground and belowground organs
151 respectively. Crop N harvest index was calculated based on shoot N only (NHI) or total plant
152 N (NHI_{tot}):
153 $$NHI = GN / SN * 100 \quad (3)$$
154 $$NHItot = GN / (SN + RN) * 100 \quad (4)$$
155 Where GN is the amount of N in the grains.
156 In order to evaluate the efficiency of N assimilation in the crop, nitrogen utilisation efficiency
157 was calculated based on shoot N only (NUtE) or total plant N ($NUtE_{tot}$):
158 $$NUtE = GDM / SN \quad (5)$$
159 $$NUtE_{tot} = GDM / (SN + RN) \quad (6)$$
160 Where GDM is the grain dry mass.
161 The amount of N lost by roots (NLR) during the post-anthesis period was calculated as the
162 differences of N amounts in roots between anthesis (RN_{anth}) and maturity (RN_{mat}):
163 $$NLR = RN_{anth} - RN_{mat} \quad (7)$$

The amount of N potentially remobilized from senescent roots ($NRR_{0.3}$) was estimated based on the assumption that N concentration in dead root tissue was fixed at 0.3% since this value was close from the lowest root N% observed in this study:

$$NRR_{0.3} = (RDM_{anth} - RDM_{mat}) \times (RN\%_{anth} - 0.3) / 100 \quad (8)$$

Where $RN\%_{anth}$ is the N concentration in root material at anthesis.

Finally the proportion of grain N remobilized from roots (PGNR) was calculated as

$$PGNR = GN_{mat} / NRR_{0.3} \quad (9)$$

The N nutrition Index (NNI) was estimated at anthesis as proposed by Justes et al. (1994).

$$N_t = 5.35 \times SDM^{-0.442} \quad (10)$$

Where N_t is the critical N concentration (%DM) and SDM expressed in $t.ha^{-1}$. NNI is then calculated as the ratio between actual shoot N concentration and N_t.

$$NNI = SN\% / N_t \quad (11)$$

3. Results

3.1. Climate

The first part of the crop cycle occurred under relatively dry conditions (Fig. 1). During the September-February period rainfall was about 30 % below the 20-year average for the same period. Oppositely the following part of the cycle occurred under extremely wet conditions. From March to July rainfall reached 390 mm representing a 40% excess compared to the 20-year average. Consequently the whole post-anthesis period was characterized by an absence of water limitation.

3.2. Crop structure at anthesis

The genotypes used in the present experiment exhibited a large variation in term of developmental rate. Anthesis date varied from May 19th (Récital) to June 4th (Beaver and

188 Consort; Table 2). Tiller number at anthesis was affected by N treatment (P=0.048) but no
189 genotype effect was found (Table 2). On average, tiller number at anthesis was 442 and 654
190 tiller m^{-2} under LN and HN, respectively. At anthesis, SDM was strongly affected by N
191 treatment with an 18% increase under HN compared to LN averaged across all genotypes.
192 Statistically significant differences between genotypes were also measured. In particular,
193 Récital and Renan, the two earliest genotypes, had high anthesis SDM under both N
194 treatments. Comparatively RDM was not affected by N treatment with 190 g DM m^{-2} on
195 average over all combinations (Table 2). Genotypic effect was particularly strong with three
196 genotypes having low RDM under both N treatments (Arche, CF99102 and CF 9107). No
197 correlation was found between SDM and RDM at anthesis (data not shown) with r^2 of 0.001
198 and 0.02 under LN and HN, respectively. RDM was strongly and positively associated with
199 tiller number (Fig. 2) with about 50% of the RDM variation explained by tiller number under
200 both N treatments. The slope of the regression for LN and HN treatments were not
201 statistically different (common slope: 0.22 gDM $tiller^{-1}$) but the intercept was significantly
202 lower under HN than LN. Comparatively, tiller number did not explain SDM at anthesis
203 (Fig.2; r^2 = 0.004 and 0.02 under LN and HN, respectively). The allocation between shoot and
204 root of both DM (Fig. 3) and N were correlated with crop N status but only under LN. Under
205 LN, R/S ratio varied between 0.18 and 0.30 while NNI varied between 0.38 and 0.55 (Fig. 3),
206 the latter values typically found under strong N restrictions. The predictive power of NNI was
207 even stronger for the N allocation with RN/SN values ranging between 0.07 and 0.13 (data
208 not shown). Both relations were poorly explicative under HN (Fig. 3). This was particularly
209 caused by four outliers (genotypes Beaver, Rialto, Savannah and Soissons) with high
210 allocation of DM and N to roots. These outliers were not explained by relatively higher
211 variation coefficients than other genotypes (data not shown). If these outliers are removed,

average R/S for the 12 remaining genotypes for HN was 0.19 and RN/SN was 0.08. Both values were similar to the lower range of the values observed for LN (Fig. 3).

RDM variation during the post-anthesis period was negatively correlated with RDM at anthesis under both N treatments (Fig. 4, $r^2 = 0.38$ and 0.67 under LN and HN respectively) and ranged from -5% to -54%. The proportional loss of belowground biomass increased with the initial belowground biomass present at anthesis.

3.3. Nitrogen remobilization from the belowground compartment and effects on nitrogen allocation calculations

NHI at anthesis was both under strong N treatment and genotype effects (Table 3). On average over genotypes NHI equaled 79 and 72% under LN and HN, respectively. Accounting for belowground N in the calculation led to an about 3 percent point decrease in the calculated NHI_{tot}. Nevertheless this did not impact significantly genotypes ranking for this trait. Spearman's rank test gave correlation Rs values of 0.83 and 0.97 for LN and HN, respectively, indicating strong rank correlations between the two calculations. Results were equivalent for NUtE calculations with a small impact of accounting for belowground N pool. $NUtE_{tot}$ was on average 1.7 percent point lower than NUtE. Again this has no impact on genotypes ranking (Rs values of 0.96 and 0.98) under LN and HN, respectively. Calculation of N potentially lost by roots during the post anthesis period (NLR) indicated that only a small N amount was lost as senesced material or remobilized during this period. The largest part of this N amount was caused by a decreased in RDM and not by a decrease in remaining biomass decrease in N concentration. Indeed, RN% variation during post anthesis under LN was nil (0.41% at both anthesis and maturity) and only marginal under HN (0.83 and 0.79 at anthesis and maturity, respectively). Calculating potentially remobilized N from senesced roots is hazardous since the N concentration of senesced root material was not measured and

may vary with time, type of roots, treatment and genotype. Nevertheless, N remobilization from roots ($NRR_{0.3}$) was estimated based on a putative N concentration in the senesced root material of 0.3%, a value close from the lowest root N concentration observed in the present experiment but much lower than reported values for dead fine roots (Gordon and Jackson, 2000). Even based on the assumption of a 0.3% N in dead roots, extremely small amount of potentially remobilized N from roots were calculated. $NRR_{0.3}$ values were on average 0.1 and 0.4 gN m^{-2} for LN and HN, respectively, corresponding to 0.8 and 2.3% of the total grain N at maturity. Genetic variation observed for both the quantity and the proportion of N remobilized from roots were not correlated with any of the traits of interest such as grain N quantity or concentration (data not shown). In addition remobilization efficiency of the belowground plant material was not related whatsoever with remobilization efficiency of aboveground vegetative organs.

4. Discussion

The aim of this study was to analyze the potential impact of the belowground compartment on the N economy of wheat. Based on previous results in controlled conditions showing that the N economy of the whole plant was affected to a great extent by the N amount in the roots (Andersson et al., 2005), our main objective was to test, under field conditions it the genetic variability associated with N remobilized from roots was detectable. Indeed, large genetic variation associated with this process might strongly impact the assessment of the genetic diversity of agronomical traits of interest such as NUE, post-anthesis N uptake and grain N. It is clear that data obtained in a single environment cannot reveal the full extent of genetic variation among a set of genotypes. However, this information can help determine if putatively observed genetic variability relies on intrinsic genetic differences of the root

compartment or if these differences follow a generic response function to traits obtained at the whole plant level.

4.1. Root sampling methodology

In the present experiment root biomass was only sampled in the 0-30 cm horizon. Clearly the full root biomass was not harvested by this method since the maximum rooting depth was evaluated to 90 cm at the experimental site. This would clearly be a strong limitation if the objective was to compare genotypes in term of functional processes such as water and N extraction capacity. Here, the key aspect was to retrieve a large proportion of the total root biomass to assess differences of biomass and nitrogen allocation pattern between genotypes. Kätterer et al. (1993) studied the root biomass of a winter wheat genotype under four management treatments including dry and irrigated crops. On average, after anthesis about 85% of the root biomass was found in the top 30 cm horizon. This proportion tended to increase in the well irrigated treatment. Sidique et al. (1990) also observed very high proportion of the total root biomass in the first 30 cm. A root dry matter profile was available for three genotypes and showed that more than 90% of the total root biomass was in this horizon. Similar figures can be found in Wechsung et al. (1999). Xue et al. (2003) reported lower proportion of root biomass (about 60%) in the first 30 cm, but in a soil with a maximum rooting depth of 2 m thus more than twice as deep as our soil. We therefore believe that the sampling method used in the present experiment allowed harvesting a very significant proportion of the total root biomass and that it is very unlikely that significant bias would hamper genotype comparisons. Kätterer et al. (1993) reported roots biomass values at anthesis of 92 g m^{-2} while values presented by Siddique et al. (1990) at the same developmental stage are three times higher (310 g DM m^{-2}). With average values of about 200 g DM m^{-2} the values

285 of the present study tend to confirm that the sampled biomass is representative of the whole
286 soil profile.
287

288 *4.2. Root biomass allocation and dynamics*

289 The present data clearly shows the effect of the genetic differences in tiller numbers on both
290 root biomass and allocation. Comparatively, both aboveground biomass and yield were
291 independent of tiller number in the present study. Tiller number optimization has been
292 identified as a potential candidate trait for yield increase (Reynolds et al., 2009). Under
293 extremely limiting conditions such as strong terminal drought, low tiller number may be a
294 promising trait for water economy and final grain yield (Dugan et al., 2005; Munns and
295 Richards, 2007). The introduction of a reduced tillering (tin) gene in wheat lines has
296 nevertheless been shown to have extremely contrasted effects on yield depending on the
297 environment and genetic background but with a tendency towards grain yield reduction
298 (Mitchell et al., 2012). In the present growing conditions, with elite material, it seems that the
299 observed genetic variations in tiller number is only a phenotypic expression of the strong
300 phenotypic plasticity of wheat, a crop that is able to express yield in a range of ways through
301 strong compensations between yield components (Lawless et al., 2005; Sinclair and Jamieson,
302 2008). In particular the tiller size/density compensation process, well characterized in
303 perennial grasses (Matthews, 1996) seems to have operated in our conditions. Nodal root
304 emission is strongly synchronized with leaf and tiller production (Klepper et al 1984). This
305 coordination does not necessarily induce a metric relationship between tiller number and root
306 biomass. Individual root can vary in diameter, length or density and have a specific branching
307 pattern that potentially breaks this relationship. In addition, low tillering can be seen as a
308 possibility for plants to allocate more resources towards root development (Duggan et al.,

2005). Nevertheless, the data presented here show that the tillering dynamic of the crop has a strong influence on root biomass at anthesis.

A second strong determinant of biomass allocation to roots at anthesis is the crop nitrogen status. The effect of the plant nitrogen status on the R/S ratio of plant has received much attention in particular for modeling purpose (Hilbert, 1990; Gleeson, 1993; Thornley, 1995; 1998; Agren and Franklin, 2003) and clearly demonstrated that the R/S decreases with increasing N supply. These models use plant N concentration (Franklin and Agren, 2003) or C, N availability (Thornley, 1995) to predict biomass allocation and are based on the hypothesis that biomass allocation is strongly controlled at the plant level and optimized in relation to the availability of C and N. In our study, NNI was the best predictive variable for R/S. The nitrogen nutrition index as described in Gastal and Lemaire (2002) allows to quantify the N status of the crop dynamically; i.e. accounting for the decreasing crop N demand as crop gets larger (Gastal and Lemaire, 2002). Therefore, optimal crop relative growth rate is attained for a crop N concentration, called critical N concentration that decreases following a power function of crop biomass. NNI derives from a comparison between actual crop N concentration and critical N concentration at the observed biomass and allows the N status of the crop to be quantified over development and also to compare N status of crops of different biomass.

Modeling analysis clearly showed that functional equilibrium approaches predicting R/S in term of C and C co-limitation was not valid for extreme conditions of high and low N availability (Agren and Flanklin, 2003). This can be related to the apparent saturation of the R/S response to NNI for high NNI values. Indeed in the present study R/S reached a minimal

R/ value of about 0.2 in the HN treatment. A possible explanation for this saturation relies on the positive correlation between tiller density and R/S observed here caused by the strong coordination between tiller and root dynamic (Hoad et al., 2001) which may lead to a minimal R/S under given environmental conditions.

Based on the strong association observed between root biomass and crop tillering dynamic on the one hand and allocation and crop N status on the second hand, we believe that the observed genetic variability for biomass allocation to roots (Table 2), is a consequence of genetic variability for crop growth and N utilization taken as a whole rather than an intrinsic variability for biomass allocation to roots.

In term of potential nutrient remobilization to the grain during the post anthesis period, it is also important to assess the biomass variation of the root pool during this period. In wheat decrease in root mass after anthesis is generally observed due to a root death rate exceeding root production rate (Kätterer et al., 1993; Steingrobe et al., 2001). This is a common feature observed in annual plants for which the developing grain is a strong competitive sink for the carbon resource (Eissenstadt and Yanai, 1997) leading to a strong root length decline after flowering in wheat (Box and Johnson, 1987 in Eissenstadt and Yanai, 1997). In the present study we observed a strong negative correlation between root mass loss during the post-anthesis period and root biomass at anthesis. In other words, plants with high root biomass at anthesis tend to loose a greater proportion of this biomass. A possible explanation relies on the much shorter life span of fine roots compared to larger roots (Eissenstadt et al., 2000). Indeed a large part of the root biomass variability may be associated with branching variability leading to a greater proportion of fine roots in plants with high root biomass. No root separation by class size could be performed in the present study therefore the causal explanation of this process remains putative. Nevertheless, the key aspect is that the apparent

359 genotypic variation associated with root biomass loss follows general responses and probably
360 do not mean a genetic variability for root longevity *stricto sensu*.
361
362 *4.3. Root nitrogen content and remobilization*
363 In the present study no attempt was made to differentiate live from dead roots in the sampled
364 material. Therefore the analyzed material is a mixture of roots differing in age and state.
365 Nevertheless based on this coarse dataset it seems clear that net N remobilization from roots
366 during the post-flowering period in wheat grown in the field is a marginal process. First, N
367 concentration in the sampled root materials did not vary between anthesis and maturity (Table
368 3). Comparatively, other vegetative organs are a net source of N for the developing grain and
369 remobilize about 70% of the N present at anthesis (e.g. Gaju et al., 2011). The remobilization
370 pattern of these vegetative organs follows a robust first-order kinetics independent of organ
371 age, genotype and N nutrition (Bertheloot et al., 2008). Second, the estimated potential N
372 remobilization from dead roots represents less than 3% of the total grain N at maturity. Our
373 estimate of remobilized N is in addition probably overestimated since past studies focusing
374 specifically on nutrient remobilization from dying fine roots tended to show that N
375 concentration did not vary between live and dead roots material implying little if no N re-
376 translocation from senescing roots (Gordon and Jackson, 2008). Based on an experiment in
377 hydroponic conditions, Andersson and Johansson (2006) observed that N amount in the root
378 at maturity was 10-20% of total plant N thus potentially affecting NUE calculations. Here we
379 clearly show that under field conditions N amount in the roots is about 4% of total plant N
380 and affects only marginally calculated variables such as NHI or NutE with in particular no
381 effect on genotype ranking for these variables. In addition no correlation between root N
382 concentration or estimated amount of N remobilized from roots and agronomic variables of
383 interest were found. Quantitatively speaking, the amount of N potentially remobilized from

roots, in spite of its probable over estimation in the present study cannot affect significantly the amount of N in the grain. Clearly, the observed genetic variation for grain N content is not likely to be determined by variations in the root N pool.

5. Conclusions

This experiment provided strong indications that the apparent genetic variability existing for wheat root biomass and allocation is driven by crop growth pattern rather than root growth variability *per se*. Similarly, root loss during the post-anthesis period appeared to be largely driven by root biomass at anthesis. More generally root biomass dynamics seems to be determined to great extent by the crop status at anthesis. There seems to be little prospect for a further exploration of root biomass genetic variability that is independent of crop response. Given the relatively small proportion of N in the roots and the apparent low N remobilization from this pool to the grain, taking into account roots in the determination of N related traits such as NUE appears of little interest. In particular, genotype ranking for this trait is not affected by the accounting or not of this generally ignored N compartment. Of course, root architectural traits such as total root length and root vertical distribution may be of major importance for crop N, water acquisition and adaptation. Observed genetic variability for such traits may be strong determinants of wheat genotypes performance in particular in limited environment (Manshadi et al., 2010), but this was beyond the scope of the present study.

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Figures captions

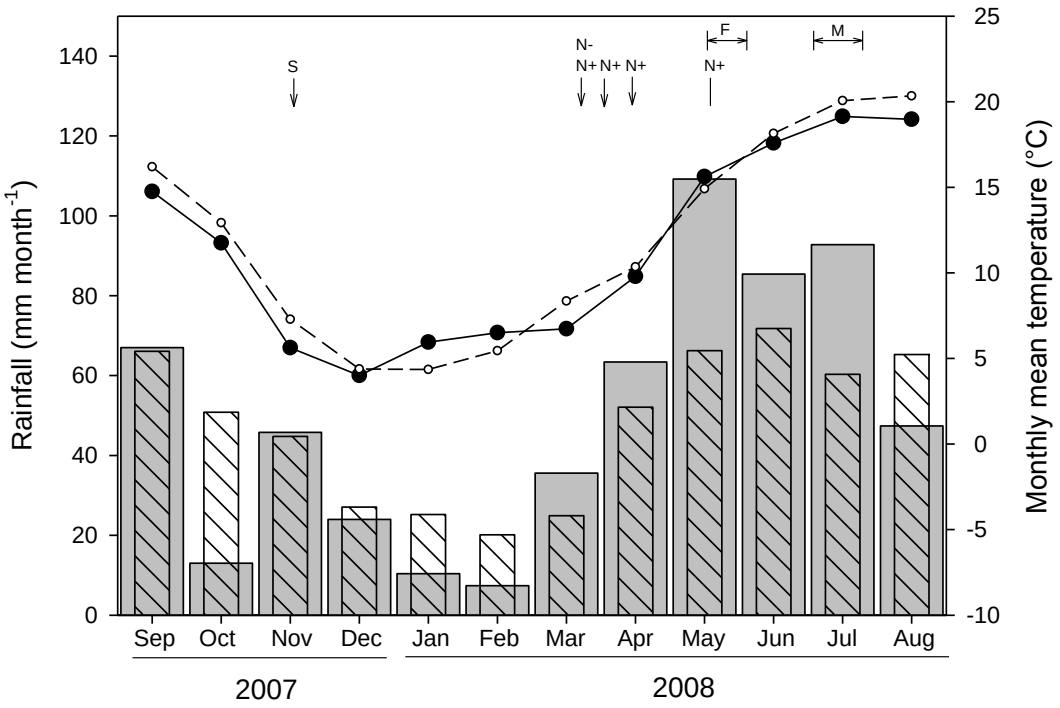
Figure 1: Monthly mean temperatures during the growing season (closed circles) and averaged over the 1990-2008 period (open circles), monthly cumulative rainfall during the growing season (grey bars) and averaged over the 1990-2008 period (open dashed bars) at Clermont-Ferrand. The letters indicate sowing (S), nitrogen applications for the HN and LN treatments, and the periods covering the flowering (F) and the maturity (M) samplings.

Figure 2: Relations between tiller density and (A) root dry mass or (B) shoot dry mass at anthesis under LN (open circles) and HN full black circles) treatments. Numbers between 1 and 16 refer to genotype code (see table 2).

Figure 3: Relation between (A) crop nitrogen nutrition index (NNI), (B) tiller density at anthesis and Root to shoot ratio at anthesis (R/S) under LN (open circles) and HN full black circles) treatments. Numbers between 1 and 16 refer to genotype code (see table 2).

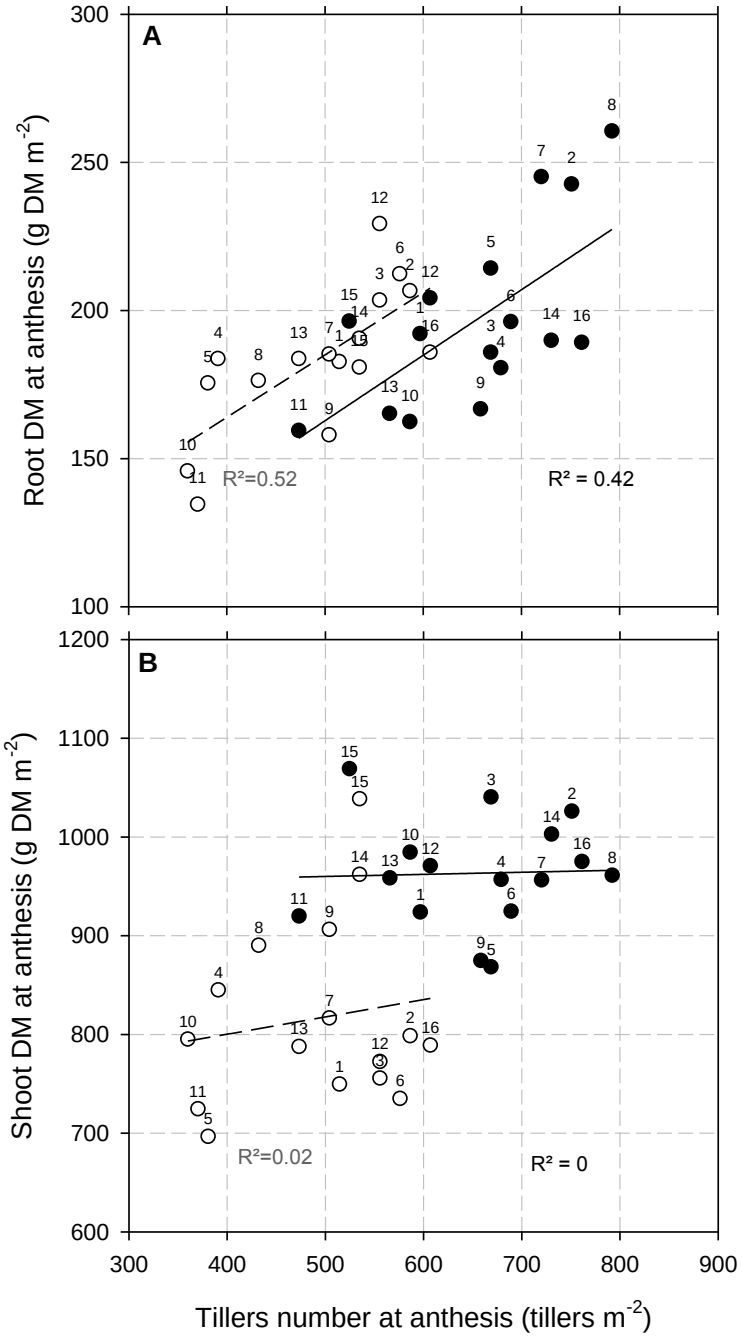
Figure 4 : Relation between belowground dry mass at anthesis and below ground dry mass variation between anthesis and maturity under LN (open circles) and HN full black circles) treatments. Numbers between 1 and 16 refers to genotype code (see table 2).

569



570

571 **Figure 1**



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573 **Figure 2**

574

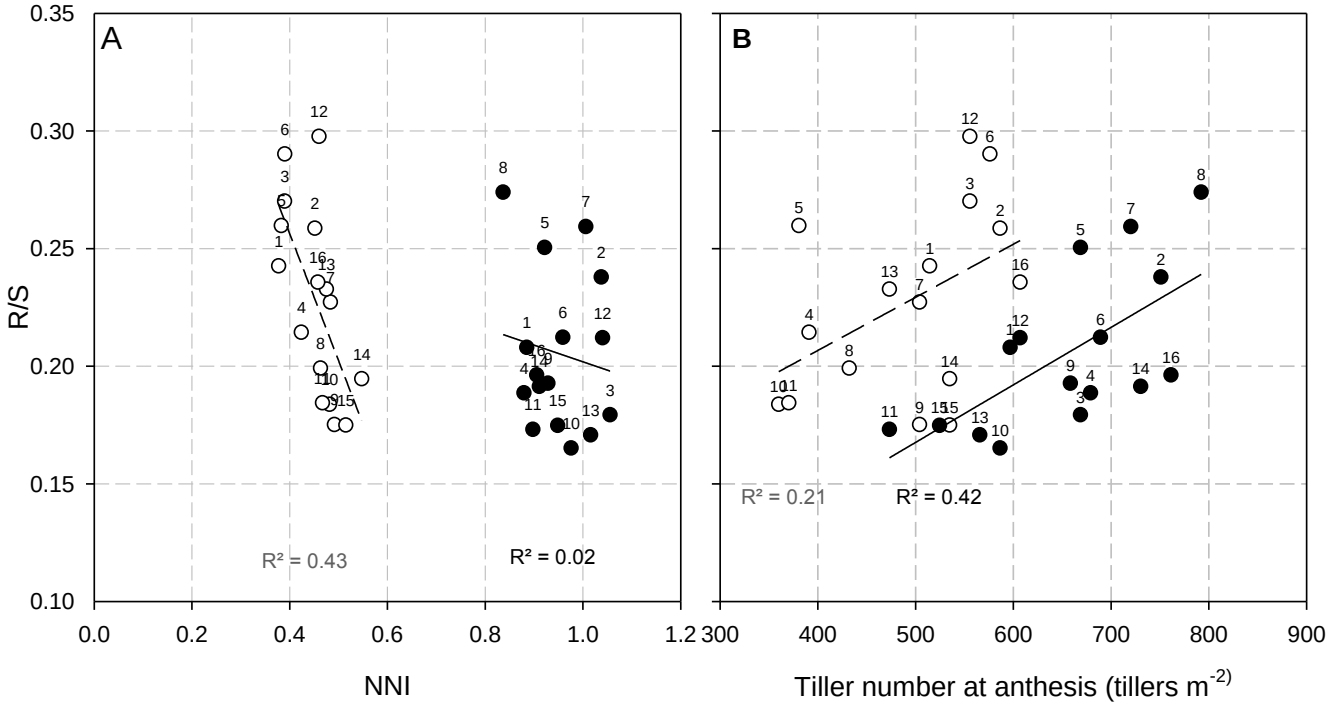
A
B
R/S
NNI
Tiller number at anthesis (tillers m-2)
R² = 0.43
R² = 0.02
R² = 0.21
R² = 0.42

Figure 3

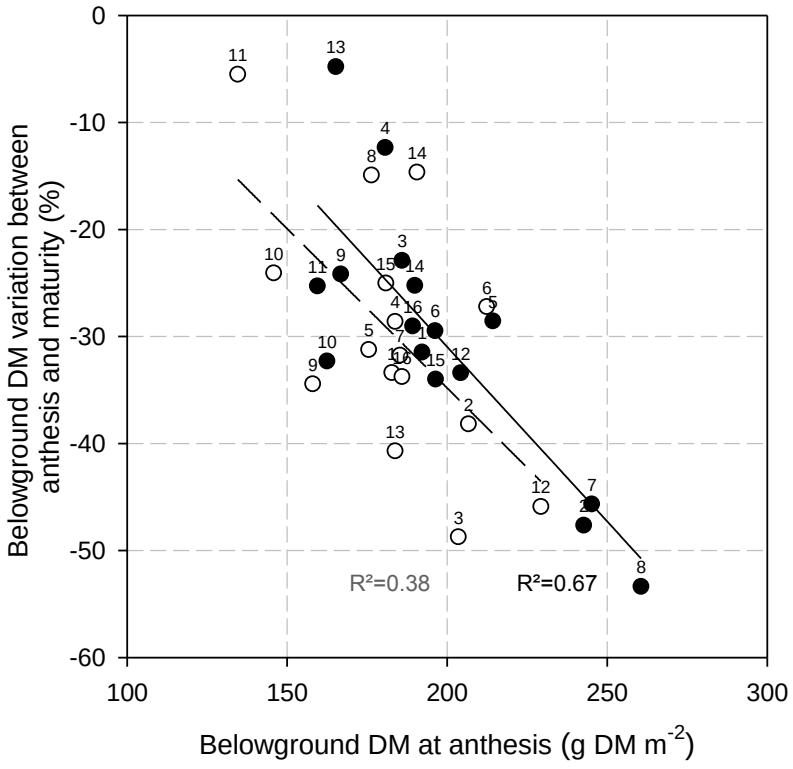
Belowground DM variation between anthesis and maturity (%)
Belowground DM at anthesis (g DM m-2)
R²=0.38
R²=0.67
0
-10
-20
-30
-40
-50
-60
100
150
200
250
300

Figure 4

585 **Tables**

586

587 **Table 1**: Soil characteristics at the Clermont-Ferrand site

Previous crop	barley
Soil textural class (USDA system)	clay loam
Soil particle size distribution (% of soil dry mass)	
Stone (> 2.0 mm)	< 2
Sand (0.05-2.0 mm)	19.8
Silt (0.002-0.05 m)	36.7
Clay (< 0.002 mm)	43.5
Maximum rooting depth (m)	0.9
Plant available soil water content (mm)	122
Apparent bulk density (t m^{-3})	1.15
Organic matter (%)	3.1
pH in water	8.1
Inorganic soil N (0-90 cm layer) at the end of winter (g N m^{-2})	6.2

588

Table 2: Anthesis date, root dry mass (RDM), shoot dry mass (SDM) and grain dry mass (GDM) at anthesis and grain maturity. Data are means of three replicates. Means for the LN and HN treatments are calculated. P values were obtained with a split plot ANOVA.

Treatment	Genotype		**Anthesis**			**Maturity**		
	Name	Code	Anthesis Date	RDM	SDM	RDM	SDM	GDM
				g DM m^{-2}	g DM m^{-2}	g DM m^{-2}	g DM m^{-2}	g DM m^{-2}
LN	Alchemy	1	03/06/08	183	750	122	1118	499
	Beaver	2	04/06/08	207	799	128	1278	590
	Consort	3	04/06/08	203	756	104	1037	493
	Paragon	4	02/06/08	184	845	131	1152	482
	Rialto	5	30/05/08	176	697	121	1054	480
	Robigus	6	02/06/08	212	735	155	1327	618
	Savannah	7	03/06/08	185	816	126	1234	592
	Soissons	8	20/05/08	176	890	150	1066	479
	Arche	9	26/05/08	158	906	104	1411	680
	CF9107	10	22/05/08	146	795	111	1099	516
	CF99102	11	26/05/08	135	725	127	1377	624
	Perfector	12	28/05/08	229	773	124	1248	595
	Quebon	13	29/05/08	184	788	109	1344	583
	Récital	14	19/05/08	191	962	163	1074	464
	Renan	15	21/05/08	181	1039	136	1260	505
	Toisondor	16	27/05/08	186	789	123	1331	649
Mean LN				***184***	***817***	***127***	***1213***	***553***
HN	Alchemy	1	03/06/08	192	924	132	1422	633
	Beaver	2	04/06/08	243	1026	127	1719	803
	Consort	3	04/06/08	186	1040	143	1750	846
	Paragon	4	02/06/08	181	957	158	1736	702
	Rialto	5	30/05/08	214	868	153	1543	730
	Robigus	6	02/06/08	196	925	138	1883	898
	Savannah	7	03/06/08	245	956	133	1705	797
	Soissons	8	20/05/08	261	961	122	1491	746
	Arche	9	26/05/08	167	875	126	1704	787
	CF9107	10	22/05/08	162	984	110	1435	734
	CF99102	11	26/05/08	159	920	119	1666	794
	Perfector	12	28/05/08	204	971	136	1827	847
	Quebon	13	29/05/08	165	958	157	1638	739
	Récital	14	19/05/08	190	1003	142	1270	614
	Renan	15	21/05/08	196	1069	130	1581	708
	Toisondor	16	27/05/08	189	975	134	1679	812
Mean HN				***197***	***963***	***135***	***1628***	***762***
Source of variance	Treatment			0.248	0.005**	0.123	0.019*	0.014*
	Genotype			0.001**	0.019*	0.397	0.010*	<0.001***
	TxG			0.251	0.425	0.464	0.865	0.886

Table 3: root N amount (RN), root N concentration (RN%) at anthesis and grain maturity. Calculated variables are nitrogen harvest index based on shoot N only (NHI) or total plant N (NHI_{tot}), N utilization efficiency based on shoot N only (NUtE) or total plant N ($NUtE_{tot}$), N lost from root between anthesis and maturity (NLR), N potentially remobilized by roots assuming 0.3% N in the dead roots ($NRR_{0.3}$) and the proportion of grain N originating from roots (PGNR). Data are means of three replicates. Means for the LN and HN treatments are calculated. P values were obtained with a split plot ANOVA.

		Anthesis		**Maturity**		**Calculations**						
Tretment	Genotype code	RN	RN%	RN	RN%	NHI	NHI_{tot}	NUtE	$NUtE_{tot}$	NLR	$NRR_{0.3}$	PGNR
		g N m^{-2}	%DM	g N m^{-2}	%DM	%		gDM gN^{-1}		g N m^{-2}		%
LN	1	0.7	0.39	0.5	0.39	76.5	73.2	47.8	45.8	0.24	0.05	0.6
	2	0.9	0.43	0.6	0.43	78.6	75.3	45.8	43.9	0.33	0.09	0.9
	3	0.7	0.35	0.4	0.41	76.2	73.2	47.7	45.8	0.34	0.00	0.0
	4	0.7	0.40	0.5	0.41	82.0	77.8	48.2	45.8	0.20	0.04	0.5
	5	0.8	0.45	0.5	0.43	79.9	76.1	45.5	43.3	0.27	0.10	1.2
	6	0.8	0.38	0.6	0.39	76.6	73.0	49.5	47.2	0.20	0.02	0.2
	7	0.8	0.43	0.6	0.45	79.1	76.1	41.7	40.0	0.21	0.08	0.7
	8	0.8	0.48	0.7	0.50	81.0	75.4	48.2	44.9	0.10	0.07	0.9
	9	0.6	0.38	0.4	0.37	80.8	78.5	51.1	49.7	0.21	0.07	0.7
	10	0.7	0.45	0.5	0.41	80.6	77.2	49.5	47.4	0.20	0.09	1.1
	11	0.6	0.46	0.5	0.40	83.0	80.1	43.6	42.1	0.08	0.05	0.5
	12	0.9	0.37	0.6	0.49	76.5	73.1	46.1	44.0	0.24	0.02	0.2
	13	0.8	0.41	0.4	0.40	77.8	75.4	41.4	40.2	0.31	0.09	0.8
	14	0.8	0.42	0.8	0.47	77.6	71.7	49.2	45.5	-0.24	0.04	0.7
	15	0.8	0.47	0.5	0.36	76.9	74.1	40.5	39.0	0.37	0.23	2.4
	16	0.8	0.43	0.4	0.34	80.8	78.3	50.2	48.7	0.38	0.19	1.9
Mean LN		***0.76***	***0.42***	***0.53***	***0.42***	***79***	***75.5***	***46.6***	***44.6***	***0.22***	***0.08***	***0.83***
HN	1	1.5	0.78	1.0	0.74	66.8	63.6	31.6	30.1	0.56	0.38	5.6
	2	2.4	0.99	1.0	0.85	69.3	66.6	31.2	30	1.35	1.00	2.6
	3	1.7	0.90	1.1	0.76	69.9	67.2	32.1	30.9	0.59	0.46	0.8
	4	1.4	0.80	1.3	0.82	69.0	65.4	28.2	26.7	0.15	0.15	5.1
	5	2.0	0.94	1.0	0.67	74.1	70.8	32.3	30.9	1.00	0.81	2.6
	6	1.6	0.83	1.0	0.72	69.5	66.8	34.6	33.2	0.64	0.47	2.4
	7	2.0	0.84	1.3	0.97	65.5	62.3	31.1	29.5	0.77	0.45	3.3
	8	2.0	0.75	1.0	0.80	75.9	72.7	33.9	32.5	0.99	0.57	1.7
	9	1.3	0.78	0.9	0.72	76.1	73.0	36.4	35.0	0.39	0.27	1.7
	10	1.3	0.80	0.9	0.81	76.9	74.0	32.9	31.6	0.41	0.29	0.6
	11	1.3	0.78	1.0	0.86	78.2	75.1	32.6	31.3	0.24	0.11	1.7
	12	1.6	0.78	1.1	0.82	68.6	65.8	31.7	30.4	0.46	0.32	2.0
	13	1.5	0.90	1.1	0.68	73.6	70.5	30.1	28.8	0.36	0.34	1.9
	14	1.6	0.86	1.4	0.99	70.4	65.4	32.6	30.3	0.26	0.26	2.3
	15	1.5	0.76	0.9	0.70	72.5	69.9	28.2	27.2	0.57	0.39	1.9
	16	1.5	0.77	1.0	0.72	73.1	70.2	34.4	33	0.5	0.34	0
Mean HN		***1.64***	***0.83***	***1.06***	***0.79***	***71.8***	***68.7***	***32.1***	***30.7***	***0.58***	***0.41***	***2.26***
Source of variance	N	0.002**	<0.001***	0.008**	0.004**	0.006**	0.005**	0.003**	0.002**	0.031*	0.025*	0.041*
	G	<0.001***	0.207	0.027*	0.019*	<0.001***	<0.001***	<0.001***	<0.001***	0.022*	0.049*	0.036*
	TxG	0.052	0.082	0.794	0.637	0.075	0.041*	0.332	0.350	0.245	0.097	0.110