Compacted soil adaptability of Brassica napus driven by root mechanical traits

Article

Accepted Version

It is advisable to refer to the publisher's version if you intend to cite from the work. See Guidance on citing.

www.reading.ac.uk/centaur

University of Reading

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

1 **Compacted soil adaptability of *Brassica napus* driven by root mechanical traits**

2 Xianjie Duan [a, b], Kemo Jin [c, *], Zhun Mao [d], Ling Liu [a, b], Yangbo He[b], Shangwen Xia [e],

3 John P. Hammond [f], Philip J. White [a, g], Fangsen Xu [a, b], Lei Shi [a, b, *]

4 [a] National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural

5 University, Wuhan 430070, China

6 [b] Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze

7 River), Ministry of Agriculture and Rural Affairs, Huazhong Agricultural University,

8 Wuhan 430070, China

9 [c] College of Resources and Environmental Sciences, National Academy of Agriculture

10 Green Development, Key Laboratory of Plant-Soil Interactions, Ministry of Education,

11 China Agricultural University, Beijing 100193, China

12 [d] Univ Montpellier, AMAP, INRAE, CIRAD, CNRS, IRD, 34000 Montpellier, France

13 [e] CAS Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical

14 Garden, Chinese Academy of Sciences, Yunnan, China

15 [f] School of Agriculture, Policy and Development, University of Reading, Reading,

16 United Kingdom

17 [g] The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

18

19 [*]Correspondence: Lei   Shi   (leish@mail.hzau.edu.cn);   Kemo   Jin

20 (kemo.jin@cau.edu.cn)

21 Address: National Key Laboratory of Crop Genetic Improvement, Huazhong

22 Agricultural University, Wuhan 430070, China

23 Tel: 0086-27-87286871

24 Fax: 0086-27-87280016

25

26   E-mail address for other authors:

27   Xianjie Duan: xianjieduan23@126.com

28   Zhun Mao: maozhun04@126.com

29 Ling Liu: hzaulingliu@webmail.hzau.edu.cn

30 Yangbo He: kathy@mail.hzau.edu.cn

31 Shangwen Xia: xsw@xtbg.org.cn

32 John P. Hammond: j.p.hammond@reading.ac.uk

33 Philip J. white: Philip.White@hutton.ac.uk

34 Fangsen Xu: fangsenxu@mail.hzau.edu.cn

Abstract

Soil compaction due to mechanized farming operations is a recurrent issue affecting crop growth and yield. Yet, how soil compaction affects plant functions and ecological strategies is poorly known. With *Brassica napus*, i.e. a widespread crop species as study object, we aim to understand (i) how soil compaction impacts root and shoot traits related to the plant's well-being, nutrient acquisition of *Brassica napus* with different mechanical robustness, as well as their trade-offs, and (ii) how such impacts vary among different cultivars. To do this, we cultivated six cultivars of *Brassica napus* in non-compacted (control) and compacted (treatment) soils, respectively, in a sand culture system. After harvesting, a series of mechanical, morphological and chemical traits of roots and/or shoots were measured. Results showed that soil compaction significantly limited root penetration depth and root system establishment in morphological traits, leading further to significant reduction in nutrients acquisition and plant biomass accumulation. However, soil compaction significantly increases the average root diameter and root/shoot ratio, and facilitate more root exudates secretion (e.g. organic acids and polysaccharides) of *Brassica napus* cultivars. The *Brassica napus* cultivars with large root mechanical traits (e.g. root tensile force, root tensile strength and modulus of elasticity) had higher root cellulose and lignin concentrations and showed a stronger response in maximum root depth and specific root length compared with *Brassica napus* cultivars with small root mechanical traits in compacted treatment, which resulted in the greater fine root length and more root exudates secretion at root-soil interface. Furthermore, deep rooting enhanced nutrients acquisition and further biomass accumulation in compacted soil. Totally, the *Brassica napus* cultivars with large root mechanical traits with more fine roots and root exudates were critical for *Brassica napus* root penetration into a deep soil layer in compacted soil.

Keywords: *Brassica napus*; Soil compaction; Root mechanical traits; Fine roots; Root exudates

Introduction

In modern agricultural system, soil compaction is mainly sourced from the improper agricultural management, such as the use of heavy machinery, soil drying, long-term no-tillage and intensive agricultural production (Shah et al., 2017; Keller et al., 2019; Mirzavand and Moradi-Talebbeigi, 2021; Ferreira et al., 2021) and is a recurrent problem worldwide. Approximately 68 million hectares of the world's land is degraded due to compaction (Flowers and Lal, 1998; Hamza and Anderson, 2005). The yield loss due to soil compaction has been estimated up to 20 % (Barken et al., 1981) or even up to 50- 75% (Flowers and Lal, 1998; Hoque and Kobata, 2000; Wolkowski and Lowery, 2008). Soil compaction causes a degraded soil structure, which could decrease soil void space available for displacement of soil particles, increase penetrating cost for plant roots (Hamza and Anderson, 2005; Batey, 2009) and lead to low connectivity and continuity of the pore space to reduce water and air transport capability of soil (Kuncoro et al., 2014; Keller et al., 2017).

Roots are the first and most direct plant organ subjected to soil compaction and their multifunctionality, such as water and nutrient uptake and scavenging, and resistance to uprooting, can be potentially affected in cultivated lands. Soil compaction can modify root morphological traits through limiting maximum rooting depth and decreasing the size of root system, reducing root elongation rate, increasing radial growth and changing the amount of root branching (Tracy et al., 2012; Correa et al., 2019). Besides limited root growth, soil compaction significantly affects the shoot performance by nutrients deficiency (Lipiec and Stępniewski, 1995; Colombi and Keller 2019). Improving the adaptation ability of roots to soil compaction in deep soil layer will provide benefit for the plant establishment in shoot and root by enhancing the water and nutrients acquisition (Jin et al., 2015; Wang et al., 2019). Yet, the impact of soil compaction on roots' functions and their adaptive strategies still remains poorly understood.

Plasticity of roots to compacted soil has been studied previously mainly associated to morphological, biochemical and mechanical traits (Jin et al., 2017; Correa et al.,

2019; Vanhees et al., 2020; Bello-Bello et al., 2022), which included (1) a plant with thicker roots tends to enhance its axial force and radial expansion in penetrating through the compacted soil (Chen and Weil, 2010; Colombi et al., 2017). Meanwhile, fine roots with small diameter relative to the small pores distribution in compacted soil could promote roots to penetrate and elongate in the textural pores spaces where there were sufficient small pores in compacted soil (Fukao and Bailey-Serres, 2004; Bodner et al., 2014). (2) The induction of organic and inorganic compounds in the root-soil interface by soil compaction could serve as the lubricant to decrease the resistance source from the friction between root surface and soil particles (Bengough and McKenzie, 1997; Groleau-Renaud et al., 1998; Iijima et al., 2004; More et al., 2020), mainly through improving the soil compression characteristics to ease penetration and enhance the recovery of root induced soil compaction (Oleghe et al., 2107); (3) plants with stronger (i.e., greater root tensile force and strength) and stiffer roots (i.e. greater modulus of elasticity) have an enhanced penetration ability against the strongly-compacted soil layers (Clark et al., 2008; Chimungu et al., 2015; Lee et al., 2020). Root thickening at the root tips can interpret the root penetration outcomes by increasing the root axial force in increased soil strength (Whiteley et al., 1982; Clark et al., 2002; Hanbury and Atwell, 2005; Jin et al., 2013).

Root mechanical traits are key metrics in studying the plant anchorage and root penetration into soil (Chimungu et al., 2015). Root functions in compressive, buckling, twisting and/or bending behaviour are important in response to soil compaction and root setting (Bourrier et al., 2013; Mao et al., 2014; Schwarz et al., 2015; Johnson et al., 2016). The root mechanical traits mostly related to the tensile force, tensile strength, modulus of elasticity and tensile strain. Wide variations in root mechanical traits among species mainly depends on root size (Gray and Barker, 2004; Ghestem et al., 2014; Mao et al., 2018; Xu et al., 2021), root moisture contents (Yang et al., 2016; Zhang et al., 2019; Ekeoma et al., 2021), root types (Loades et al., 2015; Mao et al., 2023), root structure (Genet et al., 2005; Zhang et al., 2014; Zhu et al., 2020) and root anatomy (Chimungu et al., 2015; Schneider et al., 2021). For example, the

121　　roots with multiseriate cortical sclerenchyma have greater root lignin concentration

122　　and root bending strength, and greater root penetration depth in compacted soils

123　　(Schneider et al., 2021). The impact of the root mechanical traits on the root

124　　penetration ability needs to be explored, which will be helpful for revealing the

125　　potential adaptative mechanism of roots' function traits driven by root mechanical

126　　traits in reaction to compacted soil (Stokes et al., 2009).

127　　　　Oilseed rape (*Brassica napus* L.) is the most important edible oil crops and has

128　　abundant germplasm resources in China as well as in the world (Hu et al., 2017;

129　　Friedt et al., 2018; Li et al., 2020). Most of the cultivated soil in the main planting area

130　　of *Brassica napus* in Yangtze River basin of central China are poorly drained clay soils,

131　　and have poor soil pore system (Xi, 1998; Wang et al., 2021). In addition, the

132　　frequency of mechanized harvesting of *Brassica napus* has aggravated the soil

133　　compaction recently (Zhang et al., 2006; Wang et al., 2015; Correa et al., 2019). For

134　　example, the average soil bulk density at topsoil (5-10 cm) is about 1.38 g/cm^3, while

135　　the soil bulk density up to 1.52 g/cm^3 at plow pan (Ji et al., 2013), which are much

136　　higher than the ideal soil bulk density of 1.2-1.3 g/cm^3 (Li and Zhou 1994). Oilseed

137　　rape is sensitive to soil compaction (Blake et al., 2006) and the seed yield significantly

138　　decreased under high soil compaction stress (Alakukku and Elonen, 1995; Arvidsson

139　　and Håkansson, 2014; Bogunovic et al., 2018; Orzech et al., 2021). And the

140　　adaptability of different oilseed rape species to compacted soil depends on root

141　　characteristics, such as root morphology (Wang et al., 2021), root penetration depth

142　　(Peltonen-Sainio et al., 2011), root size (Chen and Weil, 2010; Zhang et al., 2022) and

143　　root type (Chen and Weil, 2010).

144　　　　In this study, we firstly determined the variation in root mechanical traits and its

145　　effect on root depth of six *Brassica napus*, and further investigated the root

146　　morphological and biochemical traits plasticity and their effects on nutrients

147　　acquisition and plant biomass accumulation of the *Brassica napus* cultivars grown in

148　　compacted soil. Thus, the objective of the study is 1) to clarify the responses of the six

149　　*Brassica napus* cultivars to soil compaction and 2) the root plasticity to soil

150   compaction for different *Brassica napus* cultivars.

2. Materials and methods

2.1 Plant materials and growth conditions

153   In this study, six *Brassica napus* cultivars were selected from an association panel
154   collected from major breeding centers across China (Liu et al., 2016), and among
155   them, *Brassica napus* cultivars of MJDT, A148 and R2 have large root mechanical
156   traits (F, T_r and E_r) and cultivars of NY7, 11-Y7-117 and 1368 have small root
157   mechanical traits (Table S1). A sand-culture system was used to investigate the
158   response of *Brassica napus* to soil compaction in this study, which allows mechanical
159   impedance to be varied independent of aeration and water status of the growing
160   medium (Coelho Filho et al., 2013; Jin et al., 2015). Rigid plastic tubes with 45 cm in
161   length and 15 cm in diameter were placed in tanks with nutrient solution on a base.
162   Each tank contains six tubes, and each tube contains one *Brassica napus* seedling
163   (Fig. S1A). The tubes were filled with mixed quartz sand (88.89% fine sand with 0.23
164   mm particle size on average, and 11.11% coarse sand with 0.69 mm particle size on
165   average) and adequate nutrient solution. Compaction is directly proportional to soil
166   bulk density (Popova et al., 2016). Low (1.30 g cm^{-3}) and high (1.60 g cm^{-3}) bulk
167   density were quantified by adding different masses of sand soil to the tubes with the
168   same volume, which represented non-compacted and compacted treatments,
169   respectively. The changes of penetration resistance along soil profile depth in
170   non-compacted and compacted treatments were shown in Fig. 1, respectively. The
171   penetration resistance was measured by a soil compaction meter (Field Scout SC900
172   soil compaction meter, Spectrum Technologies, Inc., IL, USA) in 2.5 cm increments
173   from soil surface to 30 cm depth with four repetitions. The total porosity and particle
174   density of sand were calculated at non-compacted and compacted treatments (Table
175   1 and Table S2). The soil water potential is basically similar between two bulk density
176   treatments in the well-watered sands. Each treatment for each cultivar has four
177   replications in this study.

7

178   Seeds were sterilized using 70% (v/v) ethanol and NaOCl (2.5% active chlorine),

179   and then placed on gauze with pure water containing 0.5 µM $CaCl_2$ for germination.

180   The germinated seeds with about 1.0 cm length primary root were transplanted and

181   grown in the center of sand core. *Brassica napus* seedlings were grown in an

182   illuminated growth chamber under 16-h-light/8-h-dark photoperiod (with a photo flux

183   density of 300- 320 µmol m^{-2} s^{-1} at plant height) and 60% relative humidity. The

184   modified Hoagland nutrient solution contains 5.0 mM $Ca(NO_3)_2 \cdot 4H_2O$, 5.0 mM KNO_3,

185   2.0 mM $MgSO_4 \cdot 7H_2O$, 1.0 mM KH_2PO_4, 50 µM Fe-EDTA, 50 µM H_3BO_3, 9.5 µM

186    $MnCl_2 \cdot 4H_2O$, 0.8 µM $ZnSO_4 \cdot 7H_2O$, 0.3 µM $CuSO_4 \cdot 5H_2O$ and 0.4 µM $Na_2MoO_4 \cdot 2H_2O$.

187   A total of 60 L nutrient solution was supplied in each tank.

188   *Brassica napus* plants were harvested at 35 d after transplanting when the

189   difference in the growth phenotypes were observed between plants grown in

190   non-compacted and compacted treatments. Firstly, we tested the variations of root

191   mechanical traits and maximum root depth of the six *Brassica napus* cultivars.

192   Secondly, the root morphological traits (total root length, coarse root length, fine root

193   length, root surface area, average root diameter and specific root length), root

194   biochemical traits (organic acid concentration, xylose concentration, glucose

195   concentration and uronic acid concentration), plant biomass parameters (root and

196   shoot dry weight, root/shoot ratio), and nitrogen (N), phosphorus (P) and potassium (K)

197   concentrations and contents in shoot and root of plants were determined.

198   **2.2 Measurement of maximum root depth**

199   At harvest time, the tube grown plants were pulled out carefully from the top of the

200   sand column, and then the sand was carefully removed from the tube bottom till the

201   root tips appeared (Fig. S1B). The maximum root depth was determined by the

202   vertical distance measured from the primary root base to the root tips by ruler with the

203   accuracy of 0.1 cm.

204   **2.3 Measurements of root mechanical traits and root cellulose and lignin**

205   **content**

206   The first-order lateral roots of more than 10 cm length of the plant were sampled to

207   test root mechanical properties. Root samples were firstly preserved in an alcohol

208   solution contained 15 % ethanol and then kept in a refrigerator at 4 °C (Bischetti et al.,

209   2003). Thirty to forty-five undamaged roots were used to test the root mechanical

210   properties with a universal testing machine (model5967, Instron® Corporation) that

211   was fitted with a 50-N load cell with an accuracy of 2 mN. Root segments were

212   manually clamped with two grips and further fixed with strips of sandpaper and 502

213   Super Waterglue to increase friction. Force of loaded was recorded during tensile

214   testing with extension at the constant rate of 5 mm min^{-1} (Giadrossich et al., 2017).

215   The diameters of root segment were gauged by vernier caliper with 0.02 mm accuracy.

216   Each root segment was measured three times, in the center of the root segment, to

217   the left and right of the center adhering to both grips, respectively. The average root

218   diameter was used to calculate the root cross-sectional area (Mao et al., 2018). The

219   root tensile force (F, N) and extension (ΔL, mm) were recorded until the root segment

220   was broken. The root tensile strength (T_r, MPa) was calculated as maximum force at

221   failure divided by root cross-sectional area. The root tensile strain (ε_r, %) was

222   calculated by dividing root extension by unstrained root length. The elasticity of

223   modulus (E_r, MPa) corresponds to the slope of the curve of stress-strain within the

224   quasi-liner elastic stage of a root in tension. The calculation method of elasticity of

225   modulus was referenced from the method by Mao et al. (2018). The crude cell wall of

226   roots was extracted by 95% ethanol and ethanol-hexane (1:2) separately and then

227   dried at 55 °C in oven. And the cellulose content was measured by the phenol–sulfuric

228   acid method based on the Masuko et al. (2005) and Nielsen (2010). Total lignin

229   content was measured by the acetyl bromide method in *Brassica napus* roots based

230   on the Iiyama and Wallis (1990).

231   **2.4 Measurements of root morphological and biochemical traits**

232   The collection method of root exudates was modified from Boeuf-Tremblay et al.

233   (1995) and Pearse et al. (2006, 2007). At harvest time, the plant was lifted carefully

234   from the plastic tube and bulk soil (sand) was shaken off from the root system

235   immediately, and the sand adhering to the roots was defined as rhizosphere soil.

236   Roots were then immersed into a 200 mL container with 40 mL 0.2 mM $CaCl_2$ solution

237   for 1-2 min to remove mostly rhizosphere soil. All extracts were poured into a 50 mL

238   centrifuge tube and then centrifugated using an Eppendrof 5810R centrifuge

239   (Eppendorf, Hamburg, Germany) at 3000 g for 15 minutes to discard root debris and

240   sloughed cells. The supernatant was freeze-dried and redissolved in 8 mL of distilled

241   water.

242   The extracts of separate 2 mL were used to quantify the glucose and xylose

243   concentrations by anthrone-sulfuric acid assay, respectively (Leyva et al., 2008). A

244   hydroxybiphenyl method was used to test the uronic acid concentration with 2 mL

245   suspension (Filisetti-Cozzi and Carpita, 1991). The rest of the 2 mL extract was used

246   to analyze carboxylates by a reversed phase high-performance liquid

247   chromatography (HPLC) system on an Agilent column (Agilent 1200, equipped with a

248   C18 250×4.6 mm ion-exclusion column, Alltima, America) (Wang et al., 2007; Li et al.,

249   2016).

250   After collection of root exudates, the roots were cleaned by flow water and then

251   scanned with a modified flatbed scanner (Epson V700, Nagano-ken, Japan). The total

252   root length, fine root length, coarse root length, root surface area and average root

253   diameter were analyzed by WinRHIZO software (Regent Instruments Inc., Quebec,

254   Canada).

255 **2.5 Plant biomass and nutrients analyses**

256   Shoots and roots were dried at 80 ºC for 3 days to test the root and shoot dry weight.

257   Then, the dried samples were ground to powder and digested with sulfuric acid and

258   hydrogen peroxide in a microwave oven. The N and P concentrations were

259   determined using a fully automated flow-injection system and colorimetry (Sullivan

260   and Havlin, 1991; Alves et al., 2000). The K concentration was determined by a flame

261   photometry (Gao et al., 2005).

262 **2.6 Statistical analyses**

263   The statistical analysis of the data was conducted by SPSS software (SPSS 19.0;

264   IBM Corporation, Armonk, NY, USA). Analysis of Variance (ANOVA) was used to

265   compare the effects of soil compaction on root morphological traits, root biochemical

266   traits, plant biomass and nutrients concentrations and contents in root and shoot

267   among six *Brassica napus* cultivars. Pearson's correlation analysis was used to test

268   the linear correlations among root morphological and root biochemical traits. Principal

269   component analysis (PCA) was used to evaluate the relationships among eleven root

270   traits (including root morphological traits and root biochemical traits) in

271   non-compacted and compacted treatments, respectively. The first three principal

272   components were used to describe the relationships among eleven root traits.

273   **3. Results**

274   **3.1 Root mechanical traits and maximum root depth of *Brassica napus* in**

275   **response to compacted soil**

276   The root tensile force (F) increased significantly with increasing diameter of six

277   *Brassica napus* cultivars regardless of the compaction treatment (non-compacted

278   versus compacted) (Fig. 2a, Fig. S2a and Table S3). The root tensile strength (T_r) and

279   elasticity of modulus (E_r) of *Brassica napus* cultivars decreased with increasing

280   diameter following a non-linear relationship (Fig. 2b, c, Fig. S2b, c and Table S3).

281   There was no significant difference in root mechanical traits (F, T_r and E_r) among six

282   *Brassica napus* cultivars in non-compacted treatment (Fig. S2). However, in the

283   compacted treatment, the three LRM cultivars (MJDT, A148 and R2) had significantly

284   larger root mechanical traits (F, T_r and E_r) than SRM cultivars (NY7, 11-Y7-117 and

285   1368) (Fig. 2). There was not significant difference among six *Brassica napus*

286   cultivars for root tensile strain (ε_r) whether in non-compacted or compacted treatments

287   (Fig. S3). And we also found higher cellulose and lignin concentration in roots of LRM

288   cultivars compared with SRM cultivars, especially in compacted treatment (Fig. S4).

289   The maximum root depth (MRD) of all the six cultivars of the compacted treatment

290   was 59.4% smaller than that of the non-compacted treatment (Fig. 3). In compacted

291   treatment, LRM cultivars had a significantly larger maximum root depth compared

292 with SRM cultivars (Fig. 3), while no significant difference between LRM and SRM

293 cultivars was found in non-compacted treatment (Fig. 3).

294 **3.2 Root morphological and biochemical traits of *Brassica napus* in response to**

295 **compacted soil**

296 The effects of soil compaction on root morphological and biochemical traits of

297 *Brassica napus* were significant at P <0.05 to 0.001 (Table 2). The genotypic

298 differences among six *Brassica napus* cultivars were also observed at P < 0.05 to

299 0.01 in total root length (TRL), root surface area (RSA), specific root length (SRL) and

300 fine root length (FRL). Significant differences were also observed in glucose

301 concentration (GC), xylose concentration (XC), uronic acid concentration (UAC) and

302 organic acid concentration (OAC) of root exudates at P <0.05 to 0.001 (Table 2). The

303 interaction effects between compaction treatment and cultivars were observed only in

304 RSA and average root diameter (ARD) (P <0.05) (Table 2).

305 The mechanical impedance significantly impeded root growth and elongation in

306 TRL, RSA, SRL, FRL and CRL, but increased ARD (Fig. 4). Compared with SRM

307 cultivars, LRM cultivars were 23.4% greater in TRL, 17.8% greater in RSA, 22.8%

308 greater in FRL, 6.8% greater in CRL and 23.9% greater in SRL, but 3.6% less in ARD

309 in compacted treatment (Fig. 4). The mechanical impedance also stimulates *Brassica*

310 *napus* roots to secrete more glucose, uronic acid, xylose and organic acid

311 components in the rhizosphere (Fig. 5 and Table S4). Compared with SRM cultivars,

312 LRM cultivars were 49.0% greater in glucose, 91.7% greater in xylose, 28.3% greater

313 in uronic acid and 47.6% greater in organic acid of root exudates (Fig. 5).

314 **3.3 Trade-offs among root-related traits in response to compacted soil**

315 The principal component analysis of the eleven root functional traits of six *Brassica*

316 *napus* cultivars in non-compacted treatment explained 77.2% of the variation in the

317 first three principal components, and the first component (PC1) represented 38.6% of

318 the variability and was dominated by TRL, CRL, SRL, RSA, OAC and XC; the second

319 component (PC2) represented 22.1% of the variability and was dominated by UAC

320 and GC; the third component (PC3) accounted for 16.4% of the variability and was

321 dominated by ARD and MRD (Fig. 6a and Table S5). And the LRM cultivar was

322 clustered in the direction of OAC (e.g. LRM1), the direction of GC, UAC and MRD (e.g.

323 LRM3) and the direction of RSA and TRL (e.g. LRM2). In compacted treatment, the

324 first three traits of the PCA accounted for 39.2%, 25.8% and 10.9% of the total

325 variation, respectively. The root morphological traits (such as TRL, CRL, FRL, MRD,

326 SRL, RSA) scored high in PC1, the root biochemical traits (such as UAC, OAC, GC

327 and XC) scored high in PC2, and the ARD and MRD scored high in PC3 (Fig. 6b and

328 Table S5). Cultivars LRM1 and LRM3 were clusters in the direction of MRD, and the

329 cultivar LRM2 was cluster in the direction of FRL and SRL (Fig. 6b).

330 **3.4 Correlations between maximum root depth and root morphological,**

331 **biochemical traits of *Brassica napus* in compacted soil**

332 Across six *Brassica napus* cultivars, the MRD and CRL had significant positive

333 correlation with ARD, and the FRL and RSA both had significant positive correlations

334 with CRL in non-compacted treatment ($P < 0.01$ to 0.001, Fig. S5). However, in

335 compacted treatment, MRD had significant negative correlation with ARD ($P < 0.05$,

336 Fig. 7a). In addition, MRD had significant positive correlation with FRL ($P < 0.05$, Fig.

337 7b), but had no significant correlation with CRL ($P = 0.44$) in compacted treatment (Fig.

338 7c). Meanwhile, SRL had significant positive correlation with glucose, xylose, uronic

339 acid and organic acid concentrations in compacted treatment (Fig. S6). The maximum

340 root depth also had significant correlation with organic acid, glucose, xylose and

341 uronic acid concentrations in compacted treatment (Fig. 8).

342 **3.5 Plant biomass and nutrients acquisition of *Brassica napus* in response to**

343 **soil compaction**

344 A significant reduction of shoot dry weight (SDW), root dry weight (RDW) and

345 nutrients (N, P and K) concentrations and contents of *Brassica napus* were observed

346 in compacted treatment (Fig. 9a, b, Fig. 10 and Fig. S7). However, the root/shoot ratio

347 significantly increased in compacted treatment compared with non-compacted

348 treatment in both LRM and SRM cultivars (Fig. 9c). The genotypic differences were

349 observed among six *Brassica napus* cultivars both in SDW and RDW ($P = 0.001$, Fig.

13

9), and also in N, P and K contents of root (P =0.001) and shoot (P =0.013 to < 0.001) in both non-compacted and compacted treatments (Fig. 10). Soil compaction had significant effects on the nutrient concentrations of N, P and K in shoot and root (P < 0.001, Fig. S7). There was no significant difference in shoot N, P and K concentrations among six cultivars, however, significant differences were found in root N and K concentrations among cultivars both in non-compacted and compacted treatments (Fig. S7). Compared with SRM cultivars, LRM cultivars had significantly larger SDW and RDW, and N, P and K contents in shoot and root both in compacted and non-compacted treatments (Fig. 9 and Fig. 10); and higher N, P and K concentrations in root in compacted treatment. There was no significant difference in shoot N, P and K concentrations whether in compacted or non-compacted treatment (Fig. S7).

4. Discussion

It is an important strategy to enhance the biological potential with superior root traits to break the limitation of soil compaction stress by plants (Alameda and Villar, 2012; Grzesiak et al., 2013; Correa et al., 2019). In this study, the *Brassica napus* cultivars with large root mechanical traits (LRM) had higher root lignin and cellulose concentrations and greater rooting depth compared with *Brassica napus* cultivars with small root mechanical traits (SRM) in compacted soil (Fig. 2, Fig. 3, Fig. 11 and Fig. S4). Additionally, the former had more fine roots accompanied by more root exudates in rhizosphere than the latter in compacted treatment. These result in more nutrients uptake and higher biomass accumulation in LRM cultivars than SRM cultivars (Fig. 4, Fig. 5, Fig. 9 and Fig. 10). The penetration of roots through the compacted soil promotes plant growth while increasing soil voids due to the large taproot system of *Brassica napus* (Kautz, 2015; Semwal et al., 2020). It is beneficial for the root establishment of staple crops along the soil pores in structural soil and adherence to more water and nutrients (Gao et al., 2012; Jin et al., 2013).

Deep root development in compacted soil is associated with the root traits modification, maximum root system establishment and elongation in vertical and

379 horizontal directions (Comas et al., 2013; Pérez-Ramos et al., 2013; Zwicke et al.,

380 2015; Wu et al., 2022). However, the soil properties significantly affect deep root

381 development, especially in the drought and high soil bulk density condition associated

382 to the soil compaction (Cairns et al., 2011; Correa et al., 2019). In this study, rooting

383 depth of *Brassica napus* in compacted treatment was 59.4% smaller than in

384 non-compacted treatment (Fig. 3). In addition, LRM cultivars had larger root

385 biomechanical properties parameters including tensile force, tensile strength and

386 modulus of elasticity, and had a deeper rooting growth compared with SRM (Fig. 3).

387 These are consistent with previous studies that stiffer roots with large tensile force,

388 tensile strength and modulus of elasticity are associated with greater rooting depth in

389 strong soil (Clark et al., 2008; Chimungu et al., 2015; Lee et al., 2020). Meanwhile,

390 high cellulose or lignin concentrations in cell wall are associated with larger root

391 tensile strength and modulus of elasticity values (Marga et al., 2003; Genet et al.,

392 2005; Zhang et al., 2014), especially for lignin, deposited in the walls of secondarily

393 thickened cells, making them rigid and impervious (Degenhardt and Gimmler, 2000;

394 Zhang et al., 2011). Lee et al. (2020) has reported tree species with large root tensile

395 strength and Young's modulus has deeper and more abundant root system. We

396 hypothesized that large root mechanical traits might be attributed to the stiff root cell

397 wall structure decided by the cell wall components (Fig. 3 and Fig. S5).

398   In this study, with the increasing root diameter, the root tensile force of *Brassica*

399 *napus* grows larger in both non-compacted and compacted treatments (Fig. 2 and Fig.

400 S3). Generally, compared with fine roots, coarse roots with a large axial force and

401 radial expansion could increase root penetration probability when roots encounter a

402 strong soil layer (Whiteley et al., 1982; Clark et al., 2008). However, our results

403 showed that the trade-offs of root morphological traits with more fine roots, rather than

404 coarse roots, had significant positive correlation with root penetration depth in

405 compacted treatment (Fig. 7). Fine roots had an important function in resistance to

406 compacted soil stress, which might be depended on that (1) the shorter and narrower

407 root caps benefit to increase axial force per root cross-sectional area and facilitate

408 penetration through the dense soil layer in mechanical impedance (Souty and Rode,

409 1987; Iijima et al., 2003) and that (2) the micro-pores proportion increased in

410 compacted soil with the decreasing total soil priority, and the fine roots had a strongly

411 adaptive response to the local constriction in micropores in compacted soil (Fig. 7, Fig.

412 11 and Table 1). Additionally, a larger fine root length was found in LRM cultivars than

413 SRM cultivars in compacted treatment (Fig. 4b). Specially, LRM cultivars tend to have

414 larger SRL and finer and larger MRD in compacted treatment compared with SRM

415 cultivars (Fig. 6). Thus, we suggest that greater fine roots with large root tensile

416 strength and modulus of elasticity facilitate roots to penetrate into the deep soil layer

417 in compacted soil, and increase the soil volume exploring and nutrients acquisition by

418 proliferating more roots per unit carbon investment (Fig. 3 and Fig. 4) (Ho et al., 2005;

419 Laliberté et al., 2015).

420 In our study, greater specific root length and larger rooting depth were also found in

421 LRM cultivars than SRM cultivars in compacted soil stress (Fig. 3 and Fig. 4e). In

422 maize, roots with larger specific root length stimulated by localized fertilizer

423 application had more fine roots proliferation, which could further facilitate the roots to

424 grow into the small pores and elongate into the deep soil layers in compacted soil (Wu

425 et al., 2022). In addition, under compacted soil stress, the organic acids secretion was

426 significantly induced (Ahmed et al., 2014; Oleghe et al., 2017) and the reduction of

427 soil pore size could limit soil solution movement and restrict proton diffusion, all

428 leading to the rhizosphere acidified (McNear, 2013). Low rhizosphere soil pH could

429 regulate root proliferation and cell wall mechanical properties to contribute to the root

430 proliferation (Bloom et al., 2002). This might be supported by our results that specific

431 root length had significant positive correlation with organic acids concentration under

432 compacted soil stress (Fig. S6b). Greater specific root length could be associated with

433 more organic acids secretion in the rhizosphere, and contributed to roots proliferation

434 in compacted soil.

435 Previous studies reported that root secreted mucilage from root tip could lubricate

436 roots to reduce friction as they penetrate through deeper soil layers (Bacic et al., 1986;

437   Read and Gregory, 1997). We found that root exudates polysaccharides and organic

438   acids had significant correlations with maximum root depth in compacted treatment

439   (Fig. 7). The increase of root exudates with more sugars and organic acids can

440   decrease the penetration resistance and increase compression index of soils, and

441   facilitate the roots to grow deeper in compacted soil (Ahmed et al., 2014). In addition,

442   organic acids of exudates can disperse soil structure and decrease soil hardness

443   (Naveed et al., 2017, 2018), and thus enhancing the roots to penetrate into the soil

444   layer (Jin et al., 2013). Although sugars secretion in the soil could offset this effect to

445   stabilize soil structure (Oades, 1984), the most important function of root exudates

446   with sugars-rich mucilage formed a soil sheath to envelope the roots, and relieve the

447   friction at root-soil interface and penetrate roots deep (Bengough and McKenzie, 1997;

448   Carter et al., 2019; Liu et al., 2019). Thus, we suggest that greater root exudates

449   stimulated by mechanical impedance could facilitate roots to penetrate and elongate

450   into the deep soil layer for LRM than SRM, which was achieved by lubricating the

451   passage of biopores in the process of roots elongation (Fig. 11, Hinsinger et al., 2009;

452   Oleghe et al., 2017), and the coordination of more fine roots with more root exudates

453   in rhizosphere for LRM cultivars had the positive function in root penetration and

454   elongation in compacted soil.

455   A significant higher N, P and K contents in root and shoot, and biomass in LRM

456   cultivars than SRM cultivars were observed in compacted soil (Fig. 9, Fig. 10 and Fig.

457   11). Deeper roots and greater root proliferation in compacted soil provide benefit for

458   roots resistance to resources stress distributed in the deep soil layer, such as N and

459   water uptake (Yu et al., 2015; Battisti and Sentelhas, 2017; Xie et al., 2021; Wu et al.,

460   2022). In this study, compared with SRM cultivars, LRM cultivars had a deeper root

461   growth and greater specific root lengths, which facilitates the roots to absorbe more N,

462   P and K in compacted soil (Fig. 10), and which might be achieved by (1) greater total

463   root length and root surface area of roots dealing with the soil compaction stress

464   driven by larger root biochemical properties parameters (De Baets et al., 2008;

465   Vergani et al., 2014); and (2) more nutrients mobilization by root secreting organic

acids into the rhizosphere that increased the bioavailability of nutrients (Ström et al., 2002; Gharu and Tarafdar, 2004; Carvalhais et al., 2011; Terzano et al., 2015) and (3) a deeper root system beneficial to the nutrients absorption from the tank by shortening the distance of mass flow between roots and nutrients (Lipiec and Stępniewski, 1995; Chapman et al., 2012).

5. Conclusion

Soil compaction limited root penetration depth and root system establishment, while facilitating root exudates secretion of *Brassica napus*. LRM cultivars had higher root penetration ability, greater fine roots and more exudates, more biomass accumulation and nutrients uptake than SRM cultivars in the compacted treatments. LRM cultivars could be planted in the agricultural soils where soil compaction increases due to the intensity of agricultural activities or the pressure of heavy farm machinery.

References

Ahmed, M.A., Kroener, E., Holz, M., Zarebanadkouki, M., Carminati, A., 2014. Mucilage exudation facilitates root water uptake in dry soils. Funct. Plant Biol. 41, 1129-1137.

Alakukku, L., Elonen, P., 1995. Long-term effects of a single compaction by heavy field traffic on yield and nitrogen uptake of annual crops. Soil Till. Res. 36, 141-152.

Alameda, D., Villar, R., 2012. Linking root traits to plant physiology and growth in Fraxinus angustifolia Vahl. seedlings under soil compaction conditions. Environ. Exp. Bot. 79, 49-57.

493   Alves, B.J.R., Zotarelli, L., Resende, A.S., Polidoro, J.C., Urquiaga, S., Boddey, R.M.,

494   2000. Rapid and sensitive determination of nitrate in plant tissue using flow

495   injection analysis. Commun. Soil Sci. Plant Anal. 31, 2739-2750.

496   Arvidsson, J., Håkansson, I., 2014. Response of different crops to soil

497   compaction—Short-term effects in Swedish field experiments. Soil Till. Res. 138,

498   56-63.

499   Bacic, A., Moody, S.F., Clarke, A.E., 1986. Structural analysis of secreted root slime

500   from maize (*Zea mays* L.). Plant Physiol. 80, 771-777.

501   Barken, L.R., Bosrresen, T., Njoss, A., 1981. Effect of soil compaction by tractor traffic

502   on soil structure, denitrification, and yield of wheat (*Triticum aestivum* L.). J. Soil

503   Sci. 38, 541– 552.

504   Batey, T., 2009. Soil compaction and soil management–a review. Soil Use Manage.

505   25, 335-345.

506   Battisti, R., Sentelhas, P.C., 2017. Improvement of soybean resilience to drought

507   through deep root system in Brazil. Agron. J. 109, 1612-1622.

508   Bello-Bello, E., López-Arredondo, D., Rico-Chambrón, T. Y., Herrera-Estrella, L., 2022.

509   Conquering compacted soils: uncovering the molecular components of root soil

510   penetration. Trends Plant Sci. 2022, S1360138522001054.

511   Bengough, A.G., McKenzie, B.M., 1997. Sloughing of root cap cells decreases the

512   frictional resistance to maize (*Zea mays* L.) root growth. J Exp. Bot. 48, 885-893.

513   Bischetti, G.B., Bonfanti, F., Greppi, M., 2003. Root tensile strength measurement:

514   testing device and protocol. Quaderni di Idronomia Montana 21, 349-360.

515   Blake, J., Spink, J., Bingham, I., 2006. Management of oilseed rape to balance root

516   and canopy growth. HGCA Rep. 402, 22.

517   Bloom, A.J., Meyerhoff, P.A., Taylor, A.R., Rost, T.L., 2002. Root development and

518   absorption of ammonium and nitrate from the rhizosphere. J. Plant Growth Regul.

519   21, 416-431.

520   Bodner, G., Leitner, D., Kaul, H.P., 2014. Coarse and fine root plants affect pore size

521   distributions differently. Plant Soil 380, 133-151.

522   Boeuf-Tremblay, V., Plantureux, S., Guckert, A., 1995. Influence of mechanical

523   impedance on root exudation of maize seedlings at two development stages.

524   Plant Soil 172, 79-287.

525 Bogunovic, I., Pereira, P., Kisic, I., Sajko, K., Sraka, M., 2018. Tillage management

526   impacts on soil compaction, erosion and crop yield in Stagnosols (Croatia).

527   Catena 160, 376-384.

528 Bourrier, F., Kneib, F., Chareyre, B., Fourcaud, T., 2013. Discrete modeling of granular

529   soils reinforcement by plant roots. Ecol. Eng. 61, 646-657.

530 Cairns, J.E., Impa, S.M., O'Toole, J.C., Jagadish, S.V.K., Price, A.H., 2011. Influence

531   of the soil physical environment on rice (*Oryza sativa* L.) response to drought

532   stress and its implications for drought research. Field Crops Res. 121, 303-310.

533 Carter, A.Y., Hawes, M.C., Ottman, M.J., 2019. Drought-tolerant barley: I. Field

534   observations of growth and development. Agronomy 9, 221.

535 Carvalhais, L.C., Dennis, P.G., Fedoseyenko, D., Hajirezaei, M.R., Borriss, R., von

536   Wirén, N., 2011. Root exudation of sugars, amino acids, and organic acids by

537   maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. J.

538   Plant Nutr. Soil Sci. 174, 3-11.

539 Chapman, N., Miller, A.J., Lindsey, K., Whalley, W.R., 2012. Roots, water, and nutrient

540   acquisition: let's get physical. Trends Plant Sci. 17, 701-710.

541 Chen, G., Weil, R.R., 2010. Penetration of cover crop roots through compacted soils.

542   Plant Soil 331, 31-43.

543 Chen, G.H., Weil, R.R., 2010. Penetration of cover crop roots through compacted

544   soils. Plant Soil 331, 31-43.

545 Chimungu, J.G., Loades, K.W., Lynch, J.P., 2015. Root anatomical phenes predict

546   root penetration ability and biomechanical properties in maize (*Zea mays*). J. Exp.

547   Bot. 66, 3151-3162.

548 Clark, L.J., Cope, R.E., Whalley, W.R., Barraclough, P.B., Wade, L.J., 2002. Root

549   penetration of strong soil in rainfed lowland rice: comparison of laboratory

550   screens with field performance. Field Crops Res. 76, 189-198.

551 Clark, L.J., Price, A.H., Steele, K.A., Whalley, W.R., 2008. Evidence from

552   near-isogenic lines that root penetration increases with root diameter and

553   bending stiffness in rice. Funct. Plant Biol. 35, 1163-1171.

554   Coelho Filho, M.A., Colebrook, E.H., Lloyd, D., Webster, C.P., Mooney, S.J., Phillips,

555   A.L., Hedden, P., Whalley, W.R., 2013. The involvement of gibberellin signalling

556   in the effect of soil resistance to root penetration on leaf elongation and tiller

557   number in wheat. Plant Soil 371, 81-94.

558   Colombi, T., Keller, T., 2019. Developing strategies to recover crop productivity after

559   soil compaction—A plant eco-physiological perspective. Soil Till. Res. 191,

560   156-161.

561   Colombi, T., Kirchgessner, N., Walter, A., Keller, T., 2017. Root tip shape governs root

562   elongation rate under increased soil strength. Plant Physiol. 174, 2289-2301.

563   Comas, L.H., Becker, S.R., Cruz, V.M.V., Byrne, P.F., Dierig, D.A., 2013. Root traits

564   contributing to plant productivity under drought. Front. Plant Sci. 4, 442.

565   Correa, J., Postma, J.A., Watt, M., Wojciechowski, T., 2019. Soil compaction and the

566   architectural plasticity of root systems. J. Exp. Bot. 70, 6019-6034.

567   De Baets, S., Poesen, J., Reubens, B., Wemans, K., De Baerdemaeker, J., Muys, B.,

568   2008. Root tensile strength and root distribution of typical Mediterranean plant

569   species and their contribution to soil shear strength. Plant Soil 305, 207-226.

570   Degenhardt, B., Gimmler, H. 2000. Cell wall adaptations to multiple environmental

571   stresses in maize roots. J. Exp. Bot. 51, 595-603.

572   Ekeoma, E.C., Boldrin, D., Loades, K.W., Bengough, A.G., 2021. Drying of fibrous

573   roots strengthens the negative power relation between biomechanical properties

574   and diameter. Plant Soil 469, 321-334.

575   Ferreira, C J.B., Tormena, C.A., Severiano, E.D.C., Zotarelli, L., Betioli Júnior, E.,

576   2021. Soil compaction influences soil physical quality and soybean yield under

577   long-term no-tillage. Arch. Agron. Soil Sci. 67, 383-396.

578   Filisetti-Cozzi, T.M., Carpita, N.C., 1991. Measurement of uronic acids without

579   interference from neutral sugars. Anal. Biochem. 197, 157-162.

580   Flowers, M.D., Lal, R., 1998. Axle load and tillage effects on soil physical properties

581   and soybean grain yield on a mollic ochraqualf in northwest Ohio. Soil Till. Res.

582   48, 21-35.

583 Friedt, W., Tu, J., Fu, T., 2018. Academic and economic importance of *Brassica napus*
584   rapeseed. In The *Brassica napus* genome. Springer, 1-20, Cham, Germany.

585 Fukao, T., Bailey-Serres, J., 2004. Plant responses to hypoxia–is survival a balancing
586   act?. Trends Plant Sci. 9, 449-456.

587 Gao, W., Watts, C.W., Ren, T., Whalley, W.R., 2012. The effects of compaction and
588   soil drying on penetrometer resistance. Soil Till. Res. 125, 14-22.

589 Gao, X., C. Ma, S. Du., 2005. Techniques on formula fertilization by soil testing.
590   Chinese Agriculture Press, 94-96, Beijing, China.

591 Genet, M., Stokes, A., Salin, F., Mickovski, S.B., Fourcaud, T., Dumail, J.F., Van Beek,
592   R., 2005. Plant Soil 278, 1-9.

593 Gharu, A., Tarafdar, J.C., 2004. Influence of organic acids on mobilization of inorganic
594   and organic phosphorus in soil. J. Indian Soc. Soil Sci. 52, 248-253.

595 Ghestem, M., Cao, K.F., Ma, W.Z., Rowe, N., Leclerc, R., Gadenne, C., Stokes, A.,
596   2014. A framework for identifying plant species to be used as 'ecological
597   engineers' for fixing soil on unstable slopes. Plos One 9. e95876.

598 Giadrossich, F., Schwarz, M., Cohen, D., Cislaghi, A., Vergani, C., Hubble, T., Phillips,
599   C., Stokes, A., 2017. Methods to measure the mechanical behaviour of tree roots:
600   a review. Ecol. Eng. 109, 256-271.

601 Gray, D.H., Barker, D., 2004. Root-soil mechanics and interactions. In: Bennett JJ,
602   Simon A (eds) Riparian vegetation and fluvial geomorphology. Water Science and
603   Application 8. American Geophyisical Union, New York, pp 113–123.

604 Groleau-Renaud, V., Plantureux, S., Guckert, A., 1998. Influence of plant morphology
605   on root exudation of maize subjected to mechanical impedance in hydroponic
606   conditions. Plant Soil 201, 231-239.

607 Grzesiak, S., Grzesiak, M. T., Hura, T., Marcińska, I., Rzepka, A., 2013. Changes in
608   root system structure, leaf water potential and gas exchange of maize and
609   triticale seedlings affected by soil compaction. Environ. Exp. Bot. 88, 2-10.

610 Hamza, M.A., Anderson, W.K., 2005. Soil compaction in cropping systems: A review
611   of the nature, causes and possible solutions. Soil Till. Res. 82, 121-145.

612 Hanbury, C.D., Atwell, B.J., 2005. Growth dynamics of mechanically impeded lupin

613   roots: does altered morphology induce hypoxia?. Ann. Bot. 96, 913-924.

614   Hinsinger, P., Bengough, A.G., Vetterlein, D., Young, I.M., 2009. Rhizosphere:

615   biophysics, biogeochemistry and ecological relevance. Plant Soil 321, 117-152.

616   Ho, M.D., Rosas, J.C., Brown, K.M., Lynch, J.P., 2005. Root architectural tradeoffs for

617   water and phosphorus acquisition. Funct. Plant Biol. 32, 737-748.

618   Hoorman, J.J., Sá, J.C.M., Reeder, R., 2011. The biology of soil compaction. Science

619   68, 49-57.

620   Hoque, M., Kobata, T., 2000. Effect of soil compaction on the grain yield of rice (*Oryza*

621   *sativa* L.) under water-deficit stress during the reproductive stage. Plant Prod. Sci.

622   3, 316–322.

623   Hu, Q., Hua, W., Yin, Y., Zhang, X.K., Liu, L.J., Shi, J.Q., Zhao, Y.G., Qin, L., Chen, C.,

624   Wang, H.Z., 2017. Rapeseed research and production in China. Crop J. 5, 127–

625   135.

626   Iijima, M., Barlow, P. W., Bengough, A.G., 2003. Root cap structure and cell

627   production rates of maize (*Zea mays*) roots in compacted sand. New Phytol. 160,

628   127-134.

629   Iijima, M., Higuchi, T., Barlow, P.W., 2004. Contribution of root cap mucilage and

630   presence of an intact root cap in maize (*Zea mays*) to the reduction of soil

631   mechanical impedance. Ann. Bot. 94, 473-477.

632   Iiyama, K., Wallis, A.F., 1990. Determination of lignin in herbaceous plants by an

633   improved acetyl bromide procedure. J. Sci. Food Agr. 51, 145-161.

634   Ji, B., Zhao, Y., Mu, X., Liu, K., Li, C., 2013. Effects of tillage on soil physical

635   properties and root growth of maize in loam and clay in central China. Plant Soil

636   Environ. 59, 295-302.

637   Jin, K., Shen, J., Ashton, R.W., Dodd, I.C., Parry, M.A., Whalley, W.R., 2013. How do

638   roots elongate in a structured soil?. J. Exp. Bot. 64, 4761-4777.

639   Jin, K., Shen, J., Ashton, R.W., White, R.P., Dodd, I.C., Phillips, A.L., Whalley, W.R.,

640   2015. The effect of impedance to root growth on plant architecture in wheat. Plant

641   Soil 392, 323-332.

Johnson, D.M., Wortemann, R., McCulloh, K.A., Jordan-Meille, L., Ward, E., Warren, J.M., Palmroth, S., Domec, J.C., 2016. A test of the hydraulic vulnerability segmentation hypothesis in angiosperm and conifer tree species. Tree Physiol. 36, 983-993.

Kautz, T., 2015. Research on subsoil biopores and their functions in organically managed soils: A review. Renew. Agr. Food Syst. 30, 318-327.

Keller, T., Colombi, T., Ruiz, S., Manalili, M. P., Rek, J., Stadelmann, V., Wunderli, H., Breitenstein, D., Reiser, R., Oberholzer, H.-R., Schymanski, S., Romero-Ruiz, A., Linde, N., Weisskopf, P., Walter, A., Or, D., 2017. Long-term soil structure observatory for monitoring post-compaction evolution of soil structure. Vadose Zone J. 16, 1-16.

Keller, T., Sandin, M., Colombi, T., Horn, R., Or, D., 2019. Historical increase in agricultural machinery weights enhanced soil stress levels and adversely affected soil functioning. Soil Till. Res. 194, 104293.

Kuncoro, P.H., Koga, K., Satta, N., Muto, Y., 2014. A study on the effect of compaction on transport properties of soil gas and water I: Relative gas diffusivity, air permeability, and saturated hydraulic conductivity. Soil Till. Res. 143, 172-179.

Laliberté, E., Lambers, H., Burgess, T.I., Wright, S.J., 2015. Phosphorus limitation, soil‐borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands. New Phytol. 206, 507-521.

Lee, J.T., Chu, M.Y., Lin, Y.S., Kung, K.N., Lin, W.C., Lee, M.J., 2020. Root traits and biomechanical properties of three tropical pioneer tree species for forest restoration in landslide areas. Forests 11, 179.

Leyva, A., Quintana, A., Sánchez, M., Rodríguez, E.N., Cremata, J., Sánchez, J.C., 2008. Rapid and sensitive anthrone–sulfuric acid assay in microplate format to quantify carbohydrate in biopharmaceutical products: method development and validation. Biologicals 36, 134-141.

Li, C., Zhou, S., 1994. Effects of soil bulk density on the growth of maize seeding stage. Acta Agriculturae Boreali-Sinica (In Chinese), 9, 49-54.

Li, H., Xia, H., Mei, Y., 2016. Modeling organic fouling of reverse osmosis membrane:

672   From adsorption to fouling layer formation. Desalination 386, 25-31.

673 Li, L.X., Chen, B.Y., Yan, G.X., Gao, G.Z., Xu, K., Xie, T., Zhang, F.G., 2020. Proposed

674   strategies and current progress of research and utilization of oilseed rape

675   germplasm in China. J. Plant Genet. Resour. 21, 1–19.

676 Lipiec, J., Stępniewski, W., 1995. Effects of soil compaction and tillage systems on

677   uptake and losses of nutrients. Soil Till. Res. 35, 37-52.

678 Liu, S., Fan, C.C., Li, J.N., Cai, G.Q., Yang, Q.Y., Wu, J., Yi, X.Q., Zhang, C.Y., Zhou,

679   Y.M., 2016. A genome-wide association study reveals novel elite allelic variations

680   in seed oil content of *Brassica napus*. Theor. Appl. Genet. 129, 1203-1215.

681 Liu, T.Y., Ye, N., Song, T., Cao, Y., Gao, B., Zhang, D., Zhu, F.Y., Chen, M., Zhang, Y.J.,

682   Xu, W.F., Zhang, J., 2019. Rhizosheath formation and involvement in foxtail millet

683   (*Setaria italica*) root growth under drought stress. J. Integr. Plant Biol. 61,

684   449-462.

685 Loades, K.W., Bengough, A.G., Bransby, M.F., Hallett, P.D., 2015. Effect of root age

686   on the biomechanics of seminal and nodal roots of barley (*Hordeum vulgare* L.) in

687   contrasting soil environments. Plant Soil 395, 253-261.

688 Mao, Z., Roumet, C., Rossi, L.M.W., Merino-Martin, L., Nespoulous, J., Taugourdeau,

689   O., Boukcim, H., Fourtier, S., Del Rey-Granado, M., Ramel, M., Ji, K., Zuo, J.,

690   Fromin, N., Stokes, A., Fort, F., 2023. Intra- and inter-specific variation in root

691   mechanical traits for twelve herbaceous plants and their link with the root

692   economics space. Oikos e09032.

693 Mao, Z., Wang, Y., McCormack, M. L., Rowe, N., Deng, X.B., Yang, X.D., Xia S.W.,

694   Nespoulous, J., Sidle, Roy.C., Guo, D.L., Stokes, A., 2018. Mechanical traits of

695   fine roots as a function of topology and anatomy. Ann. Bot. 122, 1103-1116.

696 Mao, Z., Yang, M., Bourrier, F., Fourcaud, T., 2014. Evaluation of root reinforcement

697   models using numerical modelling approaches. Plant Soil, 381, 249-270.

698 Marga, F., Gallo, A., Hasenstein, K.H., 2003. Cell wall components affect mechanical

699   properties: studies with thistle flowers. Plant Physiol. Biochem. 41, 792-797.

700 Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S. I., Lee, Y. C., 2005.

701   Carbohydrate analysis by a phenol–sulfuric acid method in microplate format.

702   Anal. Biochem. 339, 69-72.

703   McNear, D.H.Jr., 2013. The rhizosphere-roots, soil and everything in between. Nat.
704   Educ. Knowl. 4, 1.

705   Mirzavand, J., Moradi-Talebbeigi, R., 2021. Relationships between field management,
706   soil compaction, and crop productivity. Arch. Agron. Soil Sci. 67, 675-686.

707   Moradi, E., Rodrigo-Comino, J., Terol, E., Mora-Navarro, G., da Silva, A.M.,
708   Daliakopoulos, I.N., Khosravi, H., Fernandez, M.P., Cerda, A., 2020. Quantifying
709   soil compaction in persimmon orchards using ISUM (Improved Stock Unearthing
710   Method) and core sampling methods. Agriculture-Basel 10, 266.

711   More, S.S., Shinde, S.E., Kasture, M.C., 2020. Root exudates a key factor for soil and
712   plant: An overview. Pharma Innov. J. 8, 449-459.

713   Naveed, M., Brown, L.K., Raffan, A.C., George, T.S., Bengough, A.G., Roose, T.,
714   Sinclair, I., Koebernick, N., Cooper, L., Hackett, C.A., Hallett, P.D., 2017. Plant
715   exudates may stabilize or weaken soil depending on species, origin and time. Eur.
716   J. Soil Sci. 68, 806-816.

717   Naveed, M., Brown, L.K., Raffan, A.C., George, T.S., Bengough, A.G., Roose, T.,
718   Sinclair, I., Koebernick, N., Cooper, L., Hallett, P.D., 2018. Rhizosphere-scale
719   quantification of hydraulic and mechanical properties of soil impacted by root and
720   seed exudates. Vadose Zone J. 17.

721   Nielsen, S.S., 2010. Phenol-sulfuric acid method for total carbohydrates. In Food
722   analysis laboratory manual. Springer, 47-53, Boston, MA.

723   Oades, J.M., 1984. Soil organic matter and structural stability: mechanisms and
724   implications for management. Plant Soil 76, 319-337.

725   Oleghe, E., Naveed, M., Baggs, E.M., Hallett, P.D., 2017. Plant exudates improve the
726   mechanical conditions for root penetration through compacted soils. Plant Soil
727   421, 19-30.

728   Orzech, K., Wanic, M., Załuski, D., 2021. The Effects of Soil Compaction and Different
729   Tillage Systems on the Bulk Density and Moisture Content of Soil and the Yields
730   of Winter Oilseed Rape and Cereals. Agriculture 11, 666.

731 Pearse, S.J., Veneklaas, E.J., Cawthray, G., Bolland, M.D., Lambers, H., 2007.
732   Carboxylate composition of root exudates does not relate consistently to a crop
733   species' ability to use phosphorus from aluminium, iron or calcium phosphate
734   sources. New Phytol. 173, 181-190.

735 Pearse, S.J., Veneklaas, E.J., Cawthray, G., Bolland, M.D., Lambers, H., 2006.
736   *Triticum aestivum* shows a greater biomass response to a supply of aluminium
737   phosphate than Lupinus albus, despite releasing fewer carboxylates into the
738   rhizosphere. New Phytol. 169, 515-524.

739 Peltonen-Sainio, P., Jauhiainen, L., Laitinen, P., Salopelto, J., Saastamoinen, M.,
740   Hannukkala, A., 2011. Identifying difficulties in rapeseed root penetration in
741   farmers' fields in northern European conditions. Soil Use Manage. 27, 229-237.

742 Pérez-Ramos, I.M., Volaire, F., Fattet, M., Blanchard, A., Roumet, C., 2013. Tradeoffs
743   between functional strategies for resource-use and drought-survival in
744   Mediterranean rangeland species. Environ. Exp. Bot. 87, 126-136.

745 Popova, L., van Dusschoten, D., Nagel, K.A., Fiorani, F., Mazzolai, B., 2016. Plant
746   root tortuosity: an indicator of root path formation in soil with different composition
747   and density. Ann. Bot. 118, 685-698.

748 Read, D.B., Gregory, J.P., 1997. Surface tension and viscosity of axenic maize and
749   lupin root mucilages. New Phytol. 137, 623–628.

750 Schneider, H.M., Strock, C.F., Hanlon, M.T., Vanhees, D.J., Perkins, A.C., Ajmera, I.B.,
751   Sidhu, J.S., Mooney, S.J., Brown, K.M., Lynch, J.P., 2021. Multiseriate cortical
752   sclerenchyma enhance root penetration in compacted soils. P. Natl. Acad. Sci.
753   USA 118, e2012087118.

754 Schwarz, M., Rist, A., Cohen, D., Giadrossich, F., Egorov, P., Büttner, D., Stolz, B.M.,
755   Thormann, J. J., 2015. Root reinforcement of soils under compression. J. Geopys.
756   Res-Earth 120, 2103-2120.

757 Semwal, T., Mali, N., Masakapalli, S.K., Uday, K.V., 2020. Effect of plant roots on
758   permeability of soil, In geotechnical characterization and modelling: Proceedings
759   of IGC 2018. Springer, Singapore, pp. 343-352.

760 Shah, A.N., Tanveer, M., Shahzad, B., Yang, G., Fahad, S., Ali, S., Bukhari, M.A.,

761 Tung, S.A., Hafeez, A., Souliyanonh, B., 2017. Soil compaction effects on soil
762   health and cropproductivity: an overview. Environ. Sci. Pollut. Res. 24,
763   10056-10067.

764 Souty, N., Rode, C., 1987. Aspect mécanique de la croissance des racines. I.-Mesure
765   de la force de pénétration. Agronomie 7, 623-630.

766 Ström, L., Owen, A.G., Godbold, D.L., Jones, D.L., 2002. Organic acid mediated P
767   mobilization in the rhizosphere and uptake by maize roots. Soil Biol. Biochem. 34,
768   703-710.

769 Sullivan, D.M., Havlin, J.L., 1991. Flow injection analysis of urea nitrogen in soil
770   extracts. Soil Sci. Soc. Am. J. 55, 109-113.

771 Terzano, R., Cuccovillo, G., Gattullo, C.E., Medici, L., Tomasi, N., Pinton, R., Mimmo,
772   T., Cesco, S., 2015. Combined effect of organic acids and flavonoids on the
773   mobilization of major and trace elements from soil. Biol. Fertil. Soils 51, 685-695.

774 Tracy, S.R., Black, C.R., Roberts, J.A., Sturrock, C., Mairhofer, S., Craigon, J.,
775   Mooney, S.J., 2012. Quantifying the impact of soil compaction on root system
776   architecture in tomato (*Solanum lycopersicum*) by X-ray micro-computed
777   tomography. Ann. Bot. 110, 511-519.

778 Vanhees, D.J., Loades, K.W., Bengough, A.G., Mooney, S.J., Lynch, J.P., 2020. Root
779   anatomical traits contribute to deeper rooting of maize under compacted field
780   conditions. J. Exp. Bot. 71, 4243-4257.

781 Vergani, C., Schwarz, M., Cohen, D., Thormann, J.J., Bischetti, G.B., 2014. Effects of
782   root tensile force and diameter distribution variability on root reinforcement in the
783   Swiss and Italian Alps. Can. J. For. Res. 44, 1426-1440.

784 Wang, C.Y., Yan, Z.K., Wang, Z.K., Batool, M., El-Badri, A.M., Bai, F., Li, Z., Wang, B.,
785   Zhou, G.S., Kuai, J., 2021. Subsoil tillage promotes root and shoot growth of
786   rapeseed in paddy fields and dryland in Yangtze River Basin soils. Eur. J. Agron.
787   130, 126351.

788 Wang, H., Hao, J., Feng, R., Nan, Y., Yang, S., Nan, J., 2015. Microhole subsoiling
789   decreasing soil compaction, and improving yield and seed quality of cotton. Trans.
790   Chin. Soc. Agric. Eng. 31, 7-14.

791 Wang, M., Feng, W.Y., Shi, J.W., Zhang, F., Wang, B., Zhu, M.T., Li, B., Zhao, Y.L.,

792   Chai, Z.F., 2007. Development of a mild mercaptoethanol extraction method for

793   determination of mercury species in biological samples by P-replete LC-ICP-MS.

794   Talanta 71, 2034–2039.

795 Wang, M., He, D., Shen, F., Huang, J., Zhang, R., Liu, W., Zhou, Q., 2019. Effects of

796   soil compaction on plant growth, nutrient absorption, and root respiration in

797   soybean seedlings. Environ. Sci. Pollut. R. 26, 22835-22845.

798 Whiteley, G.M., Hewitt, J.S., Dexter, A.R., 1982. The buckling of plant roots. Physiol.

799   Plant. 54, 333-342.

800 Wolkowski, R., Lowery, B., 2008. Soil compaction: causes, concerns, and cures.

801   University of Wisconsin-Extension, Coop. Ext. A3367 Retrieved from

802   http://www.soils.wisc.edu/extension/pubs/A3367.pdf

803 Wu, X., Li, H., Rengel, Z., Whalley, W. R., Li, H., Zhang, F., Shen, J., Jin, K., 2022.

804   Localized nutrient supply can facilitate root proliferation and increase

805   nitrogen-use efficiency in compacted soil. Soil Till. Res. 215, 105198.

806 Xi, C.F., 1998. Soils of China (in Chinese). Chinese Agriculture Press, 162-174,

807   Beijing, China.

808 Xie, Y., Islam, S., Legesse, H. T., Kristensen, H.L., 2021. Deep root uptake of

809   leachable nitrogen in two soil types is reduced by high availability of soil nitrogen

810   in fodder radish grown as catch crop. Plant Soil 1-15.

811 Xu, H., Wang, X.Y., Liu, C.N., Chen, J.N., Zhang, C., 2021. A 3D root system

812   morphological and mechanical model based on L-Systems and its application to

813   estimate the shear strength of root-soil composites. Soil Till Res 212, 105074.

814 Yang, Y.J., Chen, L.H., Li, N., Zhang, Q.F., 2016. Effect of root moisture content and

815   diameter on root tensile properties. Plos One 11, e0151791.

816 Yu, P., Li, X., White, P.J., Li, C., 2015. A large and deep root system underlies high

817   nitrogen-use efficiency in maize production. Plos One 10, e0126293.

818 Zhang, C. B., Chen, L. H., Jiang, J., 2014. Why fine tree roots are stronger than

819   thicker roots: The role of cellulose and lignin in relation to slope stability.

820   Geomorphology 206, 196-202.

821   Zhang, C.B., Zhou, X., Jiang, J., Wei, Y., Ma, J.J., Hallett, P.D., 2019. Root moisture
822   content influence on root tensile tests of herbaceous plants. Catena 172,
823   140-147.
824   Zhang, S., Grip, H., Lövdahl, L., 2006. Effect of soil compaction on hydraulic
825   properties of two loess soils in China. Soil Till. Res. 90, 117-125.
826   Zhang, Y., Culhaoglu, T., Pollet, B., Melin, C., Denoue, D., Barriere, Y., Méchin, V.,
827   2011. Impact of lignin structure and cell wall reticulation on maize cell wall
828   degradability. J. Agric. Food Chem. 59, 10129-10135.
829   Zhang, Z.B., Yan, L., Wang, Y.K., Ruan, R.J., Xiong, P., Peng, X.H., 2022. Bio-tillage
830   improves soil physical properties and maize growth in a compacted Vertisol by
831   cover crops. Soil Sci. Soc. Am. J. 86, 324-337.
832   Zhu, J.Q., Wang, Y.Q., Wang, Y.J., Mao, Z., Langendoen, E.J., 2020. How does root
833   biodegradation after plant felling change root reinforcement to soil? Plant Soil
834   446, 211-227.
835   Zwicke, M., Picon-Cochard, C., Morvan-Bertrand, A., Prud'homme, M.P., Volaire, F.,
836   2015. What functional strategies drive drought survival and recovery of perennial
837   species from upland grassland? Ann. Bot. 116, 1001–1015.

838   Figure Captions

839   **Fig. 1.** Penetration resistance at different soil profile depth in non-compacted (a) and

840   compacted (b) treatment, respectively.

841   **Fig. 2.** Correlations between root tensile force (a), root tensile strength (b), modulus of

842   elasticity (c) and root diameter of LRM or SRM cultivars in compacted treatment. LRM,

843   *Brassica napus* cultivars with large root mechanical traits; SRM, *Brassica napus*

844   cultivars with small root mechanical traits.

845   **Fig. 3**. Maximum root depth of LRM and SRM cultivars in non-compacted (NC) and

846   compacted (C) treatments. LRM, *Brassica napus* cultivars with large root mechanical

847   traits; SRM, *Brassica napus* cultivars with small root mechanical traits. The different

848   small letters above the column indicate significant difference among four treatments at

849   P <0.05.

850   **Fig. 4.** Root morphological traits of LRM and SRM cultivars in non-compacted (NC)

851   and compacted (C) treatments. Total root length (a), fine root length (b), coarse root

852   length (c), root surface area (d), specific root length (e) and average root diameter (f).

853   LRM, *Brassica napus* cultivars with large root mechanical traits; SRM, *Brassica napus*

854   cultivars with small root mechanical traits. The different small letters above the column

855   indicate significant difference among four treatments at P <0.05.

856   **Fig. 5.** The concentrations of glucose (a), organic acids (b), uronic acid (c) and xylose

857   (d) of root exudates of LRM and SRM cultivars in non-compacted (NC) and

858   compacted (C) treatments. LRM, *Brassica napus* cultivars with large root mechanical

859   traits; SRM, *Brassica napus* cultivars with small root mechanical traits. The different

860   small letters above the column indicate significant difference among four treatments at

861   P <0.05.

862   **Fig. 6.** Principal component analysis of root morphological traits (TRL, CRL, FRL,

863   RSA, SRL, ARD and MRD) and root biochemical traits (UAC, GC, XC and OAC) of six

864   *Brassica napus* cultivars (LRM1, LRM2, LRM3, SRM1, SRM2 and SRM3) in

865   non-compacted (a) and compacted (b) treatments, respectively. TRL, total root length;

866   CRL, coarse root length; FRL, fine root length; RSA, root surface area; SRL, specific

867   root length; ARD, average root diameter; MRD, maximum root depth; GC, glucose

868 concentration; XC, xylose concentration; UAC, uronic acid concentration; OAC,

869 organic acid concentration; LRM, *Brassica napus* cultivars with large root mechanical

870 traits; SRM, *Brassica napus* cultivars with small root mechanical traits.

871 **Fig. 7.** Correlations between maximum root depth and average root diameter (a), fine

872 root length (b) and coarse root length (c) of six *Brassica napus* cultivars in compacted

873 treatment. The shaded areas indicate the 95% confidence range, derived from the

874 models.

875 **Fig. 8.** Correlations between maximum root depth and organic acids (a), glucose (b),

876 uronic acid (c) and xylose (d) concentrations of six *Brassica napus* cultivars in both

877 non-compacted (NC) and compacted (C) treatments. The shaded areas indicate the

878 95% confidence range, derived from the models. *P <0.05, **P <0.01.

879 **Fig. 9.** Shoot dry weight (a), root dry weight (b) and root/shoot ratio (c) of LRM and

880 SRM cultivars in non-compacted (NC) and compacted (C) treatments. LRM, *Brassica*

881 *napus* cultivars with large root mechanical traits; SRM, *Brassica napus* cultivars with

882 small root mechanical traits. The different small letters above the column indicate

883 significant difference among four treatments at P <0.05.

884 **Fig. 10.** The contents of nitrogen (N), phosphorus (P) and potassium (K) in shoot (a-c)

885 and root (d-f) of LRM and SRM cultivars in non-compacted (NC) and compacted (C)

886 treatments. LRM, *Brassica napus* cultivars with large root mechanical traits; SRM,

887 *Brassica napus* cultivars with small root mechanical traits. The different small letters

888 above the column indicate significant difference among four treatments at P <0.05.

889 **Fig. 11.** A proposed diagram on how root traits (especially fine roots and root

890 exudates) of *Brassica napus* contribute to root penetration through the compacted soil.

891 LRM, *Brassica napus* cultivars with large root mechanical traits; SRM, *Brassica napus*

892 cultivars with small root mechanical traits.

893

894 Table Captions

895 **Table 1** Soil bulk density and total porosity of soil in non-compacted and compacted

896 treatments.

897 **Table 2** The effects of soil compaction on the root morphological and biochemical

898 traits of six *Brassica napus* cultivars.

899

900 Supplementary information

901 **Supplementary Fig. S1**. A schematic representation of the root growth system (a)

902 and root collection system (b).

903 **Supplementary Fig. S2.** Correlation between root tensile force (a), tensile strength (b)

904 and modulus of elasticity (c) and root diameter of LRM and SRM cultivars in

905 non-compacted treatment. LRM, *Brassica napus* cultivars with large root mechanical

906 traits; SRM, *Brassica napus* cultivars with small root mechanical traits.

907 **Supplementary Fig. S3.** Correlations between root tensile strain and root diameter of

908 LRM and SRM cultivars in non-compacted (a) and compacted (b) treatments. LRM,

909 *Brassica napus* cultivars with large root mechanical traits; SRM, *Brassica napus*

910 cultivars with small root mechanical traits.

911 **Supplementary Fig. S4.** Root cellulose (a) and lignin (b) concentrations of *Brassica*

912 *napus* cultivars with LRM and SRM in non-compacted (NC) and compacted (C)

913 treatments. LRM, *Brassica napus* cultivars with large root mechanical traits; SRM,

914 *Brassica napus* cultivars with small root mechanical traits.

915 **Supplementary Fig. S5.** Correlations between maximum root depth (a), coarse root

916 length (b) and average root diameter, and correlations between fine root length (c),

917 root surface area (d) and coarse root length of six *Brassica napus* cultivars in

918 non-compacted treatment.

919 **Supplementary Fig. S6.** Correlations between glucose (a), organic acid (b), uronic

920 acid (c) and xylose (d) concentrations and specific root length of six *Brassica napus*

921 cultivars in compacted treatments. The shaded areas indicate the 95% confidence

922 range, derived from the models. *P <0.05, **P< 0.01.

923 **Supplementary Fig. S7.** The concentrations of nitrogen (N), phosphorus (P) and

924 potassium (K) in shoot (a-c) and root (d-e) of LRM and SRM cultivars in

925 non-compacted (NC) and compacted (C) treatments. LRM, *Brassica napus* cultivars

926 with large root mechanical traits; SRM, *Brassica napus* cultivars with small root

927 mechanical traits. The different small letters above the column indicate significant

928 difference among four treatments at P <0.05.

929 **Table S1** *Brassica napus* cultivars used in this study.

930 **Table S2** Particle density of sand in non-compacted and compacted treatments in this

931 study.

932 **Table S3** Fitting equations of root tensile force, root tensile strength and modulus of

933 elasticity with root diameter of LRM and SRM cultivars in non-compacted and

934 compacted treatments, respectively.

935 **Table S4** The compositions and amounts of organic acids in the rhizosphere of six

936 *Brassica napus* cultivars in non-compacted and compacted treatments.

937 **Table S5** The loading scores of eleven root-related traits in the principal component

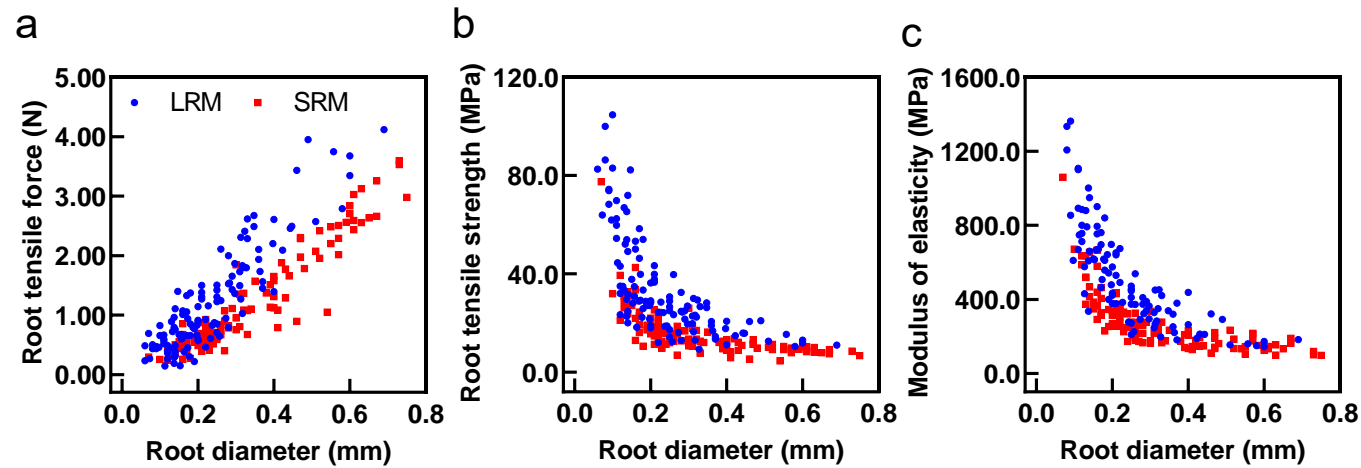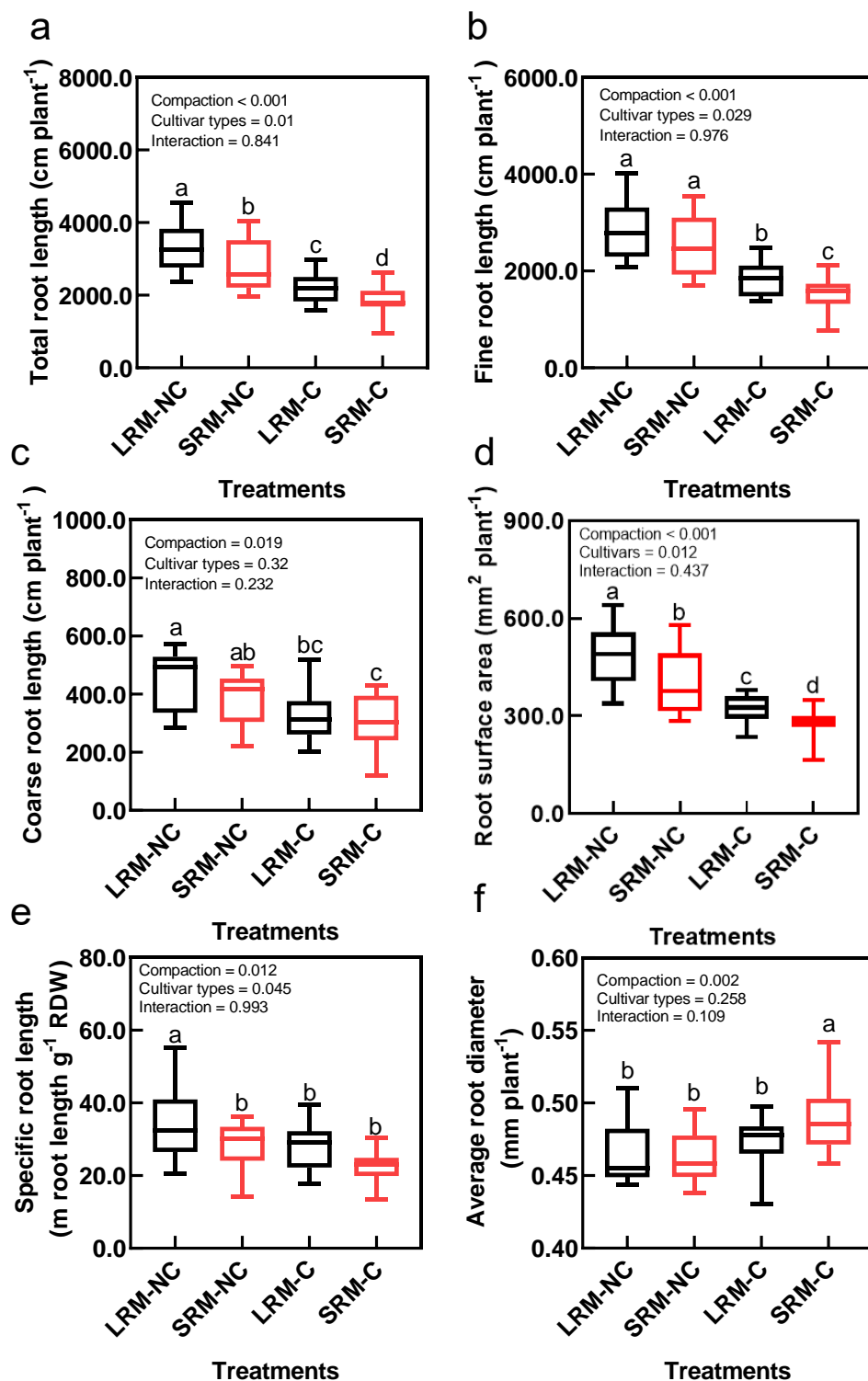938 analysis among six *Brassica napus* cultivars.

a

Soil compaction (MPa)

Non-compacted treatment

b

Soil compaction (MPa)

Compacted treatment

939

940 Fig. 1. Penetration resistance at different soil profile depth in non-compacted (a) and

941 compacted (b) treatment, respectively.

942

Fig. 2. Correlations between root tensile force (a), root tensile strength (b), modulus of elasticity (c) and root diameter of LRM or SRM

cultivars in compacted treatment. LRM, *Brassica napus* cultivars with large root mechanical traits; SRM, *Brassica napus* cultivars with

small root mechanical traits.

946

Fig. 4. Root morphological traits of LRM and SRM cultivars in non-compacted (NC)

and compacted (C) treatments. Total root length (a), fine root length (b), coarse root

length (c), root surface area (d), specific root length (e) and average root diameter (f).

LRM, *Brassica napus* cultivars with large root mechanical traits; SRM, *Brassica napus*

951 cultivars with small root mechanical traits. The different small letters above the column

952 indicate significant difference among four treatments at P <0.05.
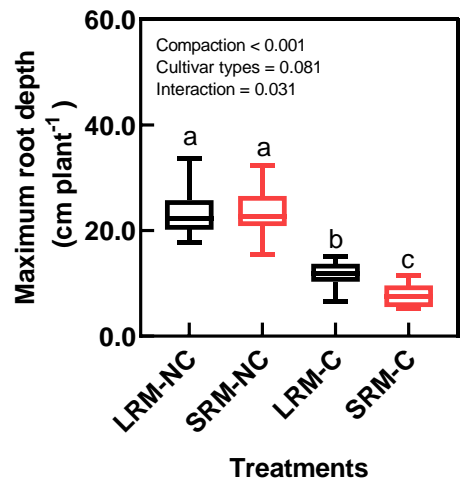

953

954 Fig. 3. Maximum root depth of LRM and SRM cultivars in non-compacted (NC) and

955 compacted (C) treatments. LRM, *Brassica napus* cultivars with large root mechanical

956 traits; SRM, *Brassica napus* cultivars with small root mechanical traits. The different

957 small letters above the column indicate significant difference among four treatments at
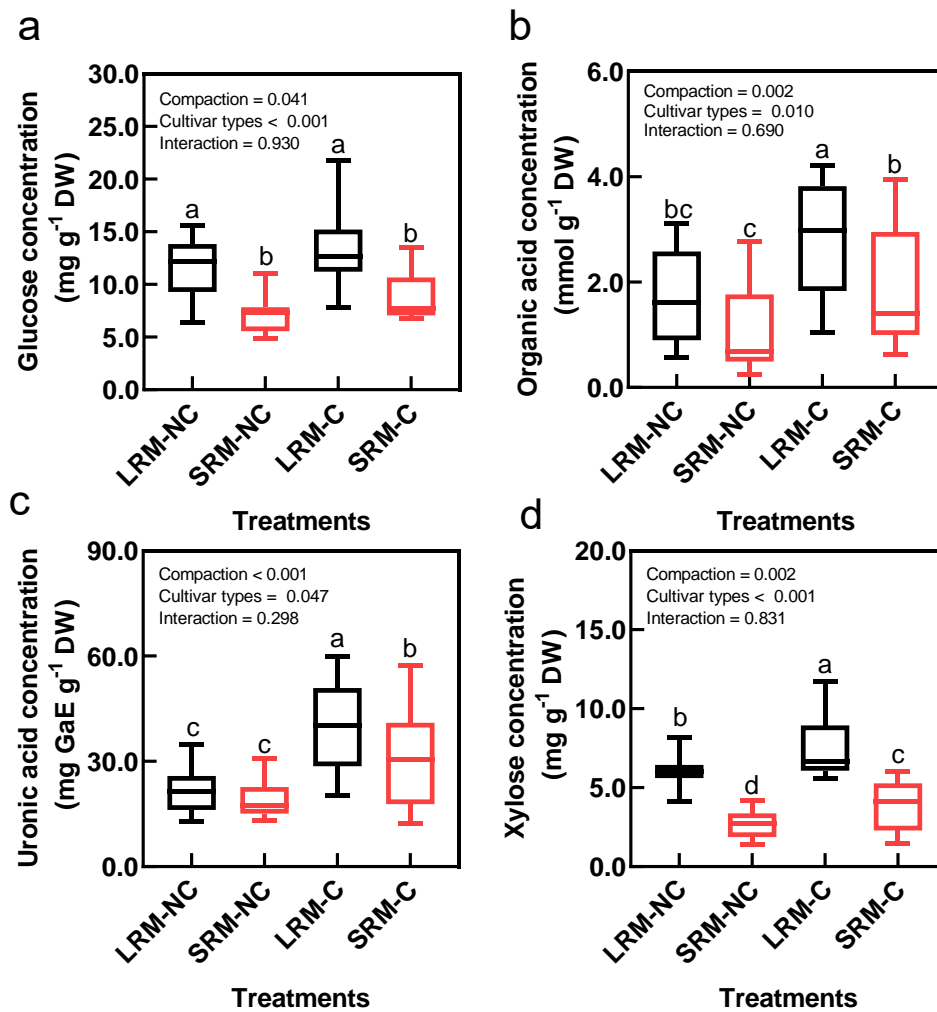
958 *P* <0.05.

959

960

Fig. 5. The concentrations of glucose (a), organic acids (b), uronic acid (c) and xylose (d) of root exudates of LRM and SRM cultivars in non-compacted (NC) and compacted (C) treatments. LRM, *Brassica napus* cultivars with large root mechanical traits; SRM, *Brassica napus* cultivars with small root mechanical traits. The different small letters above the column indicate significant difference among four treatments at *P* <0.05.
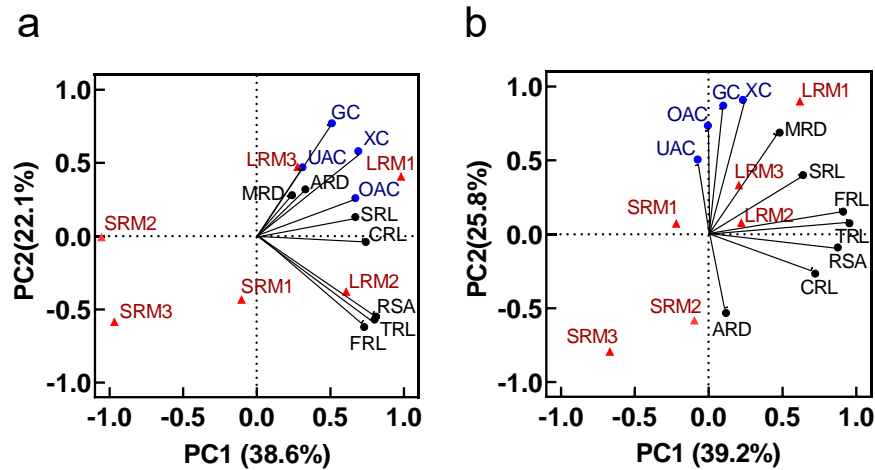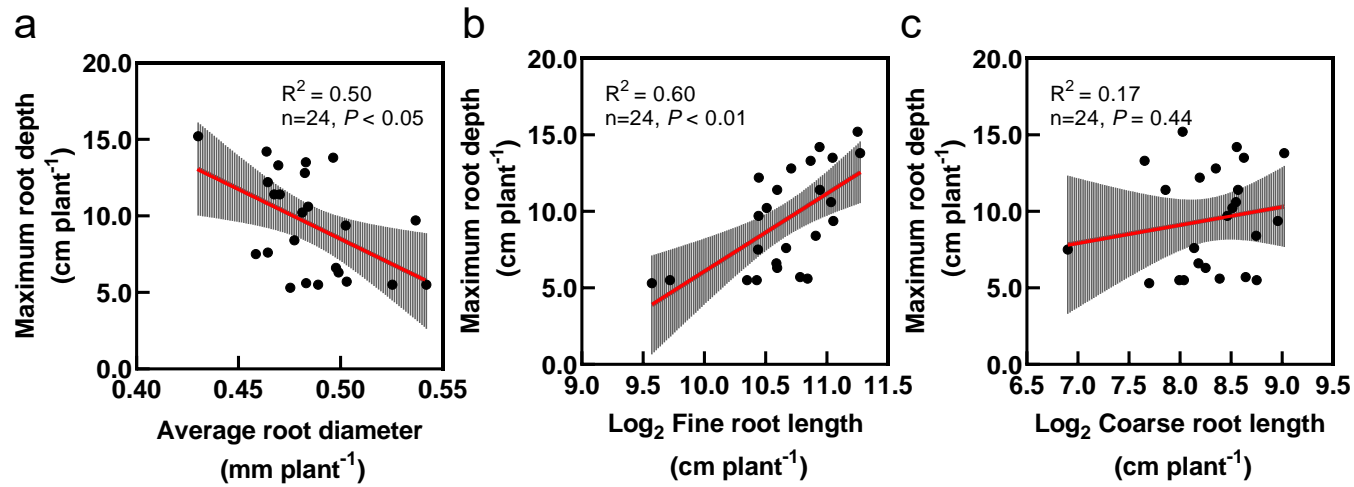
968

Fig. 6. Principal component analysis of root morphological traits (TRL, CRL, FRL, RSA, SRL, ARD and MRD) and root biochemical traits (UAC, GC, XC and OAC) of six *Brassica napus* cultivars (LRM1, LRM2, LRM3, SRM1, SRM2 and SRM3) in non-compacted (a) and compacted (b) treatments, respectively. TRL, total root length; CRL, coarse root length; FRL, fine root length; RSA, root surface area; SRL, specific root length; ARD, average root diameter; MRD, maximum root depth; GC, glucose concentration; XC, xylose concentration; UAC, uronic acid concentration; OAC, organic acid concentration; LRM, *Brassica napus* cultivars with large root mechanical traits; SRM, *Brassica napus* cultivars with small root mechanical traits.

978

979   Fig. 7. Correlations between maximum root depth and average root diameter (a), fine root length (b) and coarse root length (c) of six *Brassica*

980   *napus* cultivars in compacted treatment. The shaded areas indicate the 95% confidence range, derived from the models
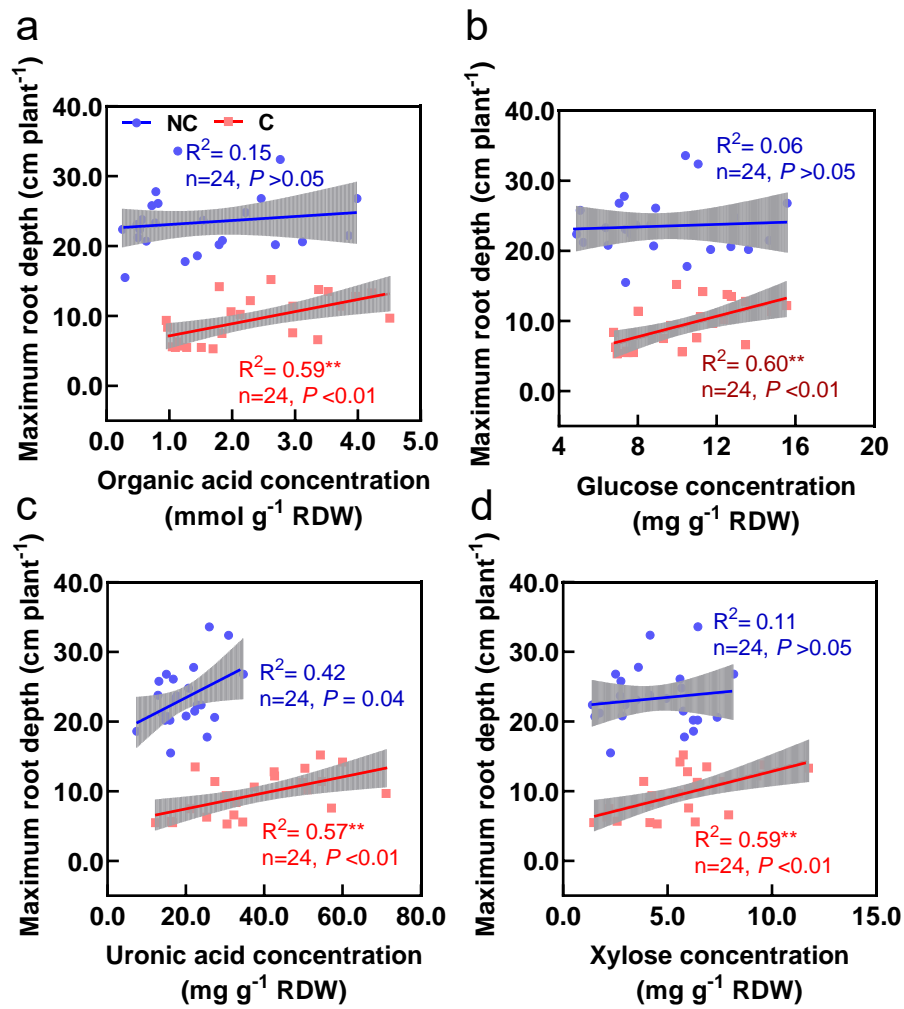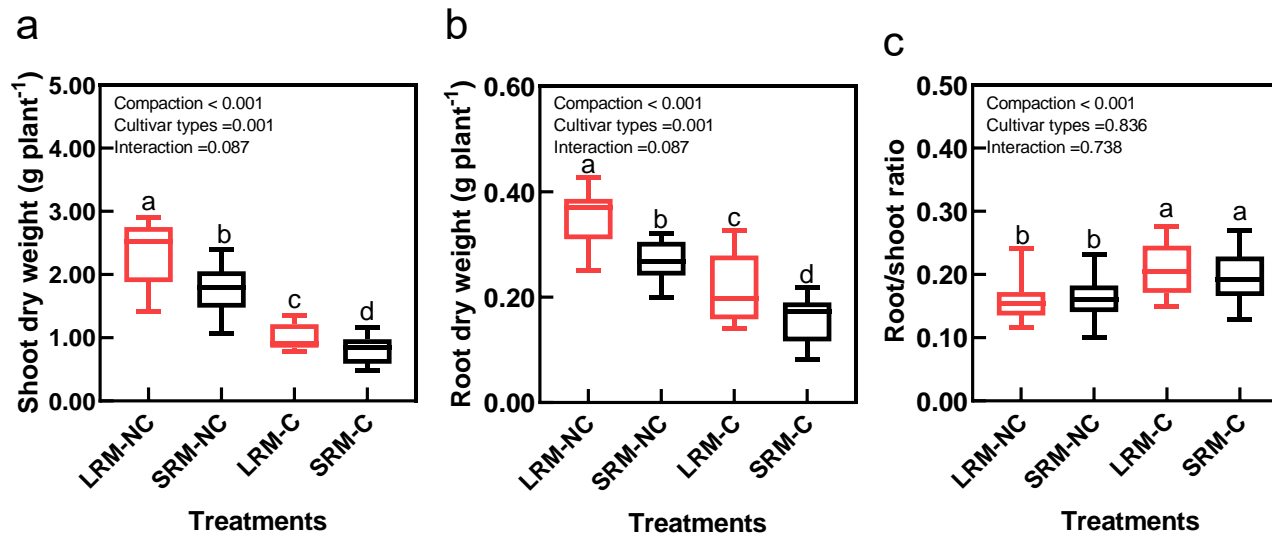
Fig. 8. Correlations between maximum root depth and organic acids (a), glucose (b), uronic acid (c) and xylose (d) concentrations of six *Brassica napus* cultivars in both non-compacted (NC) and compacted (C) treatments. The shaded areas indicate the 95% confidence range, derived from the models. $^*P$ <0.05, $^{**}P$< 0.01.

a

Shoot dry weight (g plant^{-1})

Compaction < 0.001
Cultivar types =0.001
Interaction =0.087

b

Root dry weight (g plant^{-1})

Compaction < 0.001
Cultivar types =0.001
Interaction =0.087

c

Root/shoot ratio

Compaction < 0.001
Cultivar types =0.836
Interaction =0.738

Treatments

986

Fig. 9. Shoot dry weight (a), root dry weight (b) and root/shoot ratio (c) of LRM and SRM cultivars in non-compacted (NC) and compacted (C)

treatments. LRM, *Brassica napus* cultivars with large root mechanical traits; SRM, *Brassica napus* cultivars with small root mechanical traits. The

different small letters above the column indicate significant difference among four treatments at *P* <0.05.
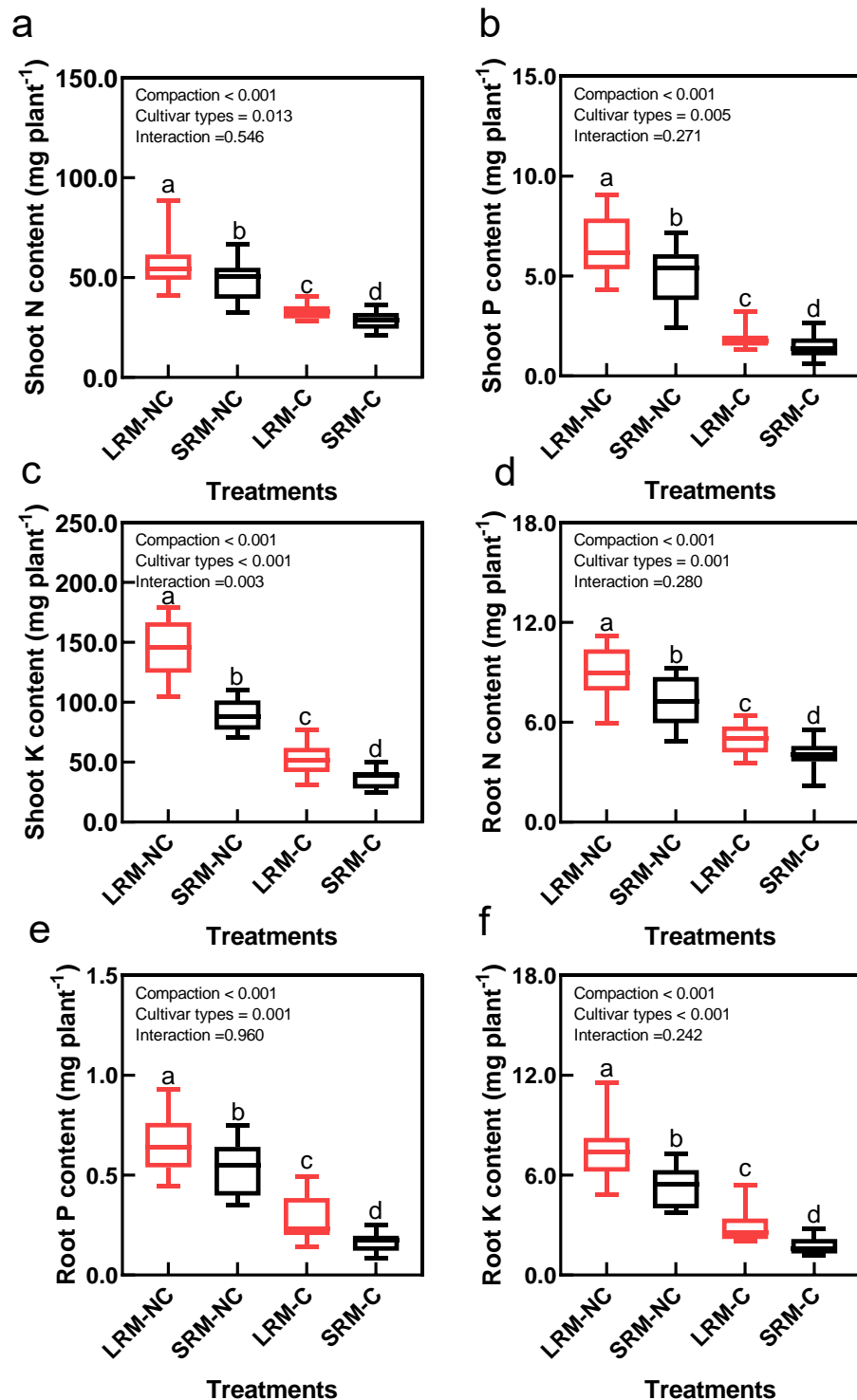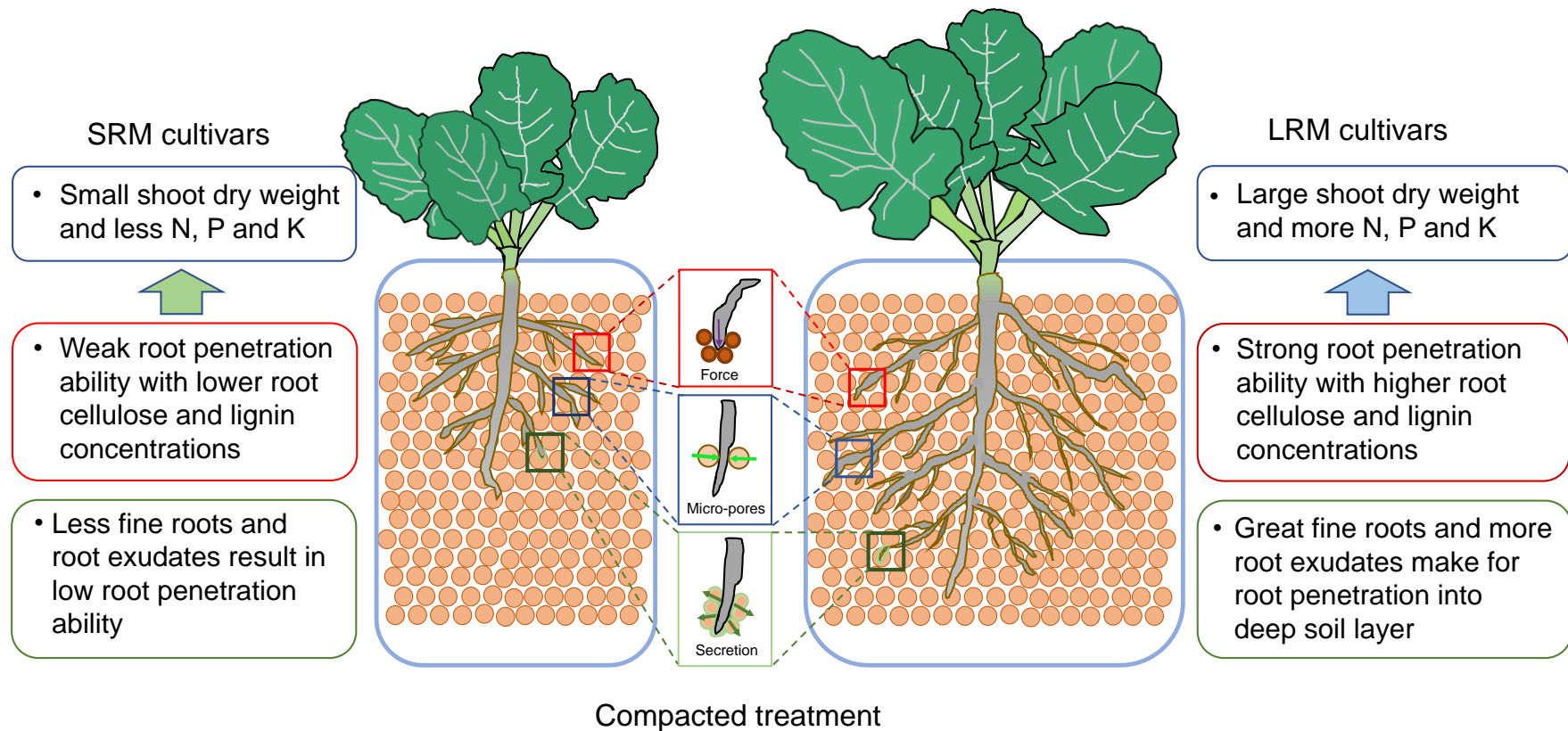
990

Fig. 10. The contents of nitrogen (N), phosphorus (P) and potassium (K) in shoot (a-c)

and root (d-f) of LRM and SRM cultivars in non-compacted (NC) and compacted (C)

treatments. LRM, *Brassica napus* cultivars with large root mechanical traits; SRM,

Brassica napus cultivars with small root mechanical traits. The different small letters

above the column indicate significant difference among four treatments at *P* <0.05.

SRM cultivars

- Small shoot dry weight and less N, P and K

- Weak root penetration ability with lower root cellulose and lignin concentrations

- Less fine roots and root exudates result in low root penetration ability

Force

Micro-pores

Secretion

LRM cultivars

- Large shoot dry weight and more N, P and K

- Strong root penetration ability with higher root cellulose and lignin concentrations

- Great fine roots and more root exudates make for root penetration into deep soil layer

Compacted treatment

996

997 Fig. 11. A proposed diagram on how root traits (especially fine roots and root exudates) of *Brassica napus* contribute to root penetration

998 through the compacted soil. LRM: *Brassica napus* cultivars with large root mechanical traits, SRM: *Brassica napus* cultivars with small

999 root mechanical traits.

1000   Table 1 Soil bulk density and total porosity of soil in non-compacted and compacted

1001   treatments.

Treatment	Soil bulk density (g cm^{-3})	Total porosity of soil (%)[1]
Non-compacted treatment	1.30	51.33
Compacted treatment	1.60	40.10

1002   Note: [1] The total porosity of soil (%) was calculated with the formula by Hao et al.

1003   (2008).

Table 2 The effects of soil compaction on the root morphological and biochemical traits of six *Brassica napus* cultivars.

Effect	Root morphological traits							Root biochemical traits			
	TRL (cm plant^{-1})	RSA (cm^2 plant^{-1})	ARD (mm plant^{-1})	SRL (m root length g^{-1} RDW)	CRL (cm plant^{-1})	FRL (cm plant^{-1})	MRD (cm plant^{-1})	GC (mg g^{-1} RDW)	XC (mg g^{-1} RDW)	UAC (mg g^{-1} RDW)	OAC (mmol g^{-1} RDW)
Compaction	38.13***	40.56***	16.67***	7.79***	5.90*	41.95***	167.02***	10.57**	10.72***	25.93***	10.37**
Cultivars	7.96**	9.34**	1.32ns	7.07*	1.01ns	3.63*	1.52ns	48.07***	75.73***	3.43*	7.35**
Compaction × Cultivars	0.93ns	4.21*	4.43*	0.04ns	1.47ns	0.07ns	2.71ns	0.71ns	0.05ns	1.11ns	0.16ns

Note: TRL, total root length; RSA, root surface area; ARD, average root diameter; SRL, specific root length; CRL, coarse root length; FRL, fine root length; MRD, maximum root depth; GC, glucose concentration; XC, xylose concentration; UAC, uronic acid concentration; OAC, organic acid concentration and RDW, root dry weight. A two-way ANOVA was conducted to test the significance of genotypes, treatments and their interaction on the investigated traits. ns, no significant differences, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.