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1 Genetic variability in biomass allocation to roots in wheat is mainly related to crop tillering
2 dynamics and nitrogen status

3

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16

17

18 **Abstract**

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

38

39 Wheat; gentic variability; root; NUE

40

41

42

43 **1. Introduction**

44 Until very recently, productivity has been the major target of agronomical sciences. The
45 growing demand for low input agriculture, driven both by economical and environmental
46 considerations emphasizes now the improvement of resource use efficiency. Nitrogen (N)
47 fertilizers play a major role in crop productivity, but are also one of the main sources of
48 agricultural pollution either through nitrous oxide (N₂O) volatilization or nitrate (NO₃⁻)
49 leaching (IPCC, 2001; DECC, 2010). N fertilization represents also a major cost for farmers.
50 Increasing N use efficiency (NUE) received therefore much attention in the recent years
51 whether through improved agronomical practices (Cui et al, 2011) or plant breeding (eg Hirel
52 et al. 2007; Foulkes et al., 2009; Gaju et al., 2011). This quest for highly efficient wheat
53 cultivars and agronomical practices is made more complex by the fact that not only grain
54 yield but also grain protein concentration (GPC), the major determinant of wheat end-use
55 quality (eg Shewry 2007; Oury et al., 2010) should be improved. The involvement of N in
56 both biomass accumulation and grain protein concentration determination creates a need for
57 studying the complex interactions between carbon and N metabolisms that determine crop
58 productivity and quality (Triboi and Triboi-Blondel, 2002).

59

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

68 order kinetic that is independent of organ age, genotype and N nutrition (Bertheloot et al.,
69 2008). Genetic variability for N remobilization efficiency has also been characterized
70 (Barbotin et al 2005, Uauy et al., 2006; Kichey et al 2007) showing relatively large genetic
71 variation for this trait and strong interaction with the environment and N fertilizer regimes.

72

73 Oppositely little is known about the N allocation and remobilization pattern in the wheat root
74 system. In controlled conditions, wheat roots have the ability to remobilize N to the grain
75 (Andersson et al., 2005) and are the last organs to senesce (Peoples and Dalling, 1988). In
76 these conditions, N partitioning to the roots and redistribution of N from the roots to the grain
77 may play an important role for the nitrogen budget of the whole plant (Andersson et al., 2005)
78 since a large part (10 to 20%) of the total plant N at maturity appears to remain in the roots
79 (Andersson and Johansson, 2006). This may have several consequences since most studies
80 occult the root compartment. First, accounting for the root compartment may affect to some
81 extent whole plant NUE if it is associated to different shoot/root allocations (Andersson et al.,
82 2005). Substantial genetic variation has already been observed for this trait in wheat (Siddique
83 et al., 1990; Hoad 2001). Second, if variations in shoot/root allocation were associated to
84 large differences in root N content and remobilization patterns, it may impact other
85 agronomical traits such as grain protein concentration. The aim of this study was to analyze
86 the N allocation to roots in wheat and the net N remobilization from roots during the post-
87 anthesis period under field conditions. A particular emphasis was put on the evaluation of the
88 genetic variability associated with these processes. Root biomass, relative allocation and mass
89 loss were studied on 16 bread wheat cultivars grown in the field at two N levels. Root N
90 content and remobilization were quantified in order to evaluate the potential role of N
91 remobilization from roots in the N economy of the crop.

92

93 2. Materials and Methods

94 2.1. Plant material and growing conditions

95 Sixteen genotypes of winter bread wheat (Table 2) were studied in a field experiment carried
96 out at Clermont-Ferrand, France (45°47' N, 3°10' E, 329 m elevation) during the 2007-2008
97 growing season. This panel of genotypes represents a large part of the genetic variability for
98 nitrogen use efficiency and its components (Le Gouis *et al.*, 2000; Gaju *et al.*, 2011) and for
99 the deviation from the negative correlation between grain yield and protein concentration (Oury
100 *et al.*, 2003) reported in UK and France elite germplasms. Soil characteristics are presented in
101 Table 2. Crops were sown at a density of 250 seeds m⁻² on 06 November 2007. A high (HN)
102 and a low (LN) N treatments were applied. For the HN treatments, the rates of N fertilisation
103 were determined using the balance sheet method (Rémy & Hébert, 1977) to optimize grain
104 yield. N was applied in four splits as ammonium nitrate granules (33.5% N) with 4, 8, 8, and 4 g
105 N m⁻² being applied when Rialto, which has a rate of development in the middle of the range of
106 the sixteen cultivars used in this study (Table 2), reached growth stages (GS; Zadocks *et al.*,
107 1974) GS21 (beginning tillering), GS31 (ear at 1cm), GS39 (male meiosis), and GS61
108 (anthesis) respectively. In the LN treatment, 4 g N m⁻² were applied in one split when Rialto
109 reached GS31. The experimental field was not irrigated. All other crop inputs including weed,
110 disease and pest control, and potassium, phosphate and sulfur fertilizers, were applied at
111 levels to prevent nutrients or weeds, diseases and pests from limiting yield. The experimental
112 design was a split-plot in which N treatment was randomized on main plots, cultivars were
113 randomized on the sub-plots and each treatment was replicated three times. Sub-plot size was
114 7 × 1.5 m with an inter-row spacing of 0.17 m.

115

116 *2.2. Plant sampling*

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

128

129 *2.3. Root sampling*

130 Root sampling was performed with a square-shaped soil corer (height = 40 cm, section = 18 ×
131 18 cm). The corer was positioned on the soil, centered on a plant row and drove into the soil
132 to a depth of 30 cm with a sledge hammer. The soil core was then retrieved, soaked into 10 L
133 of water to facilitate soil disaggregation and washed abundantly with water above a 1 mm
134 mesh-size sieve until roots were totally free of soil.

135

136 *2.4. Plant tissues dry mass and nitrogen concentration,*

137 Lamina, stem, chaff, grains and roots dry mass (DM) of the main shoots were determined
138 after oven drying at 80°C for 48h. Samples were then ground and their total N concentration
139 (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/shoot ratio (R/S) as follows:

$$146 \quad R/S = SDM / RDM \quad (1)$$

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:

$$149 \quad RN/SN = RN / SN \quad (2)$$

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}):

$$153 \quad NHI = GN / SN * 100 \quad (3)$$

$$154 \quad NHI_{tot} = GN / (SN + RN) * 100 \quad (4)$$

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}):

$$158 \quad NUtE = GDM / SN \quad (5)$$

$$159 \quad NUtE_{tot} = GDM / (SN + RN) \quad (6)$$

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}):

$$163 \quad NLR = RN_{anth} - RN_{mat} \quad (7)$$

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

$$167 \quad NRR_{0.3} = (RDM_{anth} - RDM_{mat}) \times (RN\%_{anth} - 0.3) / 100 \quad (8)$$

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

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

$$170 \quad PGNR = GN_{mat} / NRR_{0.3} \quad (9)$$

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

$$172 \quad N_t = 5.35 \times SDM^{-0.442} \quad (10)$$

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

$$175 \quad NNI = SN\% / N_t \quad (11)$$

176 **3. Results**

177 *3.1. Climate*

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

184

185 *3.2. Crop structure at anthesis*

186 The genotypes used in the present experiment exhibited a large variation in term of
187 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,

212 average R/S for the 12 remaining genotypes for HN was 0.19 and RN/SN was 0.08. Both
213 values were similar to the lower range of the values observed for LN (Fig. 3).
214 RDM variation during the post-anthesis period was negatively correlated with RDM at
215 anthesis under both N treatments (Fig. 4, $r^2 = 0.38$ and 0.67 under LN and HN respectively)
216 and ranged from -5% to -54%. The proportional loss of belowground biomass increased with
217 the initial belowground biomass present at anthesis.

218

219 *3.3. Nitrogen remobilization from the belowground compartment and effects on nitrogen*
220 *allocation calculations*

221 NHI at anthesis was both under strong N treatment and genotype effects (Table 3). On
222 average over genotypes NHI equaled 79 and 72% under LN and HN, respectively.
223 Accounting for belowground N in the calculation led to an about 3 percent point decrease in
224 the calculated NHI_{tot} . Nevertheless this did not impact significantly genotypes ranking for this
225 trait. Spearman's rank test gave correlation R_s values of 0.83 and 0.97 for LN and HN,
226 respectively, indicating strong rank correlations between the two calculations. Results were
227 equivalent for $NUtE$ calculations with a small impact of accounting for belowground N pool.
228 $NUtE_{tot}$ was on average 1.7 percent point lower than $NUtE$. Again this has no impact on
229 genotypes ranking (R_s values of 0.96 and 0.98) under LN and HN, respectively. Calculation
230 of N potentially lost by roots during the post anthesis period (NLR) indicated that only a small
231 N amount was lost as senesced material or remobilized during this period. The largest part of
232 this N amount was caused by a decreased in RDM and not by a decrease in remaining
233 biomass decrease in N concentration. Indeed, RN% variation during post anthesis under LN
234 was nil (0.41% at both anthesis and maturity) and only marginal under HN (0.83 and 0.79 at
235 anthesis and maturity, respectively). Calculating potentially remobilized N from senesced
236 roots is hazardous since the N concentration of senesced root material was not measured and

237 may vary with time, type of roots, treatment and genotype. Nevertheless, N remobilization
238 from roots ($NRR_{0.3}$) was estimated based on a putative N concentration in the senesced root
239 material of 0.3%, a value close from the lowest root N concentration observed in the present
240 experiment but much lower than reported values for dead fine roots (Gordon and Jackson,
241 2000). Even based on the assumption of a 0.3% N in dead roots, extremely small amount of
242 potentially remobilized N from roots were calculated. $NRR_{0.3}$ values were on average 0.1 and
243 0.4 gN m^{-2} for LN and HN, respectively, corresponding to 0.8 and 2.3% of the total grain N at
244 maturity. Genetic variation observed for both the quantity and the proportion of N
245 remobilized from roots were not correlated with any of the traits of interest such as grain N
246 quantity or concentration (data not shown). In addition remobilization efficiency of the
247 belowground plant material was not related whatsoever with remobilization efficiency of
248 aboveground vegetative organs.

249

250 **4. Discussion**

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

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

263

264 *4.1. Root sampling methodology*

265 In the present experiment root biomass was only sampled in the 0-30 cm horizon. Clearly the
266 full root biomass was not harvested by this method since the maximum rooting depth was
267 evaluated to 90 cm at the experimental site. This would clearly be a strong limitation if the
268 objective was to compare genotypes in term of functional processes such as water and N
269 extraction capacity. Here, the key aspect was to retrieve a large proportion of the total root
270 biomass to assess differences of biomass and nitrogen allocation pattern between genotypes.
271 Kätterer et al. (1993) studied the root biomass of a winter wheat genotype under four
272 management treatments including dry and irrigated crops. On average, after anthesis about
273 85% of the root biomass was found in the top 30 cm horizon. This proportion tended to
274 increase in the well irrigated treatment. Siddique et al. (1990) also observed very high
275 proportion of the total root biomass in the first 30 cm. A root dry matter profile was available
276 for three genotypes and showed that more than 90% of the total root biomass was in this
277 horizon. Similar figures can be found in Wechsung et al. (1999). Xue et al. (2003) reported
278 lower proportion of root biomass (about 60%) in the first 30 cm, but in a soil with a maximum
279 rooting depth of 2 m thus more than twice as deep as our soil. We therefore believe that the
280 sampling method used in the present experiment allowed harvesting a very significant
281 proportion of the total root biomass and that it is very unlikely that significant bias would
282 hamper genotype comparisons. Kätterer et al. (1993) reported roots biomass values at anthesis
283 of 92 g m⁻² while values presented by Siddique et al. (1990) at the same developmental stage
284 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.,

309 2005). Nevertheless, the data presented here show that the tillering dynamic of the crop has a
310 strong influence on root biomass at anthesis.

311

312

313 A second strong determinant of biomass allocation to roots at anthesis is the crop nitrogen
314 status. The effect of the plant nitrogen status on the R/S ratio of plant has received much
315 attention in particular for modeling purpose (Hilbert, 1990; Gleeson, 1993; Thornley, 1995;
316 1998; Agren and Franklin, 2003) and clearly demonstrated that the R/S decreases with
317 increasing N supply. These models use plant N concentration (Franklin and Agren, 2003) or
318 C, N availability (Thornley, 1995) to predict biomass allocation and are based on the
319 hypothesis that biomass allocation is strongly controlled at the plant level and optimized in
320 relation to the availability of C and N. In our study, NNI was the best predictive variable for
321 R/S. The nitrogen nutrition index as described in Gastal and Lemaire (2002) allows to
322 quantify the N status of the crop dynamically; i.e. accounting for the decreasing crop N
323 demand as crop gets larger (Gastal and Lemaire, 2002). Therefore, optimal crop relative
324 growth rate is attained for a crop N concentration, called critical N concentration that
325 decreases following a power function of crop biomass. NNI derives from a comparison
326 between actual crop N concentration and critical N concentration at the observed biomass and
327 allows the N status of the crop to be quantified over development and also to compare N
328 status of crops of different biomass.

329

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

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

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

343

344 In term of potential nutrient remobilization to the grain during the post anthesis period, it is
345 also important to assess the biomass variation of the root pool during this period. In wheat
346 decrease in root mass after anthesis is generally observed due to a root death rate exceeding
347 root production rate (Kätterer et al., 1993; Steingrobe et al., 2001). This is a common feature
348 observed in annual plants for which the developing grain is a strong competitive sink for the
349 carbon resource (Eissenstadt and Yanai, 1997) leading to a strong root length decline after
350 flowering in wheat (Box and Johnson, 1987 in Eissenstadt and Yanai, 1997). In the present
351 study we observed a strong negative correlation between root mass loss during the post-
352 anthesis period and root biomass at anthesis. In other words, plants with high root biomass at
353 anthesis tend to loose a greater proportion of this biomass. A possible explanation relies on
354 the much shorter life span of fine roots compared to larger roots (Eissenstadt et al., 2000).
355 Indeed a large part of the root biomass variability may be associated with branching
356 variability leading to a greater proportion of fine roots in plants with high root biomass. No
357 root separation by class size could be performed in the present study therefore the causal
358 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

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

387

388 **5. Conclusions**

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

404

405

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408 Messaoud did all plant sampling, biometric measurements and elemental analysis. The
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410

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542 .

543

544 **Figures captions**

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

550

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

554

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

558

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

562

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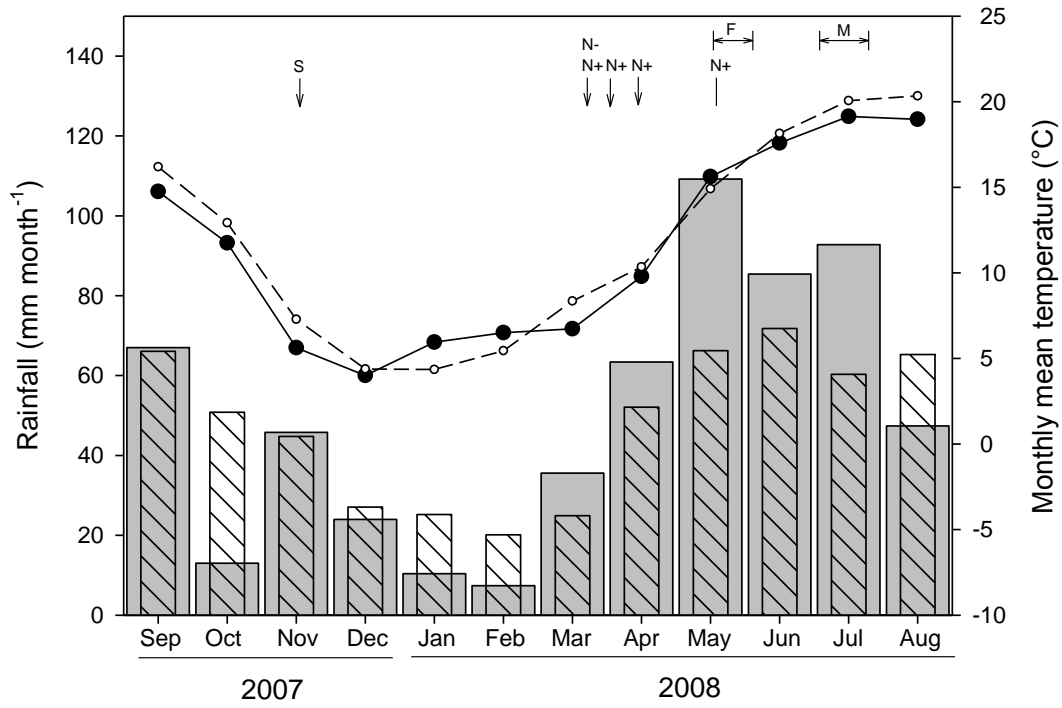
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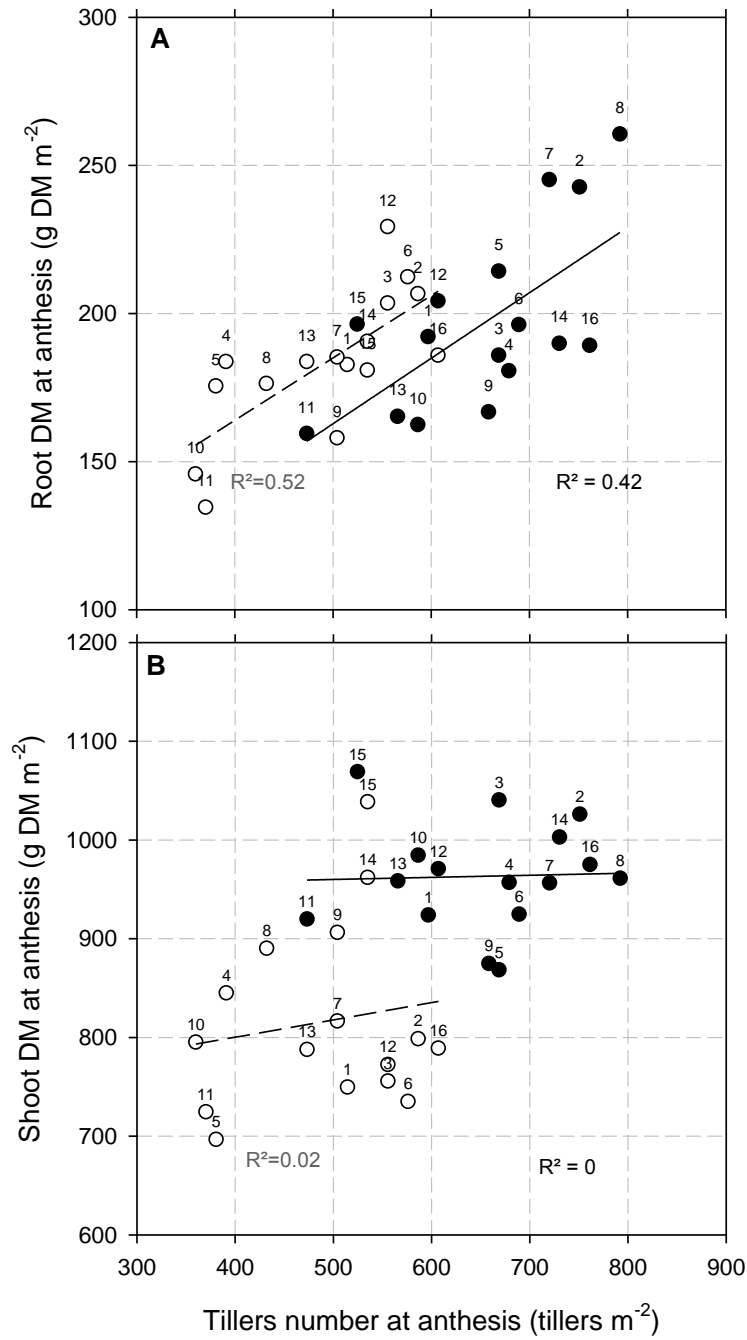
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571 **Figure 1**



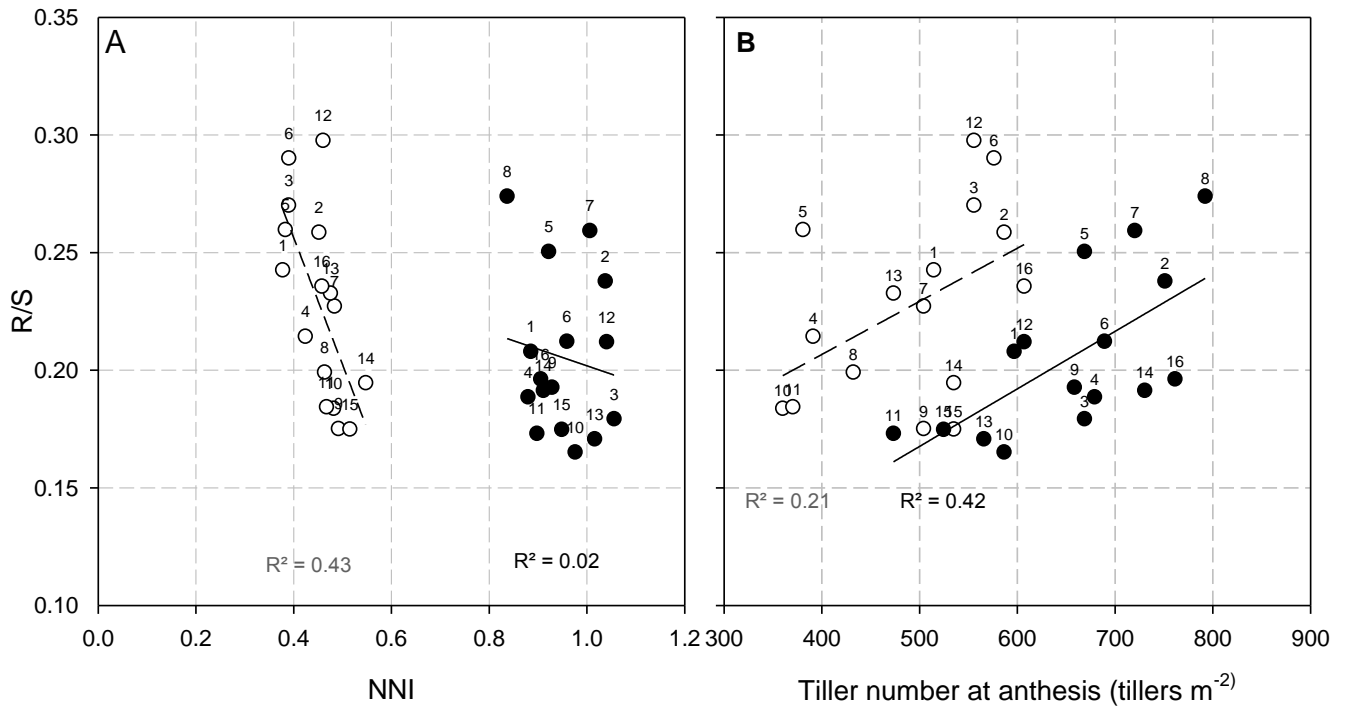
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573 **Figure 2**

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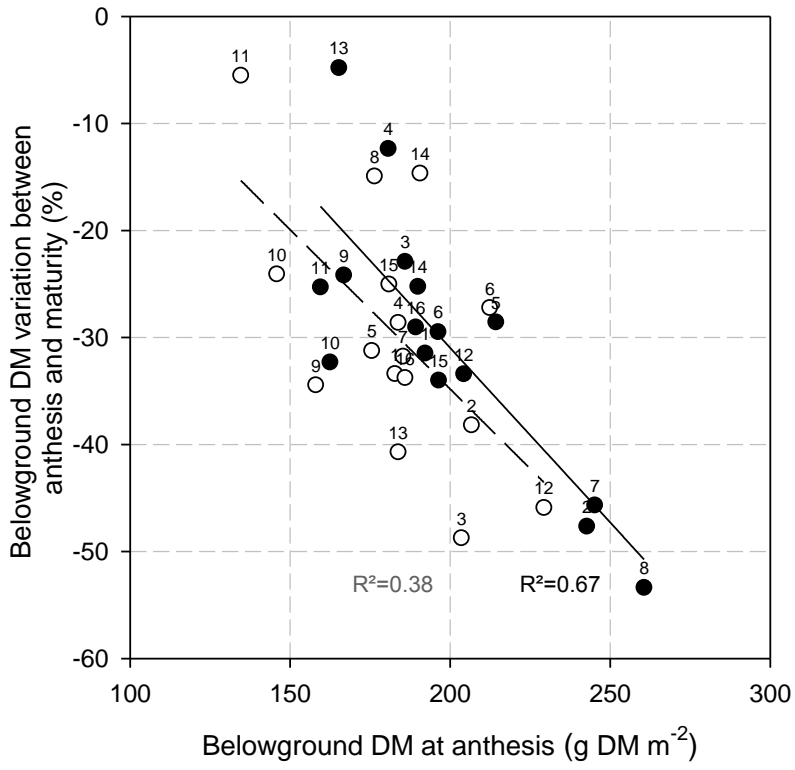


577

578 **Figure 3**

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581

582 **Figure 4**

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584

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.

Treatment	Genotype code	Anthesis		Maturity		Calculations						
		RN g N m ⁻²	RN% %DM	RN g N m ⁻²	RN% %DM	NHI %	NHI _{tot}	NUtE gDM	NUtE _{tot} gN ⁻¹	NLR g N m ⁻²	NRR _{0.3}	PGNR %
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