1 **Title**: Performance of eleven winter wheat varieties in a long term experiment on mineral

2 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

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73 performances of the varieties may change as a function of their genetic background

74 (Ceccarelli, 1996; Charles et al., 2006; El Bassam, 1998; Ruiz et al., 2008; Vlachostergios and

75 Roupakias, 2008). Today, varieties are usually selected under high or medium nitrogen

76 conditions. Several studies suggest that traits selected under nitrogen rich conditions may not

77 be the same as those required for high performance under nitrogen limiting conditions

78 (Ceccarelli, 1996; El Bassam, 1998; Ruiz et al., 2008; Vlachostergios and Roupakias, 2008).

79 Consequently, in order to improve nutrient use efficiency in wheat, the performance of

80 genotypes under contrasting soil fertility conditions needs to be understood.

81 A few studies have investigated varietal responses when only very low nitrogen is available

82 thus identifying useful genetic traits for low input systems (Dawson et al., 2008). Under most

83 low input conditions, the seasonal course of nitrogen availability in soil is the determining

84 factor of the progression of nitrogen accumulation in plants, followed by their capacity of N

85 remobilisation from vegetative biomass and post-anthesis accumulation (Berry et al., 2002;

86 Dawson et al., 2008; Masclaux et al., 2001). These processes may even be more important in

87 production systems using organic amendments, because the release of organic nitrogen

88 depends on the dynamics of mineralisation, which in turn depends on climatic and other

89 environmental conditions. Chemical fertilisers, on the other hand, release nitrogen rather

90 quickly. The release of organic nitrogen is therefore slow and spread over a longer period of

91 time (Berry et al., 2002). In low input systems in stockless farms, soil organic matter

92 decreases gradually on a long term perspective (Maltas et al., 2012b; Riley et al., 2008). Yet,

93 the decrease in soil organic matter affects the dynamic of nitrogen availability in the soil. It is

94 thus important to identify wheat genotypes able to cope with these conditions.

95 Consequently, there is a need to better select plants adapted to limited nitrogen conditions, to

96 the form of its supply (mineral, organic), to its nature in soil (high, low organic matter

97 content) and to the dynamics of its availability to the crop. For instance, it has been suggested

98 that varieties absorbing nitrogen early in the season perform better in conditions with low

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99 nitrogen availability (Baresel et al., 2008). Breeding for nitrogen efficient varieties that

100 perform well under nitrogen limiting or organic conditions has to account for the genetic basis

101 of the crop but also all cultivation and environmental factors that influence the absorption and

102 the allocation of nitrogen in the plant. This raises the question of the need for a specific

103 breeding scheme for organic production methods as varieties have to cope with stronger

104 stresses and higher environmental variability in contrast to high input systems (Lammerts van

105 Bueren et al., 2002; Löschenberger et al., 2008; Müllner et al., 2014; Reid et al., 2011; Wolfe

106 et al., 2008).

107 To address the question of varietal responses to low input and organic fertilisation, a study

108 was made within a long-term field experiment of nitrogen fertilisation and organic

109 enrichment, established in 1976 in Switzerland. This experiment offered the unique

110 opportunity to study eleven European winter wheat varieties (*Triticum aestivum* L.) in

111 unusually contrasting environments of different soil fertility within a single field and over two

112 years. The objectives of this study were 1. to investigate the varietal responses to various

113 sources and amounts of nitrogen fertilisation and various soil organic matter content in the

114 same soil, 2. to better understand the agronomic traits that characterise the behaviour of

115 modern varieties under these different conditions, their capacity to exploit soil nitrogen

116 mineralisation, and the dynamics of resource acquisition, 3. to evaluate the need to undertake

117 specific selection for low input systems and 4. to identify the crucial traits necessary for the

118 selection of best adapted varieties. For this, varietal responses in terms of yield, nitrogen

119 content, nitrogen accumulation and nitrogen related traits were systematically explored.

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122 **2. Material and methods**

123 *2.1. Experimental site*

124 The experiment was carried out at the Agroscope Changins (46° 24' N, 06° 14' E, 430 m

125 above sea level) in Nyon, Switzerland, during the years 2005-2006 and 2006-2007. The soil is

126 a well-drained brown soil (Calcaric Cambisol) with 14% clay and 39% silt, and a depth of 100

127 cm. For this site, the average total annual precipitation is 999 mm and the mean temperature

128 10.2°C (30-year averages, 1981-2010). While 2005 was a rather dry year, 2006 and 2007

129 were in line with the mean long term values (2005: 707 mm, 10.3°C; 2006: 930 mm, 11.0°C;

130 2007: 992 mm, 11.1°C). The total precipitation and mean temperature during the two growing

131 periods (February to June) were similar in 2006 and 2007 (2006: 472 mm, 9.8°C; 2007: 450

132 mm, 11.9°C), though the distribution of precipitation differed (Table 1).

133 The long term experiment was established in 1976 in Changins. It follows a split plot design

134 with four replicates, six main treatments of organic enrichment, and four sub treatments of

135 mineral nitrogen fertilisation (Maltas et al., 2012a).

136 This study focused exclusively on two levels of organic enrichment and three levels of

137 mineral nitrogen fertilisation. The two main treatments were 1. no organic enrichment

138 (Mineral) and 2. 70 t/ha manure every three years (Manure). The sub treatments represented

139 three levels of mineral nitrogen fertilisation: 1. no nitrogen supply (noN); 2. suboptimal

140 fertilisation, nitrogen supply 40 kg/ha below the optimal dose (lowN); 3. over fertilisation,

141 nitrogen supply 40 kg/ha above the optimal dose (highN). The long term treatments and sub

142 treatments modified soil characteristics substantially (Maltas et al., 2012b; Vullioud et al.,

143 2006). In particular, soil organic matter content, its C/N ratio and total soil nitrogen increased

144 significantly in the Manure treatment compared to the Mineral one (Maltas et al., 2012b).

145 The crop rotation of the long term experiment alternates winter and spring crops, with 67% of

146 cereals (winter wheat, spring barley and oat), complemented with winter rapeseed and maize.

147 At harvest, cereal straw is removed while rapeseed and maize residues are incorporated into

148 the soil. This standard rotation was interrupted in 2005 after a maize crop to allow the

149 settlement of the present study.

150

2.2. Wheat varieties

151 The long term experiment was used to test eleven recently released winter wheat varieties.

152 The varieties originated from different European countries and breeders and bore different

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154 genetic backgrounds (Table 2). They are all registered in the European variety catalogue.

155 Pireneo and Aszita are organic varieties, and all other Swiss varieties are recommended for

156 low input and integrated systems. Varieties were chosen according to a relative similarity in

157 precocity and maturation to avoid impact on development, growth and yield performance. The

158 varieties covered a large diversity in terms of physiological traits and baking quality.

159

2.3. Experimental setup

161 The experimental setting consisted of the eleven winter wheat varieties sown in strips across

162 the nitrogen fertilisation subplots of the long term experiment. The plots (3 m x 1.5 m for each

163 single plot) were machine sown in mid-October (12 October 2005 and 27 October 2006) at a

164 rate of 350 seeds/m^2. The optimal nitrogen dose for the mineral treatment was computed

165 according to the Swiss fertilisation guidelines (Ryser et al., 2001). It reached 160 kgN/ha and

166 140 kgN/ha respectively for the first and the second year. The dose was higher in the first year

167 to compensate for the nitrogen immobilisation induced by the incorporation of maize straw. N

168 fertiliser was machine applied as NH_4NO_3 in a two- or three-way split during the growing

169 season (Zadoks growth stages 25, 30, 40 in 2006, and 25, 30, 37 in 2007) (Table 1). The last

170 farmyard manure was applied before the 2005 maize in the treatment Manure.

171 Phosphorus and potassium were supplied in order to warrant non limiting conditions on all

172 main treatments, according to the Swiss fertilisation guidelines (Ryser et al., 2001), and taking

173 into account phosphorus and potassium from organic origins. Herbicides were applied

174 depending on weed pressure, and standard phytosanitary protection was applied according to

175 integrated crop protection principles (Häni et al., 1990). Crops received no growth regulator

176 to avoid hormonal effect on biomass growth.

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

178

2.4. Dry matter and nitrogen related traits

180 At harvest (Zadoks growth stage 89), grain yield (DMgrain), adjusted at 0% humidity, grain N

181 concentration (%N), and thousand kernel weight (TKW), adjusted at 0% humidity, were

182 assessed for each plot. At anthesis (Zadoks growth stage 61-65), plants were removed from

183 the third row in each plot, over a length of 0.5 m. These samples were used to determine the

184 total aboveground dry matter and the corresponding N concentration. At harvest, additional

185 data on total aboveground dry matter (DMmat) and N concentration in straw were collected in

186 a similar way. Nitrogen concentrations in biomass and in kernels were determined by near

187 infrared spectrometry using a NIRS6500 (FOSS NIRSystems, Inc., Laurel, Md, USA).

188 Kernel number per square meter (KN) was obtained by dividing yield per square meter by

189 thousand kernel weight. Nitrogen uptake of grain (Ngrain), and of total aboveground biomass

190 at anthesis (Nanth) and maturity (Nmat), were calculated based on their respective N

191 concentration and dry weight. Similarly, harvest index (HI) and nitrogen harvest index (NHI)

192 were calculated as the ratio of DMgrain to DMmat and Ngrain to Nmat, respectively. In

193 addition, six traits linked to nitrogen accumulation and use were derived:

194 - N post-anthesis accumulation (NpA): Nmat - Nanth

195 - fraction of total N uptake accumulated after anthesis (fNpA): (Nmat - Nanth) / Nmat

196 - N use efficiency (NUE): DMgrain / N supply

197 - N utilisation efficiency (NutE): DMgrain / Nmat

198 - N uptake efficiency (NupE): Nmat / N supply

199 - N use efficiency for protein (NUEP): Ngrain / N supply

200 Several methods for estimating N supply (Nsup) exist (Limon-Ortega et al., 2000). Here,

201 Nsup was defined as the sum of mineral N fertilisation and of the maximal whole plant total N

202 uptake observed in the sub treatment with no nitrogen fertilisation (Nmat at noN), and at the

203 respective main treatment levels (Mineral or Manure) and replicates. However, it has been

204 shown that, though the choice of the method influences the values of NUE and NupE, it has

205 little effect on the relative comparisons of this value between treatments (Bingham et al.,

206 2012).

207

208 *2.5. Data analysis*

209 An analysis of variance was performed on each trait. The full experimental design

210 corresponded to a split-strip plot with four replications, with organic enrichment (two levels)

211 as the main plots, nitrogen fertilisation (three levels) and varieties (eleven levels) as the

212 orthogonal strips.

213 A principal component analysis (PCA) was performed to investigate how varieties were

214 characterised by the measured traits. This analysis was performed on the correlation matrix of

215 the mean variety values (mean over all replicates, treatments and years), with the R package

216 vegan (Oksanen et al., 2011).

217 N supply allowed sorting the treatments relative to the nitrogen made available for the crop.

218 This value was used to situate the individual varietal responses (*i.e.* deviation from the variety

219 mean) for the different traits studied, as a function of nitrogen availability. Independently for

220 each variety, a linear regression of its deviation from the variety mean trait was adjusted, as a

221 variant of the procedure proposed by Finlay and Wilkinson (1963).

222 Pearson correlations were performed between yield (DMgrain), grain N concentration (%N),

223 and the other traits, to assess the relationship between varietal characteristics and

224 performance. Correlations were computed on the mean values of each variety across the four

225 replicates, for each treatment independently.

9

226 The relative contributions of N uptake (NupE) and utilisation (NutE) efficiency to the

227 variation in N use efficiency (NUE) among the varieties, in each treatment, were analysed

228 using the method described in Moll et al. (1982). This method allows determining the

229 contribution of each component of a trait to the mean squares of the compound trait. The

230 analysis is performed on the logarithms of the values in order to linearise the product. The

231 mean values for each variety across the four replicates were used for the analysis. In addition,

232 to better understand how grain nitrogen concentration is built up, four other relationships were

233 analysed with this same method:

234 1. NUE = NupE x NutE (on logarithms)

235 2. Ngrain = DMgrain x %N (on logarithms)

236 3. DMgrain = DMmat x HI (on logarithms)

237 4. Ngrain = Nmat x NHI (on logarithms)

238 5. Nmat = Nanth + NpA

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

240

241

242 **3. Results**

243 Grain yield, nitrogen concentration and uptake were significantly influenced by the factor

244 year (Table 3). As the factor year interacted also with several other factors (Table 3), the

245 results were generally analysed separately for each year and then synthesised.

246

247 *3.1 Overall response of wheat to the long term field trial treatments*

248 3.1.1 Yield and nitrogen utilisation

249 Grain yield (DMgrain) and nitrogen content (%N and Ngrain) were significantly influenced

250 by the type of organic enrichment and the amount of nitrogen fertiliser (Table 3). They rose

251 with increasing quantities of nitrogen fertiliser and with the addition of manure (Table 4). Yet,

252 organic enrichment and nitrogen fertilisation strongly interacted, especially for %N (Table 3,

253 Figure 1).

254 All the traits concerning dry matter as well as nitrogen accumulation and use were

255 significantly influenced by the level of nitrogen fertilisation, except the fraction of total

256 nitrogen accumulated after anthesis (fNpA) which was not influenced by any of the treatments

257 (Supplementary data Table S1 and Figures S1 and S2). Organic enrichment had an effect on

258 the kernel number (KN), dry matter at maturity (DMmat), nitrogen uptake at anthesis (Nanth)

259 and at maturity (Nmat), nitrogen post-anthesis accumulation (NpA) and N utilisation

260 efficiency (NutE). In treatments with organic enrichment in combination with mineral

261 nitrogen fertilisation, positive and significant interactions were observed for the use of

262 nutrients by the plant, in particular NUE, NutE, NupE and NUEP, similar to observations

263 made for %N (Table S1 and Figures S1 and S2).

264

265 3.1.2 Total nitrogen uptake at maturity as a characterisation of available nitrogen

266 Each combination of organic enrichment and nitrogen fertilisation treatments was

267 characterised by its mean nitrogen uptake Nmat, related to the amount of nitrogen available

268 for the crop. Nmat ranged from 38 kgN/ha to 217 kgN/ha in 2006, and from 60 kgN/ha to 198

269 kgN/ha in 2007. In both years, the treatments were ranked in the same order relative to Nmat:

270 1. Mineral-noN, 2. Manure-noN, 3. Mineral-lowN, 4. Manure-lowN, 5. Mineral-highN, 6.

271 Manure-highN.

272 The nitrogen uptake at maturity (Nmat) with Manure-lowN and Mineral-highN was very

273 similar, in both years (non-significant differences: 154 kgN/ha and 165 kgN/ha in 2006 (lsd

274 5% = 15), and 159 kgN/ha and 179 kgN/ha in 2007 (lsd 5% = 20)). It is conceivable, that

275 these two treatments provided almost the same amount of nitrogen to the plants, yet from

276 different sources (organic + mineral or only mineral). The difference in added nitrogen

277 between these two treatments represented 80 kgN/ha.

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278 These two treatments also resulted in comparable grain yields (DMgrain; respectively 6.13

279 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% =

280 0.82)) (Figure 1) but not the same grain N concentrations (%N; respectively 1.98% and 2.18%

281 in 2006 (lsd 5% = 0.07), and 2.18% and 2.47% in 2007 (lsd 5% = 0.06)). Only 2006 gave

282 similar Ngrain (respectively 120 kgN/ha and 126 kgN/ha in 2006 (lsd 5% = 9), and 136

283 kgN/ha and 154 kgN/ha in 2007 (lsd 5% = 17)).

284

285 *3.2 Individual response of the wheat varieties*

286 3.2.1 Yield and nitrogen performance of individual varieties

287 Yield (DMgrain) and nitrogen concentration (%N) and uptake (Ngrain) differed significantly

288 between varieties (Table 3). On average (on all treatments and years), the lowest DMgrain

289 was achieved by Aszita (4.21 t/ha) and the highest by Tapidor (5.47 t/ha), whereas the lowest

290 %N was observed for Tapidor (1.90 %N), and the highest for Aszita (2.33 %N) (Table 4 and

291 Supplementary data Table S2). The lowest Ngrain was measured for Toras (99 kgN/ha), and

292 the highest for Farandole (108 kgN/ha). The rankings of varieties in terms of DMgrain and

293 %N was generally similar in both years (Figure 2A). A negative relationship between

294 DMgrain and %N was observed, on average, and within each treatment (Figure 2A).

295 No significant differences were observed between varieties for N accumulation at anthesis

296 (Nanth) nor for post-anthesis accumulation (NpA) and fNpA, while differences were present

297 at maturity (Nmat) (Table S1). A significant effect of the factor variety was observed on all

298 the other traits of dry matter and nitrogen accumulation and use (Table S1).

299

300 3.2.2 Grouping of varieties

301 Three main groups of varieties were delineated according to their performances (Figure 2A).

302 A first group consisted of the varieties Arina, Aszita and Titlis (#2, #3 and #4) with low grain

303 yield (DMgrain) and high grain nitrogen concentration (%N). A second group was composed

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304 of Pireneo and Zinal (#1 and #5) with intermediate values. The third group contained the

305 varieties Ephoros, Pegassos, Toras, Caphorn, Farandole and Tapidor (#6, #7, #8, #9, #10 and

306 #11), characterised by high DMgrain and low %N.

307 The principal component analysis confirmed the classification into three groups and allowed a

308 more precise characterisation of the groups (Figure 2B). The first two axes of the PCA

309 together accounted for 86% (axis 1: 55%; axis 2: 31%) of the total variance. The first group

310 (Arina #2 – Aszita #3 – Titlis #4) was characterised by a high %N and DMmat as well as by a

311 low harvest index (HI), nitrogen harvest index (NHI), NutE and NUE. In contrast, the third

312 group (Ephoros #6 – Pegassos #7 – Toras #8 – Caphorn #9 – Farandole #10 – Tapidor #11)

313 was characterised by an elevated harvest and nitrogen harvest indices, and high nitrogen

314 utilisation and use efficiencies. However, the third group was rather inhomogeneous for

315 Nmat, Ngrain, NupE and NUEP, with low (Ephoros #6 – Toras #8) or high (Farandole #10

316 and Tapidor #11) values. Looking at the same criteria, these two latter varieties were also

317 comparable to the second group (Pireneo #1 – Zinal #5).

318

319 3.2.3 Stability of the performances of the varieties with increasing nitrogen inputs

320 According to the analysis of variance, the factor variety tended to interact with the factors

321 organic enrichment and nitrogen fertilisation (Table 3). This means that the performance of

322 the single varieties was not consistent along the increasing nitrogen supply but depended also

323 on its origin (mineral or organic).

324 The varietal response to nitrogen supply Nsup is highlighted in Figure 3 and in supplementary

325 data Table S3. Here, the performance of each varietiy in relation to all other varieties is

326 displayed on the gradient line from low to high nitrogen supplies, for yield, N concentration

327 and N accumulation. For yield (DMgrain), the varieties of the first group showed significantly

328 decreasing performances, with respect to the average of all varieties, with increasing nitrogen

329 supply. This suggests that their yield benefited less from the increase of available nitrogen

13

330 compared to the other varieties. The other varieties exhibited a steady increase of yield with

331 increasing Nsup, yet were either lower than the average yield (second group Pireneo #1 and

332 Zinal #5) or higher (highest values for Tapidor #11 and Farandole #10). Only the variety

333 Toras (#8) showed a significant increase from a very low yield in the absence of mineral

334 fertilisation to a yield over the mean by intensive fertilisation.

335 With respect to grain nitrogen concentration (%N), Pireneo (#1) and especially Titlis (#4)

336 increased more than the average with increasing nitrogen supply (Figure 3). In contrast,

337 Ephoros (#6) and Toras (#8) (both from the third group) were unable to follow the average

338 increase in %N with increasing availability of nitrogen. The other varieties exhibited mostly

339 very stable responses along the increase of Nsup, performing either above (Aszita #3, Arina

340 #2, Zinal #5) or below the mean values curve (Pegassos #7, Caphorn #9, Tapidor #11).

341 For grain N uptake (Ngrain), Zinal (#5) showed higher accumulation of nitrogen with

342 increasing nitrogen availability compared to the mean value (Figure 3) than all other varieties.

343 Concerning the dynamics of nitrogen accumulation, the varieties exhibited very similar

344 responses at anthesis (Nanth) to the increase of available nitrogen (except Farandole #10 but

345 this effect is due to an outlier value) (Table S3). The accumulation of nitrogen at maturity

346 (Nmat) was also very similar in all varieties, except for Zinal (#5) that increased accumulation

347 with more nitrogen at disposal, and Pegassos (#7) with poor reaction to the available nitrogen

348 (Table S3). For post-anthesis accumulation (NpA), Ephoros (#6) did not react to increased

349 nitrogen availability.

350 The direct comparison of the performance of the varieties in the two most extreme systems in

351 terms of nitrogen input, Mineral-noN and Manure-highN, suggested however that the ranking

352 of the varieties was in principle consistent at low and at high nitrogen inputs.

353 Strong correlations between the varieties for %N at low and high nitrogen inputs were

354 observed (Pearson's coefficient of correlation r: 0.87, p<0.001 in 2006 and r=0.95, p<0.001 in

355 2007). The correlation was also significant for yield in 2006 (r=0.75, p=0.005), but not in

14

356   2007 (r=0.38, p=0.25), though a single outlier (Toras #8) was responsible for this non-

357   significant correlation (without Toras: r=0.79, p=0.006). The more pronounced correlations

358   for N concentration than for yield suggested that genetic determinism is stronger for this trait.

359

360   3.2.4 Varietal response to distinct sources of nitrogen

361   As pointed out previously, the two treatments Mineral-highN and Manure-lowN supplied

362   similar amounts of nitrogen to the plants, which showed similar mean yield and grain N

363   uptake values. When comparing the varieties, it was possible to highlight those varieties

364   reacting more strongly to the source of nitrogen (mineral only or organic + mineral). Figure 4

365   displays how the performances of the varieties deviate from the general pattern. Concerning

366   yield (DMgrain), Tapidor (#11) in 2006 and Pegassos (#7) in 2007 diverged more than the

367   other varieties, showing a preference to mixed sources of nitrogen (organic + mineral in the

368   Manure-lowN treatment) (Figure 4, upper row). For grain N concentration (%N), Zinal (#5)

369   showed a divergent reaction in both years, towards mixed sources in 2006 and towards

370   mineral nitrogen in 2007 (Figure 4, lower row). Here again, the response of %N was more

371   consistent than that of DMgrain.

372

373   *3.3 Relationship between varietal traits and performance*

374   3.3.1 Correlations of varietal traits with yield and nitrogen concentration

375   Among the studied traits, harvest index (HI) and kernel number (KN) were strongly positively

376   correlated with yield (DMgrain) and negatively with %N (Supplementary data Table S4).

377   Nitrogen utilisation efficiency (NutE) also displayed strong positive correlations with

378   DMgrain and negative correlations with %N for all six treatments, whereas N uptake

379   efficiency (NupE) was not correlated with either variable (except with yield at lowN in 2007).

380   Nitrogen use efficiency for protein (NUEP) was generally correlated positively with

381   DMgrain, but not with %N. Interestingly, the three traits associated with the dynamics of

15

382 nitrogen accumulation (Nanth, NpA, fNpA) were correlated neither with DMgrain nor with

383 %N (except in a few situations) (Table S4).

384

385 3.3.2 Relative contributions of varietal traits

386 The amount of nitrogen accumulated in the grain (Ngrain) is the product of N concentration

387 (%N) and yield (DMgrain). In all treatments (except Mineral-noN in 2006 and Mineral-highN

388 in 2007), variations in %N contributed less to the Ngrain than did DMgrain (Table 5). Grain

389 dry matter (DMgrain) is itself a function of the total dry matter at harvest in the whole plant

390 (DMmat) and of the harvest index (HI). Variations in DMgrain among varieties were better

391 explained by variations in HI than in DMmat. Since the nitrogen harvest index (NHI) was

392 relatively stable, the differences in grain nitrogen uptake (Ngrain) depended on the quantity of

393 nitrogen accumulated at maturity (Nmat). Depending on the nitrogen treatment, the variation

394 of nitrogen at maturity (Nmat) was alternatively more influenced by the quantity of nitrogen

395 at anthesis (Nanth) or by the post-anthesis nitrogen accumulation (NpA) (Table 5). In 2006,

396 Nanth contributed more to Nmat in the Mineral treatments and NpA more in the Manure

397 treatments, thus showing not only a clear influence of the presence of organic enrichment to

398 the dynamics of nitrogen absorption, but also an effect of the year (climate). In 2007 the

399 pattern was less clear, but with a greater influence of Nanth on Nmat in all cases except

400 Mineral-lowN and Manure-highN (Table 5).

401 The variation in nitrogen use efficiency (NUE) was more influenced by nitrogen utilisation

402 efficiency (NutE) than by nitrogen uptake efficiency (NupE), in all treatments (Table 5). This

403 effect was stronger at high than at low nitrogen fertilisation levels.

404

405

406 **4. Discussion**

407 The availability of nitrogen is fundamental for the expression of the yield and the baking

408 quality potential in wheat. Under nitrogen limiting conditions, each wheat genotype must

409 choose to attribute the available nitrogen either for yield or for quality. In the present work,

410 the interplay between the form of added nitrogen and the wheat genotype has been

411 investigated in a long term experimental scheme. The principal aim was to understand which

412 proportion of the available nitrogen is taken up and allocated in the different plant metabolic

413 compartments. Before dissecting genotype effects of nitrogen metabolism, it was necessary to

414 characterise the gross availability of nitrogen in the single long term treatments.

415

416 *4.1 Response of wheat to organic enrichment, nitrogen fertilisation and their interaction*

417 Most of the traits, in particular grain yield and nitrogen, were strongly influenced by both the

418 organic enrichment and the nitrogen fertilisation treatments. As expected, the more nitrogen is

419 readily available, the higher is the yield and the grain N concentration. The long term

420 experiment made the study of different types of readily and delayed nitrogen availability

421 possible, especially in treatments with organic and mineral nitrogen allowances. The

422 dynamics of the organic matter and the availability of nitrogen in these different treatments

423 have been characterised in previous studies (Maltas et al., 2012ab). The addition of mineral

424 nitrogen tended to increase the soil organic matter (values ranging, in 2004, from 1.45% in

425 Mineral-noN to 1.75% in Mineral-highN, and from 1.98% in Manure-lowN to 2.23% in

426 Manure-highN), which is likely to be due to the greater amount of residues and root biomass

427 produced in fertilised plots, which then has a positive influence on crop growth (Maltas et al.,

428 2012b).

429 The addition of manure also significantly affected plant performance, even though manure

430 was incorporated before the preceding crop in spring 2005. Here, the positive effect relies

431 essentially on the indirect and long lasting effects of manure addition and not on the direct

432 input of easily available nitrogen. Indeed, the availability of nitrogen for crops after manure

17

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.

18

458   To understand how nitrogen was used, three groups of varieties were identified, based on their

459   yield and N concentration. Within each group, the response to the increase of nitrogen was

460   consistent. The groups corresponded largely to the baking quality classes shown in Table 2,

461   further validating this cataloguing. The group composed of Arina, Aszita and Titlis showed a

462   decreasing yield response, with the increasing nitrogen supply. However, in comparison with

463   high yielding varieties, they remained low yielding varieties at low input too. Obviously, the

464   interaction was not strong enough to inverse the ranking of the varieties at low input. In

465   contrast, this group of varieties was characterised by high N concentration, which is a sign of

466   high bread making quality. Yet, Titlis exhibited a strongly positive response of grain N

467   concentration with the increase of N supply. This could be explained by its really stable grain

468   nitrogen uptake, which allowed it to concentrate nitrogen as its yield response slowly

469   decreased. Thus, Titlis is a more responsive variety, in terms of quality, which benefits from

470   highly fertile conditions.

471   In the second group (intermediate yield and N concentration), Zinal (quality class 1) showed a

472   capacity of post-anthesis nitrogen assimilation superior to the mean and increasing with N

473   supply, leading to high total and grain N uptake at high N supply. This is thus typically an

474   interesting variety, responding when nitrogen is available at high doses, and able to profit

475   from nitrogen available late in the season.

476   In the third group (high yield and low N concentration, quality classes 3-5), Ephoros had a

477   decreasing response of N concentration when nitrogen supply increased. This was linked to a

478   weak response of nitrogen post anthesis accumulation and total nitrogen uptake at maturity,

479   leading to low nitrogen use efficiency with high nitrogen supply. This variety responded

480   weakly to an increase of nitrogen supply. A more responsive variety was Tapidor, which

481   exhibited a strong increase in its number of grains leading to high yields with high nitrogen

482   supply. The choice of the best variety will thus depend on the level of soil fertility available

483   for its growth, the timing of its availability and on the importance of quality stability.

484

4.3 Efficiency of breeding for low input systems

485

The results showed a strong interaction between the genotype and the environment for both

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yield and nitrogen concentration potentials. Despite this interaction, the ranking of the

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varieties in the different treatments hardly changed. High yielding varieties under high input

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conditions also exhibited high yields under low input conditions. The same was true for the N

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concentration and several other traits, revealing thereby non-crossover interactions (cf.

490

Ceccarelli, 1996). Interestingly, the varieties Aszita and Pireneo were selected under low

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input conditions while all other varieties were selected under medium to high input levels.

492

The present results do not support the hypothesis that varieties specifically selected for low

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input are better adapted to low input conditions. Many similar findings have been reported

494

(Barraclough et al., 2010; Guarda et al., 2004; Hasegawa, 2003; Le Gouis et al., 2000; Wang

495

et al., 2011), and advocate the fact that specific selection programs for low input systems may

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not be necessary. In contrast, other studies show opposite results postulating that direct

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selection in low input environments helps to breed better adapted varieties (Brancourt-Hulmel

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et al., 2005; Ceccarelli, 1996; Dawson et al., 2008; El Bassam, 1998; Müllner et al., 2014;

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Presterl et al., 2002; Ruiz et al., 2008). Results from this study suggest that breeding may be

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carried out under any fertility conditions. Yet, testing advanced breeding lines and varieties

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systematically under a wide range of contrasting environments is still recommended,

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including high as well as low input conditions. This procedure, combined with baking quality

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assessments, will allow characterization of the nitrogen allocation of the variety and its

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aptitude for low input agriculture.

505

The treatments Mineral-highN and Manure-lowN provided comparable levels of plant total

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nitrogen uptake, thus differing basically by the source of nitrogen but not by the amount. The

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results showed only slight differences in terms of grain yield and N concentration that,

508

moreover, were not consistent through the years. Nevertheless, high yielding varieties tended

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510 to better use mixed sources of nitrogen. It is however likely that potential effects are masked

511 by environmental factors in particular by variability between the years. In contrast, other

512 studies have given evidence to differential responses of varieties to organic fertilisation

513 conditions (Baresel et al., 2008; Dawson et al., 2008; Löschenberger et al., 2008; Murphy et

514 al., 2007; Reid et al., 2009, 2011; Vlachostergios and Roupakias, 2008; Wolfe et al., 2008). In

515 addition, when breeding for low input conditions or for organic agriculture, other traits have

516 also to be included in the selection scheme such as disease resistance, resilience to abiotic

517 stress and competitiveness against weeds.

518

4.4 Development of selection criteria

520 In order to improve nutrient use efficiency of crops and thus yield and N concentration, it is

521 necessary to identify appropriate targets of selection (Foulkes et al., 2009). Many studies have

522 found genetic variability among traits involved in nitrogen processes, and genetic associations

523 between these traits and the focal properties (Austin et al., 1977; Barraclough et al., 2010,

524 2014; Bogard et al., 2010; Foulkes et al., 2009; Gaju et al., 2011; Le Gouis et al., 2000). In the

525 present study, almost all traits responded to a variety effect, suggesting genetic determinism.

526 Nitrogen use efficiency, nitrogen utilisation efficiency and harvest index were strongly

527 correlated with yield and N concentration in grain. However, these correlations were observed

528 within all treatments, whatever the level of nitrogen fertilisation and organic enrichment.

529 These traits can therefore not be used to target specifically high performance (yield or %N) in

530 low input or organic environments.

531 The contributions of nitrogen uptake efficiency and nitrogen utilisation efficiency to nitrogen

532 use efficiency were also explored in detail (following Moll et al., 1982). It was found that

533 nitrogen utilisation efficiency plays a more important role than nitrogen uptake efficiency in

534 nitrogen use efficiency, and this in all environments. This stresses the paramount importance

535 of grain biomass production. Several other authors support this finding (Barraclough et al.,

536 2010; Bingham et al., 2012; Gaju et al., 2011). However, contradictory results about the

537 importance of uptake and use efficiency have been reported in different experiments and for

538 different species. Indeed, other studies attribute a more important role to nitrogen uptake

539 efficiency for the determination of nitrogen use efficiency, especially at low nitrogen levels

540 (Dhugga and Waines, 1989; Le Gouis et al., 2000; Moll et al., 1982; Ortiz-Monasterio et al.,

541 1997; Wang et al., 2011).

542 Present results showed that for grain nitrogen uptake, dry matter accumulation was more

543 important than nitrogen concentration. Furthermore, these results postulated a major role of

544 nitrogen accumulated in the whole plant at maturity and of the harvest index for the

545 determination of grain nitrogen concentration at harvest. In these experiments, organic

546 enrichment has been observed to change the respective contribution of nitrogen accumulated

547 at anthesis and post-anthesis in total N uptake at maturity. In 2006, late accumulated nitrogen

548 had a major role in the presence of manure, compared to the mineral treatments. This is

549 probably explained by the increased rate of organic nitrogen mineralisation when

550 temperatures rise in spring, which makes more nitrogen available for the crops after anthesis.

551 This effect might have been more noticeable in 2006 than in 2007 because the last manure

552 application was in spring 2005.

553

554 *4.5 Conclusions*

555 In the present experiment, the allocation of nitrogen for yield or for grain nitrogen (i.e.

556 quality) has been investigated on an array of eleven modern wheat varieties in a 40-year long

557 term field experiment. The experiment offered the conditions to compare the effect of mineral

558 and organic nitrogen fertilisation in an experimentally robust scheme. Overall, the varietal

559 responses depended strongly on the organic enrichment and mineral nitrogen fertilisation.

560 Transition from high input production to low input or organic production would potentially

561 alter the performance of varieties, mostly selected at high input levels, since the availability of

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562 nitrogen is delayed, compared to production systems exclusively fertilised with mineral

563 nitrogen. Results presented here indicate that even if an important variability between

564 varieties was observed, their ranking in terms of yield and nitrogen accumulation in grain was

565 the same in nitrogen depleted and in nitrogen fertilised plots. Thus, varieties efficient in high

566 input environments tended also to outcompete the other varieties in low input or organic

567 fertilised environments. In conclusion, these results suggest that selection of wheat for

568 nitrogen efficiency can be carried out under any nitrogen fertilisation regime. Yet, selection

569 for low input agriculture must also integrate other traits, such as disease resistance, resilience

570 to abiotic stresses and competitiveness against weeds.

571

580

Supplementary Data

582 **Table S1**: Analysis of variance for the studied traits.

583 **Table S2**: Mean values of grain yield, grain nitrogen concentration (%N) and uptake (Ngrain)

584 for each variety in each treatment.

585 **Table S3**: For each variety, linear regression coefficients of its difference from the mean

586 treatment values on the nitrogen supply Nsup.

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

23

588   **Figure S1:** Distribution of the studied traits as a function of organic enrichment and nitrogen

589   fertilization treatments in 2006.

590   **Figure S2**: Distribution of the studied traits as a function of organic enrichment and nitrogen

591   fertilization treatments in 2007.

References

Annichiarico, P., 2002. Genotype x environment interaction - Challenges and opportunities for plant breeding and cultivar recommendations. Food and Agriculture Organization of the United Nations, Rome.

Austin, R.B., Ford, M.A., Edrich, J.A., Blackwell, R.D., 1977. The nitrogen economy of winter wheat. J. Agric. Sci. 88, 159-167.

Baresel, J.P., Zimmermann, G., Reents, H.J., 2008. Effects of genotype and environment on N uptake and N partition in organically grown winter wheat (Triticum aestivum L.) in Germany. Euphytica 163, 347-354.

Barraclough, P.B., Howarth, J.R., Jones, J., Lopez-Bellido, R., Parmar, S., Shepherd, C.E., Hawkesford, M.J., 2010. Nitrogen efficiency of wheat: Genotypic and environmental variation and prospects for improvement. Eur. J. Agron. 33, 1-11.

Barraclough, P.B., Lopez-Bellido, R., Hawkesford, M.J., 2014. Genotypic variation in the uptake, partitioning and remobilisation of nitrogen during grain-filling in wheat. Field Crop Res. 156, 242-248.

Berry, P.M., Sylvester-Bradley, R., Philipps, L., Hatch, D.J., Cuttle, S.P., Rayns, F.W., Gosling, P., 2002. Is the productivity of organic farms restricted by the supply of available nitrogen? Soil Use Manage. 18, 248-255.

Bingham, I.J., Karley, A.J., White, P.J., Thomas, W.T.B., Russell, J.R., 2012. Analysis of improvements in nitrogen use efficiency associated with 75 years of spring barley breeding. Eur. J. Agron. 42, 49-58.

Bogard, M., Allard, V., Brancourt-Hulmel, M., Heumez, E., Machet, J.M., Jeuffroy, M.H., Gate, P., Martre, P., Le Gouis, J., 2010. Deviation from the grain protein concentration-grain yield negative relationship is highly correlated to post-anthesis N uptake in winter wheat. J. Exp. Bot. 61, 4303-4312.

617 Brancourt-Hulmel, M., Heumez, E., Pluchard, P., Beghin, D., Depatureaux, C., Giraud, A., Le

618   Gouis, J., 2005. Indirect versus direct selection of winter wheat for low-input or high-

619   input levels. Crop Sci. 45, 1427-1431.

620 Ceccarelli, S., 1996. Adaptation to low high input cultivation. Euphytica 92, 203-214.

621 Charles, R., Jolliet, O., Gaillard, G., Pellet, D., 2006. Environmental analysis of intensity level

622   in wheat crop production using life cycle assessment. Agric. Ecosyst. Environ. 113,

623   216-225.

624 Dawson, J.C., Huggins, D.R., Jones, S.S., 2008. Characterising nitrogen use efficiency in

625   natural and agricultural ecosystems to improve the performance of cereal crops in low-

626   input and organic agricultural systems. Field Crop Res. 107, 89-101.

627 Dhugga, K.S., Waines, J.G., 1989. Analysis of nitrogen accumulation and use in bread and

628   durum wheat. Crop Sci. 29, 1232-1239.

629 Dick, R.P., 1992. A review: long-term effects of agricultural systems on soil biochemical and

630   microbial parameters. Agric. Ecosyst. Environ. 40, 25-36.

631 Eghball, B., Power, J.F., 1999. Phosphorus- and nitrogen-based manure and compost

632   applications: Corn production and soil phosphorus. Soil Sci. Soc. Am. J. 63, 895-901.

633 El Bassam, N., 1998. A concept of selection for 'low input' wheat varieties. Euphytica 100,

634   95-100.

635 Finlay, K.W., Wilkinson, G.N., 1963. The analysis of adaptation in a plant-breeding

636 programme. Aust. J. Agric. Res. 14, 742-754.

637 Flood, R.G., Martin, P.J., 2001. Nitrogen accumulation and distribution at anthesis and

638   maturity in ten wheats grown at three sites in north-western Victoria. Aust. J. Exp.

639   Agric. 41, 533-540.

640 Foulkes, M.J., Hawkesford, M.J., Barraclough, P.B., Holdsworth, M.J., Kerr, S., Kightley, S.,

641   Shewry, P.R., 2009. Identifying traits to improve the nitrogen economy of wheat:

642   Recent advances and future prospects. Field Crop Res. 114, 329-342.

643 Frink, C.R., Waggoner, P.E., Ausubel, J.H., 1999. Nitrogen fertiliser: Retrospect and

644 prospect. Proc. Natl. Acad. Sci. U. S. A. 96, 1175-1180.

645 Gaju, O., Allard, V., Martre, P., Snape, J.W., Heumez, E., Le Gouis, J., Moreau, D., Bogard,

646 M., Griffiths, S., Orford, S., Hubbart, S., Foulkes, M.J., 2011. Identification of traits to

647 improve the nitrogen-use efficiency of wheat genotypes. Field Crop Res. 123, 139-

648 152.

649 Guarda, G., Padovan, S., Delogu, G., 2004. Grain yield, nitrogen-use efficiency and baking

650 quality of old and modern Italian bread-wheat cultivars grown at different nitrogen

651 levels. Eur. J. Agron. 21, 181-192.

652 Häni, F., Popow, G., Reinhard, H., Schwarz, A., Tanner, K., Vorlet, M., 1990. Protection des

653 plantes en production intégrée. LMZ Centrale des moyens d'enseignement agricole,

654 Zollikofen, 334 p.

655 Hasegawa, H., 2003. High-yielding rice cultivars perform best even at reduced nitrogen

656 fertiliser rate. Crop Sci. 43, 921-926.

657 Hirel, B., Le Gouis, J., Ney, B., Gallais, A., 2007. The challenge of improving nitrogen use

658 efficiency in crop plants: towards a more central role for genetic variability and

659 quantitative genetics within integrated approaches. J. Exp. Bot. 58, 2369-2387.

660 Huggins, D.R., Pan, W.L., 1993. Nitrogen efficiency component analysis: an evaluation of

661 cropping system differences in productivity. Agron. J. 85, 898-905.

662 Khan, S.A., Mulvaney, R.L., Ellsworth, T.R., Boast, C.W., 2007. The myth of nitrogen

663 fertilisation for soil carbon sequestration. J. Environ. Qual. 36, 1821-1832.

664 Ladha, J.K., Pathak, H., Krupnik, T.J., Six, J., van Kessel, C., 2005. Efficiency of fertiliser

665 nitrogen in cereal production: Retrospects and prospects. Adv. Agron. 87, 85-156.

666 Lal, R., 2009. Challenges and opportunities in soil organic matter research. Eur. J. Soil Sci.

667 60, 158-169.

668 Lammerts van Bueren, E.T., Struik, P.C., Jacobsen, E., 2002. Ecological concepts in organic

669   farming and their consequences for an organic crop ideotype. Neth. J. Agric. Sci. 50,

670   1-26.

671 Le Gouis, J., Beghin, D., Heumez, E., Pluchard, P., 2000. Genetic differences for nitrogen

672   uptake and nitrogen utilisation efficiencies in winter wheat. Eur. J. Agron. 12, 163-

673   173.

674 Lemaire, G., van Oosterom, E., Jeuffroy, M.-H., Gastal, F., Massignam, A., 2008. Crop

675   species present different qualitative types of response to N deficiency during their

676   vegetative growth. Field Crops Res. 105, 253-265.

677 Limon-Ortega, A., Sayre, K.D., Francis, C.A., 2000. Wheat nitrogen use efficiency in a bed

678   planting system in northwest Mexico. Agron. J. 92, 303-308.

679 Löschenberger, F., Fleck, A., Grausgruber, H., Hetzendorfer, H., Hof, G., Lafferty, J., Marn,

680   M., Neumayer, A., Pfaffinger, G., Birschitzky, J., 2008. Breeding for organic

681   agriculture: the example of winter wheat in Austria. Euphytica 163, 469-480.

682 Maltas, A., Charles, R., Bovet, V., Sinaj, S., 2012a. Long-term effect of organic fertilisers on

683   crop yield and nitrogen fertilisation. Agrarforschung Schweiz 3, 156-163.

684 Maltas, A., Oberholzer, H., Charles, R., Bovet, V., Sinaj, S., 2012b. Long-term effect of

685   organic fertilisers on soil properties. Agrarforschung Schweiz 3, 148-155.

686 Marschner, H., 1995. Mineral nutrition of higher plants. Academic Press London.

687 Masclaux, C., Quillere, I., Gallais, A., Hirel, B., 2001. The challenge of remobilisation in

688   plant nitrogen economy. A survey of physio-agronomic and molecular approaches.

689   Ann. Appl. Biol. 138, 69-81.

690 Meng, L., Ding, W.X., Cai, Z.C., 2005. Long-term application of organic manure and

691   nitrogen fertiliser on N2O emissions, soil quality and crop production in a sandy loam

692   soil. Soil Biol. Biochem. 37, 2037-2045.

693   Moll, R.H., Kamprath, E.J., Jackson, W.A., 1982. Analysis and interpretation of factors which

694   contribute to efficiency of nitrogen utilisation. Agron. J. 74, 562-564.

695   Müllner, A.E., Mascher, F., Schneider, D., Ittu, G., Toncea, I., Rolland, B., Löschenberger, F.,

696   2014. Refining breeding methods for organic and low-input agriculture: analysis of an

697   international winter wheat ring test. Euphytica 199, 81-95.

698   Murphy, K.M., Campbell, K.G., Lyon, S.R., Jones, S.S., 2007. Evidence of varietal

699   adaptation to organic farming systems. Field Crop Res. 102, 172-177.

700   Nemecek, T., von Richthofen, J.-S., Dubois, G., Casta, P., Charles, R., Pahl, H., 2008.

701   Environmental impacts of introducing grain legumes into European crop rotations.

702   Eur. J. Agron. 28, 380-393.

703   Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson,

704   G.L., Solymos, P., Stevens, M.H.H, Wagner, H., 2011. vegan: Community Ecology

705   Package. R package version 2.0-2.

706   Ortiz-Monasterio, J.I., Sayre, K.D., Rajaram, S., McMahon, M., 1997. Genetic progress in

707   wheat yield and nitrogen use efficiency under four nitrogen rates. Crop Sci. 37, 898-

708   904.

709   Presterl, T., Groh, S., Landbeck, M., Seitz, G., Schmidt, W., Geiger, H.H., 2002. Nitrogen

710   uptake and utilisation efficiency of European maize hybrids developed under

711   conditions of low and high nitrogen input. Plant Breed. 121, 480-486.

712   R Core Team, 2014. R: A language and environment for statistical computing. R Foundation

713   for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

714   Raun, W.R., Johnson, G.V., 1999. Improving nitrogen use efficiency for cereal production.

715   Agron. J. 91, 357-363.

716   Reid, T.A., Yang, R.C., Salmon, D.F., Navabi, A., Spaner, D., 2011. Realized gains from

717   selection for spring wheat grain yield are different in conventional and organically

718   managed systems. Euphytica 177, 253-266.

29

719 Reid, T.A., Yang, R.C., Salmon, D.F., Spaner, D., 2009. Should spring wheat breeding for

720 organically managed systems be conducted on organically managed land? Euphytica

721 169, 239-252.

722 Riley, H., Pommeresche, R., Eltun, R., Hansen, S., Korsaeth, A., 2008. Soil structure, organic

723 matter and earthworm activity in a comparison of cropping systems with contrasting

724 tillage, rotations, fertiliser levels and manure use. Agric. Ecosyst. Environ. 124, 275-

725 284.

726 Ruiz, M., Aguiriano, E., Carrillo, J.M., 2008. Effects of N fertilisation on yield for low-input

727 production in Spanish wheat landraces (Triticum turgidum L. and Triticum

728 monococcum L.). Plant Breed. 127, 20-23.

729 Ryser, J.P., Walther, U., Flisch, R., 2001. Données de base pour la fumure des grandes

730 cultures et des herbages. Rev. Suisse Agric. 33, 4-80.

731 Sadras, V.O., Lemaire, G., 2014. Quantifying crop nitrogen status for comparisons of

732 agronomic practices and genotypes. Field Crop Res. 164, 54-64.

733 Sakala, W.D., Cadisch, G., Giller, K.E., 2000. Interactions between residues of maize and

734 pigeonpea and mineral N fertilisers during decomposition and N mineralisation. Soil

735 Biol. Biochem. 32, 679-688.

736 Sinaj, S., Richner, W., Flisch, R., Charles, R., 2009. Données de base pour la fumure des

737 grandes cultures et des herbages. Rev. Suisse Agric. 41, 1-98.

738 Singer, J.W., Kohler, K.A., Liebman, M., Richard, T.L., Cambardella, C.A., Buhler, D.D.,

739 2004. Tillage and compost affect yield of corn, soybean, and wheat and soil fertility.

740 Agron. J. 96, 531-537.

741 Sylvester-Bradley, R., Kindred, D.R., 2009. Analysing nitrogen responses of cereals to

742 prioritize routes to the improvement of nitrogen use efficiency. J. Exp. Bot. 60, 1939-

743 1951.

744 Tilman, D., 1999. Global environmental impacts of agricultural expansion: The need for

745   sustainable and efficient practices. Proc. Natl. Acad. Sci. U. S. A. 96, 5995-6000.

746 Van Sanford, D.A., Mackown, C.T., 1986. Variation in nitrogen use efficiency among soft red

747   winter wheat genotypes. Theor. Appl. Genet. 72, 158-163.

748 Vlachostergios, D.N., Roupakias, D.G., 2008. Response to conventional and organic

749   environment of thirty-six lentil (Lens culinaris Medik.) varieties. Euphytica 163, 449-

750   457.

751 Vullioud, P., Neyroud, J.-A., Mercier, E., 2006. Efficacité de différents apports organiques et

752   d'un engrais minéral azoté à Changins (1976-2004). Rev. Suisse Agric. 38, 173-183.

753 Wang, R.F., An, D.G., Hu, C.S., Li, L.H., Zhang, Y.M., Jia, Y.G., Tong, Y.P., 2011.

754   Relationship between nitrogen uptake and use efficiency of winter wheat grown in the

755   North China Plain. Crop Pasture Sci. 62, 504-514.

756 Watson, C.A., Atkinson, D., Gosling, P., Jackson, L.R., Rayns, F.W., 2002. Managing soil

757   fertility in organic farming systems. Soil Use Manage. 18, 239-247.

758 Wolfe, M.S., Baresel, J.P., Desclaux, D., Goldringer, I., Hoad, S., Kovacs, G., Loschenberger,

759   F., Miedaner, T., Ostergard, H., van Bueren, E.T.L., 2008. Developments in breeding

760   cereals for organic agriculture. Euphytica 163, 323-346.

761 Yang, S.M., Malhi, S.S., Li, F.M., Suo, D.R., Xu, M.G., Wang, P., Xiao, G.J., Jia, Y., Guo,

762   T.W., Wang, J.G., 2007. Long-term effects of manure and fertilisation on soil organic

763   matter and quality parameters of a calcareous soil in NW China. J. Plant Nutr. Soil

764   Sci. 170, 234-243.

765 Ye, Y.L., Wang, G.L., Huang, Y.F., Zhu, Y.J., Meng, Q.F., Chen, X.P., Zhang, F.S., Cui,

766   Z.L., 2011. Understanding physiological processes associated with yield-trait

767   relationships in modern wheat varieties. Field Crop Res. 124, 316-322.

768   Zhang, H., Xu, M., Zhang, F., 2009. Long-term effects of manure application on grain yield

769   under different cropping systems and ecological conditions in China. J. Agric. Sci.

770   147, 31-42.

771 **Table and figure legends**

772

773 **Table 1**: Cumulated rainfall [mm] and growing degree days from sowing (T_{base} = 10°C) [°C]

774 at different growth stages, and nitrogen fertilisation splits and amounts [kgN/ha] for each

775 fertilisation treatments, for the two experimental years. Zadoks stage 89 corresponds to

776 harvest.

777

778 **Table 2**: Wheat varieties tested in this study. Origin: A = Austria, CH = Switzerland, D =

779 Germany, F = France. Year: year of release. Swiss class: estimation of the corresponding

780 swiss class, based on variety quality. Use: principal use of the variety.

781

782 **Table 3:** Analysis of variance (p-values) for grain yield (DMgrain), grain nitrogen

783 concentration (%N) and uptake (Ngrain), total dry matter at maturity (DMmat), nitrogen

784 uptake at anthesis (Nanth) and total nitrogen uptake at maturity (Nmat). Factors: experimental

785 year (Year), organic enrichment (Organic), mineral nitrogen fertilisation (Nitrogen) and

786 Variety.

787

788 **Table 4**: Overall mean values of grain yield, grain nitrogen concentration (%N) and uptake

789 (Ngrain), total dry matter at maturity (DMmat), nitrogen uptake at anthesis (Nanth) and total

790 nitrogen uptake at maturity (Nmat), for each factor (experimental year, organic enrichment,

791 mineral nitrogen fertilisation and variety).

792

793 **Table 5**: Decomposition analysis of varietal traits. %N: nitrogen concentration, Ngrain: grain

794 nitrogen uptake, DMgrain: grain yield, DMmat: dry matter at maturity, HI: harvest index,

795 Nmat: nitrogen uptake at maturity, NHI: nitrogen harvest index, Nanth: nitrogen accumulated

33

796 at anthesis, NpA: post-anthesis nitrogen accumulation, NUE: nitrogen use efficiency, NupE:

797 nitrogen uptake efficiency, NutE: nitrogen utilisation efficiency.

798

799 **Figure 1**: Nitrogen concentration (%N) as a function of yield for the organic enrichment and

800 nitrogen fertilisation treatments. Mean values over all the varieties and replicates are

801 presented. The shape of the symbols stands for the mineral fertilisation treatments, circle: no

802 nitrogen supply (noN), square: sub fertilisation (lowN), triangle: over fertilisation (highN).

803 Text labels specify the organic enrichment treatments, Min: Mineral and Man: Manure. Year

804 2006 values are in darkgrey, and 2007 values in white. The dashed lines represent isolines of

805 grain nitrogen uptake. The small grey symbols represent the individual variety values.

806

807 **Figure 2**: Characterisation of varieties. A. Nitrogen concentration (%N) as a function of yield

808 (mean values over all treatments and replicates). B. Principal components analysis of the

809 mean values of varieties (over all factors). Each number stands for a variety (see Table 2).

810 Descriptors: yield = grain yield, %N = grain nitrogen concentration, Ngrain = grain nitrogen

811 uptake, DMmat = total dry matter at maturity, Nmat = total nitrogen uptake at maturity, HI =

812 harvest index, NHI = nitrogen harvest index, NUE = nitrogen use efficiency, NutE = nitrogen

813 utilisation efficiency, NupE = nitrogen uptake efficiency, NUEP = nitrogen use efficiency for

814 protein.

815

816 **Figure 3**: A. Grain yield (DMgrain), B. grain nitrogen concentration (%N) and C. grain

817 nitrogen uptake (Ngrain), as a function of mean total nitrogen supply Nsup, for the two years.

818 Left side: raw values, Right side: differences from the treatment mean. Variety specific linear

819 regressions of the differences from the mean on the total nitrogen uptake are shown.

820 Significant regressions are indicated with bold lines.

821

34

Figure 4: Pairwise comparisons of treatments Mineral-highN and Manure-lowN for A. grain

823   yield in 2006, B. grain yield in 2007, C. grain nitrogen concentration (%N) in 2006 and D.

824   grain nitrogen concentration (%N) in 2007. Each number stands for a variety (see Table 2).

825   The grey lines have a slope of one, and pass through the overall mean of all the varieties.

826 **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	

827

828 **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

829 *estimation of corresponding swiss classes based on Swissgranum classification requirement
830 (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						

833 **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*

834

Table 5

|	Resultant trait	Component traits	Mineral			Manure		
			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
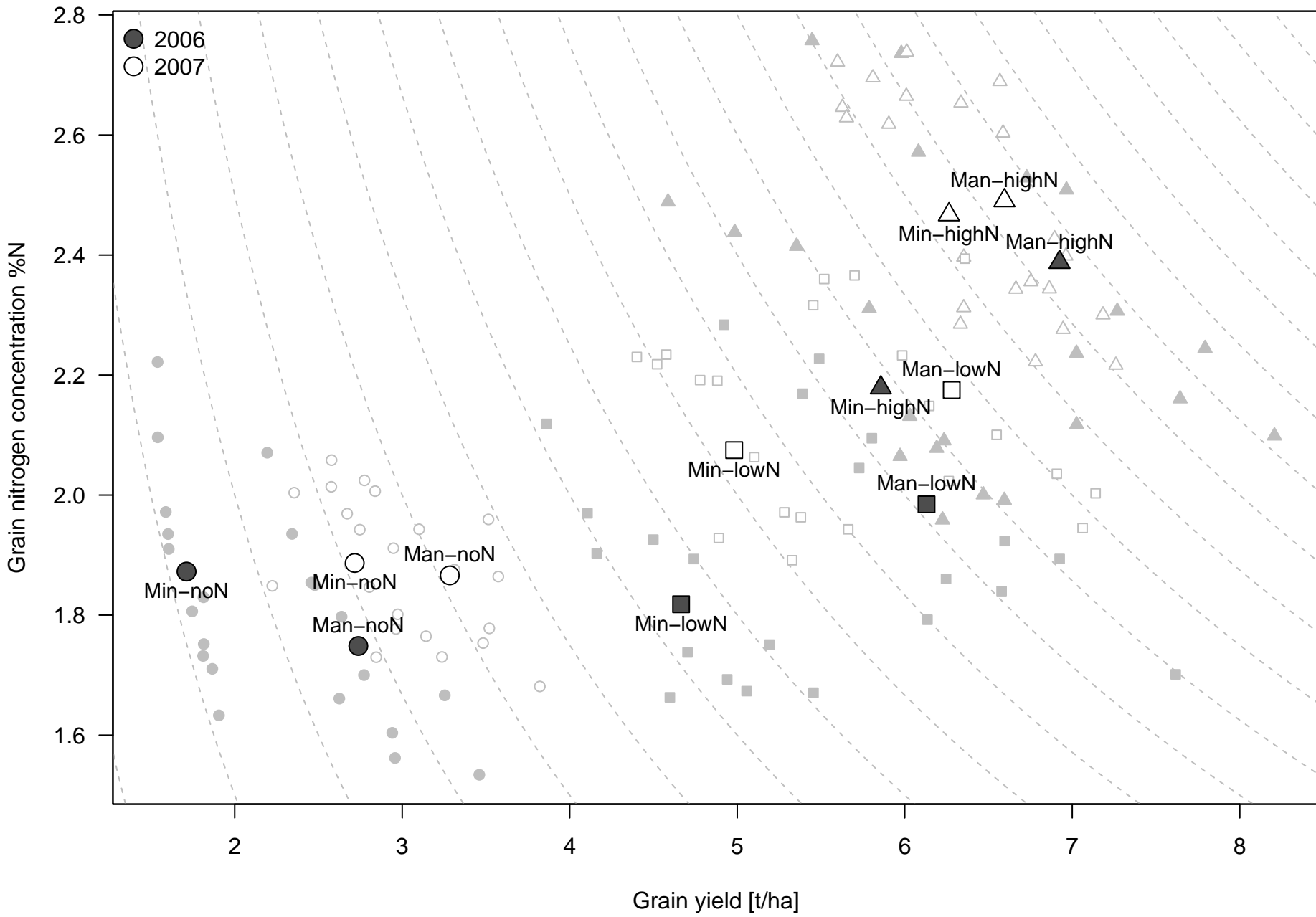
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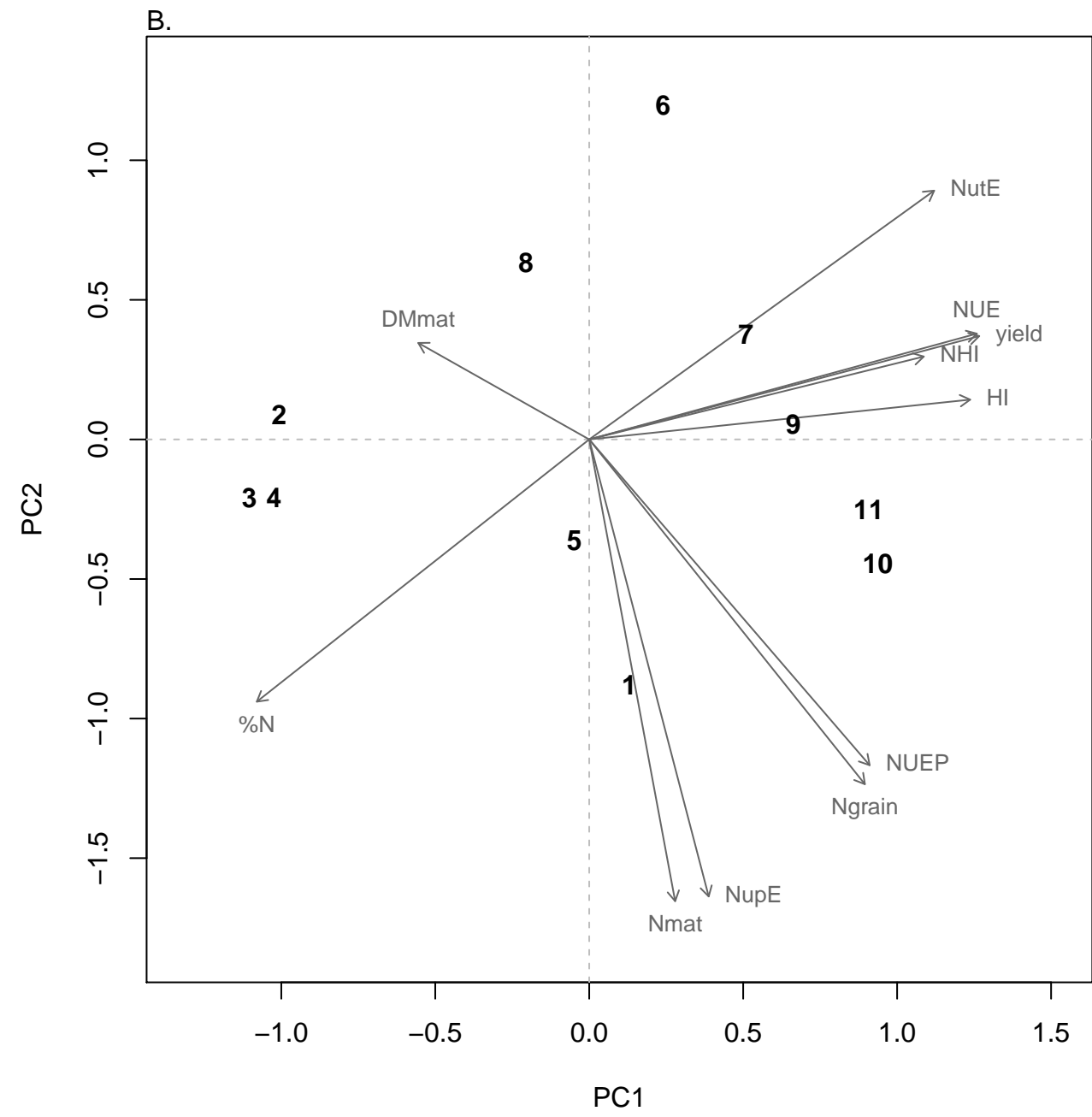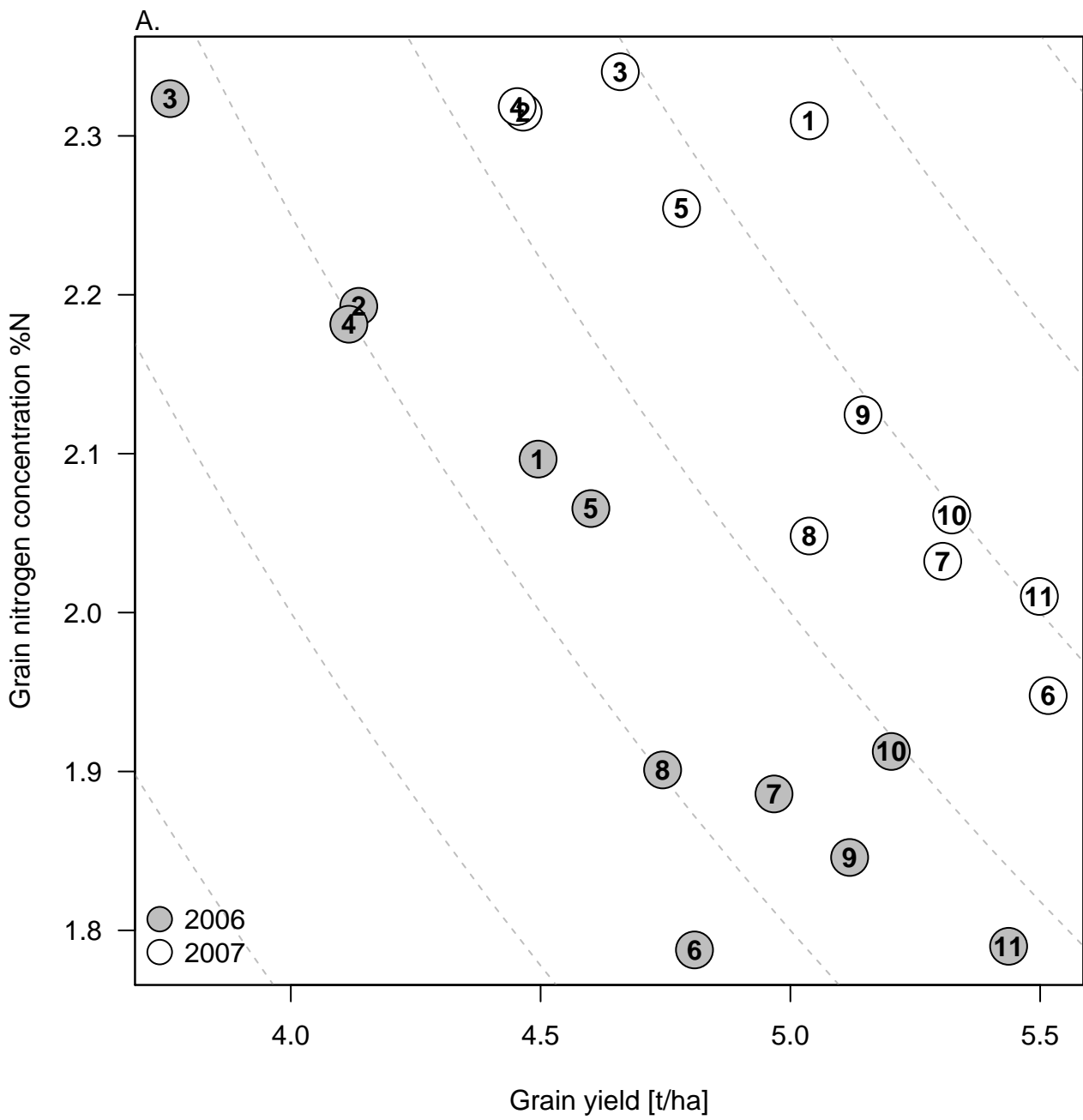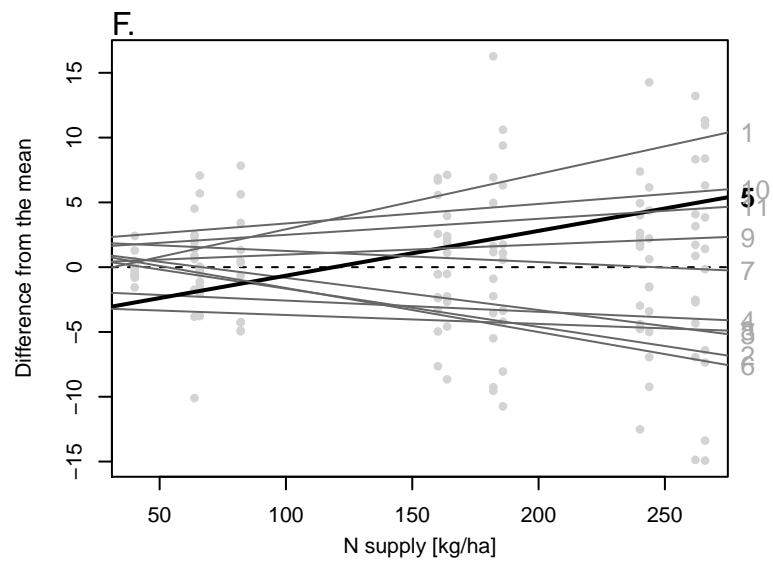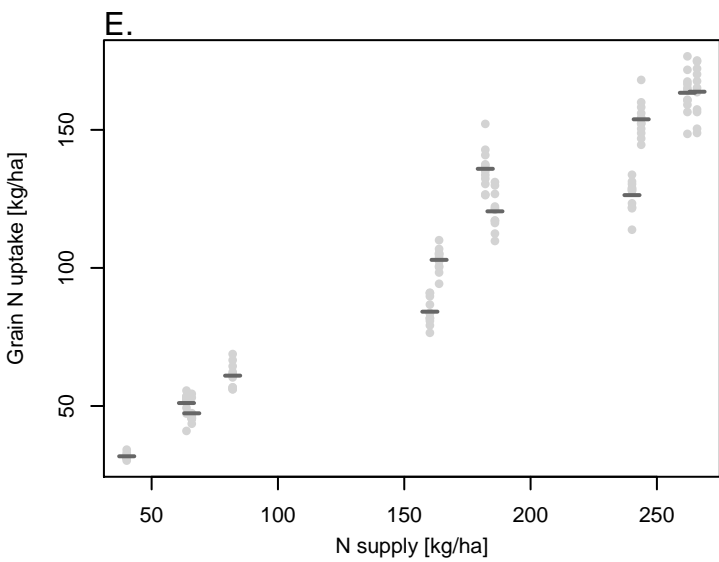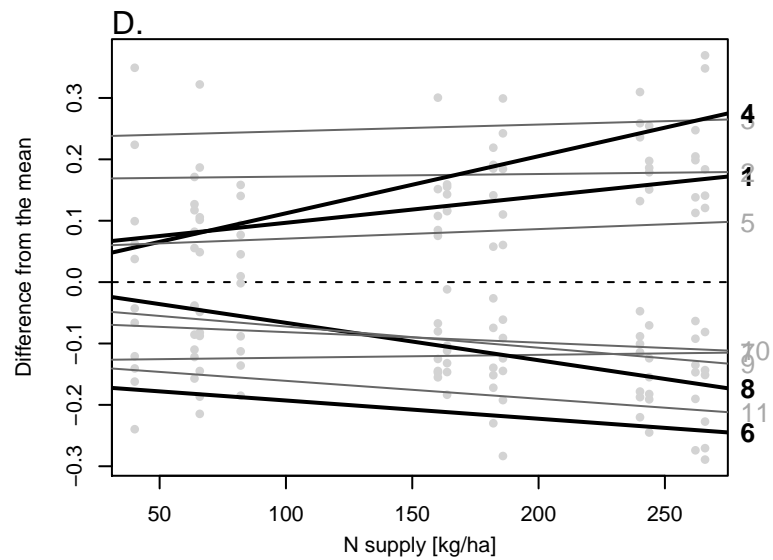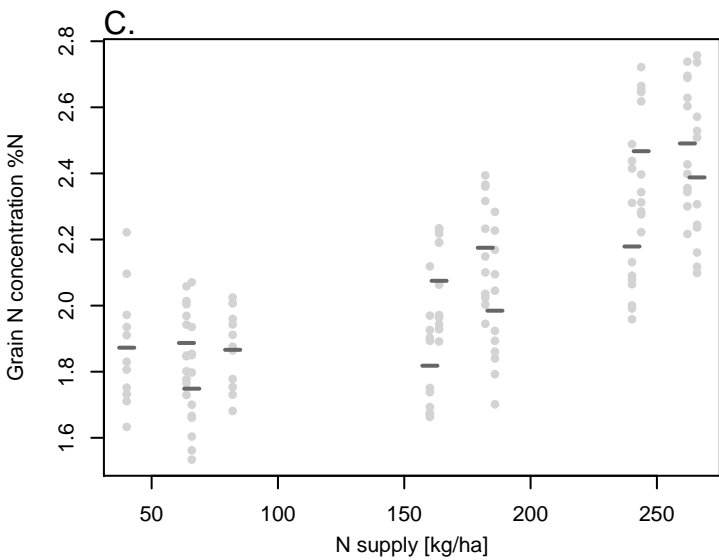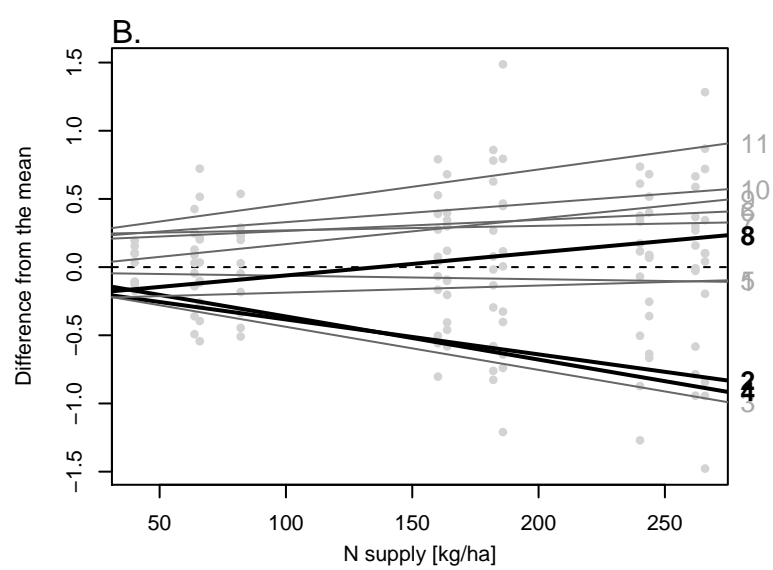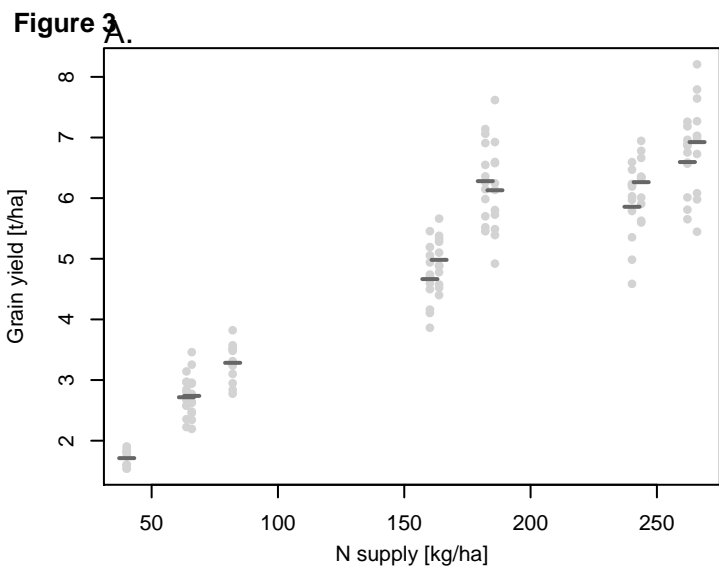
Figure 2

Figure 3

Figure 4
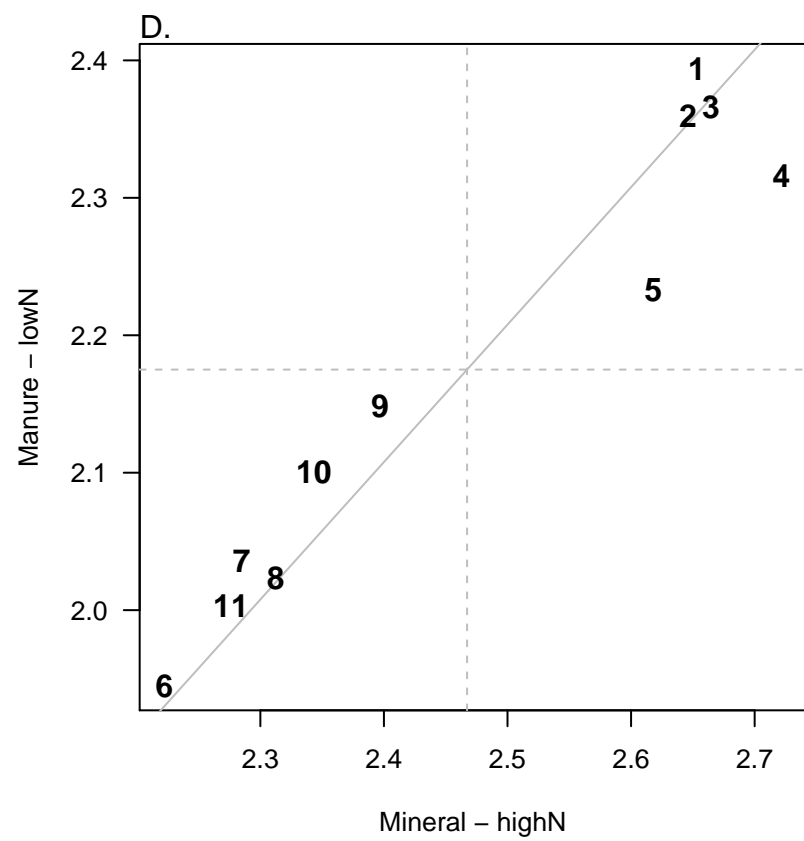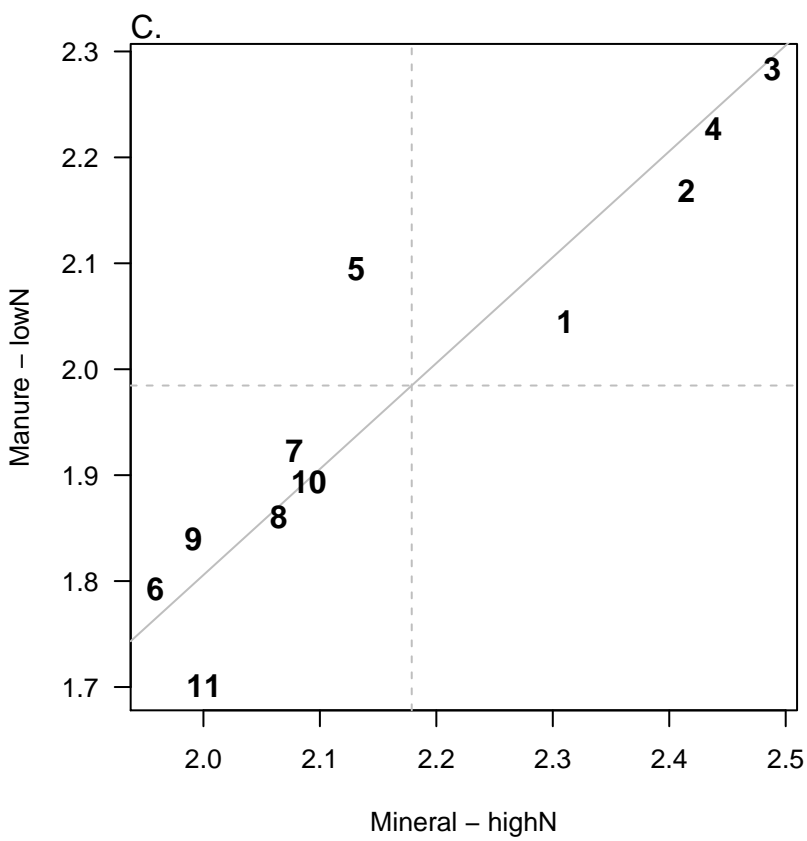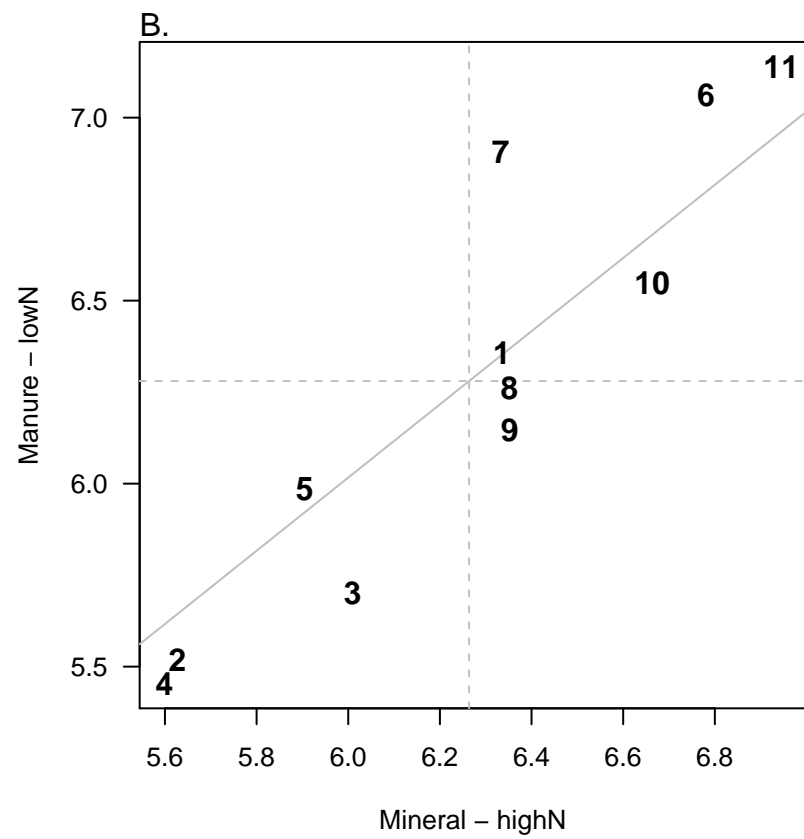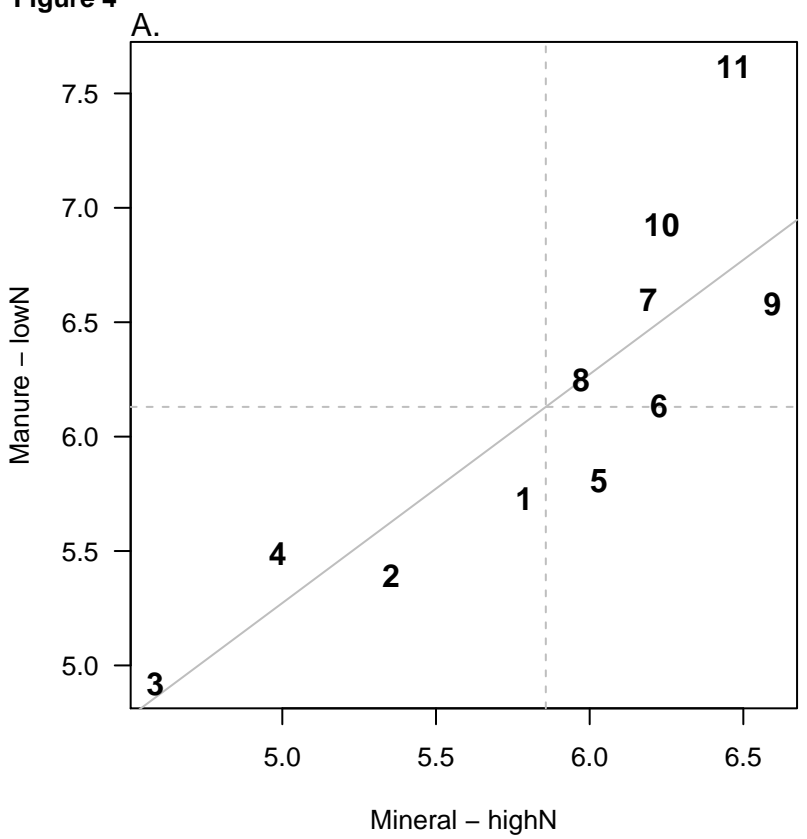
Supplementary data

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