Mulch quality regulates soil fertility and microbial communities during a crop cycle

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1   **Mulch quality regulates soil fertility and microbial communities during a crop cycle**

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9   **Abstract**

10   **Context**. The benefits of plant-based mulch extend beyond improving soil physical properties as

11   mulches hold value as a fertiliser and in maintaining robust soil microbial communities throughout

12   the crop cultivation period.

13   **Aims**. We investigated the impact of plant-based mulch quality (comprising contrasting

14   hemicellulose, cellulose, and lignin components) sourced from single and diverse species mixtures

15   on soil fertility, microbial communities, crop yield, and seed quality by growing barley from seed to

16   maturity in rhizotrons.

17   **Methods**. The treatments consisted of residue mixtures containing 17, 12, 6, and 1 grassland plant

18   species, as well as wood chips, with a Control group receiving no residues. Soil samples were

19   collected at two time points, 69 and 195 days after mulch application for nutrient and microbial

20   phospholipid fatty acid analysis (PLFA). Residues were analysed for hemicellulose, cellulose, and

21   lignin content.

22   **Key results**. We found that high-quality residues, with low C:N ratio, low recalcitrance and the

23   highest N content decomposed most quickly and increased available soil potassium concentrations

24   compared to residues of lower quality. The lower-quality residues retained a greater proportion of

25   their initial fertilisation capacity which was released gradually. There was a concomitant increase

26   in soil microbial biomass at later stages of decomposition in residues of lower quality. Residue

27   quality significantly affected crop seed quality but not yield.

28   **Conclusions.** Lower-quality residues can maintain their fertilisation capacity over a longer time

29   horizon than higher-quality residues. Plant residue mulch can influence soil microbial biomass

30   throughout the cultivation period, regardless of its initial quality, and it can affect crop quality

31   although this effect was slow and not always readily detectable.

32   **Implications.** These findings emphasise the importance of considering plant-based mulch quality

33   in enhancing soil fertility and crop quality.

36

37

Introduction

39 The impact of plant-based mulch spread on the soil surface as ground cover to prevent soil erosion

40 and evaporation on soil properties has been extensively studied with a strong focus on soil physical

41 properties (Bateman et al., 1963., Prats et al., 2017; Donjadee & Tingsanchali 2016; Jordán et al.,

42 2010; Blanco-Canqui et al., 2011; Mulumba & Lal, 2008). However, the effects on soil chemical and

43 biological properties have received less attention in the literature.

44 Current research on soil chemical properties primarily investigates the contribution of mulch to

45 carbon (C), nitrogen (N), and phosphorus (P) dynamics, while other nutrients have been relatively

46 understudied. Significant differences in C mineralisation rates have been observed based on the

47 amount of mulch applied under different environmental conditions (Dietrich et al., 2017;

48 Razafimbelo et al., 2006). Residues of high quality, characterised by a low carbon-to-nitrogen (C:N)

49 ratio, had lower N immobilisation (Abiven & Recous, 2007) and higher dry mass loss (Donjadee &

50 Tingsanchali, 2016). Conversely, straw mulch with a high C:N ratio resulted in lower soil N content

51 after the seedling stage that is the stage of the growth of young plants from seeds (Bateman et al.,

52 1963; Su et al., 2014). Consequently, in soils with low nitrate content, N availability may be limited

53 shortly after mulch application, especially with low-quality residues (Siczek & Lipiec, 2011).

54 Nitrogen mineralisation is typically lower as mulch residue particle size increases (with longer stems

55 and leaves) (Nguyen & Marschner, 2017; Singh & Khind, 1992). Utilising long-size residues is more

56 cost-effective since they do not require an additional cost (in time and energy) for particle size

57 reduction. Furthermore, they provide prolonged physical protection for the soil due to slower

58 degradation (Reichert et al., 2015; Giacomini et al., 2007). Mulch application frequency and dosage

59 also play a role, with higher nutrient concentrations observed with up to 3 applications (Pavlů et

60 al., 2016). A recent study found that the greater the application rate of plant mulch over 5 Mg ha^{-1}

61 the greater the increase in soil organic matter and total N content (Irshad et al., 2022). Other

62 researchers found that the application of different application rate of mulch materials affected

63 various plant growth parameters such as plant height and stem diameter in a maize crop (Pratama

64 et al., 2025).

65 Long-term experiments have shown that mulching, compared to bare soil with no plants growing

66 or removal of plant residues, can increase soil P and potassium (K) content (Zhou et al., 2025; Akhtar

67 et al., 2019; Lynch & Brown, 2001). The mineralisation of organic P in the soil during decomposition

68 of organic material is rapidly influenced by its fixation on soil particles or immobilisation by

69 microbes (George et al., 2018). The addition of organic matter from plant residues can influence

70 oxidation-reduction processes, acting as a chelating agent and releasing soluble forms of iron (Fe)

71 and manganese (Mn) (Singh & Khind, 1992).

72 Researchers have increasingly focused on residues derived from plant species mixtures, considering

73 them to provide greater diversity of resources and consequently providing soil ecological benefits

74 than single plant species mulch – as evidenced by the lack of monocultures in natural ecosystems.

75 The influence of plant mixtures on the decomposition process can vary, resulting in positive,

76 negative, or additive effects compared to expectations (Redin et al., 2014; Shu et al., 2021). Among

77 the commonly studied mixtures are those consisting of legume and non-legume species. Legumes

78 are utilised due to their higher N status and ability to provide readily available N for other plants

79 (Tribouillois et al., 2015). Non-legume species are often included to capture excessive N in the soil

80 solution, thereby preventing leaching losses (White et al., 2017). In some cases, species with

81 phosphorus-mobilising abilities such as white lupin (*Lupinus albus* L.) (Muller et al., 2015) and

82 crambe (*Crambe hyspanica subsp. Abyssinica* (Hochst. R.E.Fr.) Prina) (Janegitz et al., 2017) or other

83 nutrient-acquisition traits are added to cover crops to facilitate nutrient uptake by the main crop

84 through root exudates or decomposition processes (Li et al., 2014).

85 Mulch has been shown to impact the enrichment of soil microbial communities positively. Different

86 chemical compositions among mulch treatments influenced soil microbial biomass, as indicated by

4

87 carbon dioxide (CO_2) evolution (Bremer et al., 1991). The fungal-to-bacterial ratio in the soil was

88 higher when litter had a higher C:N ratio or when the soil solution pH was low, regardless of litter

89 chemistry (Grosso et al., 2016). However, in a long-term continuous residue input experiment,

90 bacteria dominated the soil microbial community despite variations in litter quality (Frasier et al.,

91 2016). Wang et al. (2015) did not observe any significant difference in the fungal-to-bacterial ratio

92 after adding residues. Additionally, although soil microbial populations were initially higher under

93 high-quality residues in the same experiment, the differences became negligible by the end of the

94 study period. The indirect effect of plant mixtures via soil microbes may provide enzymatic

95 specificity in the decomposition process (Fontaine et al., 2003), and a mixture of plant species

96 substrates is expected to increase microbial biomass and alter microbial diversity (Shu et al., 2022).

97 Farmers utilise shredded tree branches and twigs as mulch in some agricultural practices,

98 particularly in organic farming or agroforestry (Wise et al., 2007; Akinnifesi et al., 2010). These

99 residues typically have very high C:N ratios (>40) and high recalcitrance, resulting in initial N

100 immobilisation (Brust, 2019). They tend to favour fungal growth over bacterial growth (Barreiro et

101 al., 2016), but their long-term effects on chemical and biological soil properties during

102 decomposition have not been extensively investigated. Decomposition of lower-quality plant

103 residues (higher C:N ratio) favours the growth of fungi over bacteria because fungi have a higher

104 C:N ratio than bacteria (Grosso et al., 2016). However, a higher substrate C:N does not favour fungi

105 over bacteria if C availability from plant residues is low (Barreiro et al., 2016).

106 Conflicting findings have been reported on the effect of mulching on crop yield and quality (Prasad,

107 2018; Su et al., 2014). In one case, yield and nutritional value (protein content of seeds) of maize

108 were increased under sufficient application of mulch (Awopegba et al., 2017). In contrast, straw

109 mulch increased nodulation and nitrogenase activity by reducing soil compaction and increasing

110 soybean seed protein content but yield was unaffected (Siczek & Lipiec, 2011). Furthermore, it has

111 been shown that longer residue size of mulch can also contribute to the increase of seed protein

112   content (Gaitanis et al., 2023). It is well known that seed protein is important for human and animal

113   nutrition but also it can positively affect the growth of the plants in the seedling stage (Lowe & Ries,

114   1973).

115   There is a paucity of knowledge about the value and contribution of low versus high quality residue

116   mulch on soil fertility at later stages of their decomposition. Moreover, little or no attention has

117   been paid to the residual fertilising capacity of the different mulches at advanced decomposition

118   stage. It was found that residues with longer particle size or whole plants decompose more slowly

119   and results indicated that they may retain a substantial fertilising capacity for the subsequent crop

120   (Gaitanis et al., 2023). This is important considering that the ongoing decomposition possibly

121   sustains soil microbial activity until the next crop is planted. In the same experiment, residue dry

122   mass loss was highly influenced by residue quality and mainly by residue recalcitrance. The

123   decomposition rate was initially higher in residues of higher quality but this trend was reversed at

124   later stages. Moreover, there were indications that residue species richness may increase soil

125   microbial activity. However, there is a lack of knowledge on whether residues of lower quality

126   maintain higher amount of their initial fertilisation capacity than those of higher quality at later

127   stages of decomposition and even more how do they affect soil microbial community.

128   To address the existing research gap, we conducted a study investigating the impact of long-size

129   (circa 30 cm) plant-based residue mulch on soil chemical and biological properties during the later

130   stages of decomposition and its effects on crop yield and quality. Soil chemical properties included

131   total C and N, available NH_4^+ and NO_3^-, and available P, K, Mg, Fe, Mn, Zn, and Cu. Soil biological

132   properties included fungi, Gram+ and Gram- bacteria, Protozoa, and Cyanobacteria. Our

133   experimental treatments comprised the application of residues of single plant species, mixtures of

134   plant species, wood chips (consisting of shredded tree branches and twigs with their leaves), and a

135   Control treatment without mulch. The residue types were the same as those that were used in

136   Gaitanis et al. (2023), bar wood chips. Wood chips were characterised by much higher C:N ratio

137 and recalcitrance than the rest of the residue types and therefore we were expected to draw more

138 sound and secured conclusions regarding the effect of residue quality on soil fertility at later stage

139 of decomposition. In addition, the influence of residue quality and residue diversity on soil

140 microbial groups, which is a good indicator of soil fertility, was not investigated in the

141 aforementioned experiment. Considering that residues of high quality are those with low C:N ratio

142 and low recalcitrance and residues of low quality are those with high C:N ratio and high

143 recalcitrance (Gaitanis et al., 2023), we hypothesised that: (i) Residues of higher quality would

144 decompose faster initially, resulting in increased soil microbial biomass of decomposer microbes,

145 compared to residues of lower quality, (ii) at advanced stage of the degradation process there

146 would be a reverse trend in the decomposition rate, which would be higher in residues of lower

147 quality than those of higher quality, leading to significant differences in soil microbial biomass,

148 nutrient dynamics, and crop quality, (iii) increased residue diversity (plant species richness) would

149 result in increased soil microbial biomass, and (iv) residues of lower quality would maintain higher

150 quantity of their initial fertilising capacity than residues of higher quality at the end of the growing

151 season.

152

153 **Materials and Methods**

154 *Experimental design and materials*

155 The experiment was carried out at the Crop and Environment Laboratory of the University of

156 Reading in the UK, from June 2019 to December 2019. The experimental units were rhizotrons,

157 constructed using 0.5 cm PVC sheeting and filled with soil suitable for cultivating annual cereal

158 plants (Fig. 1 and 2).

159 **Fig. 1**

160 **Fig. 2**

161 Each rhizotron had a removable clear acrylic panel on one side, allowing for root growth

162 observation and periodic soil sampling. When not under observation, this side was covered with

163 Thermawrap silver foil to prevent light penetration and minimise temperature fluctuations. The

164 rhizotrons were 1 m in height to accommodate root growth at depth, 30 cm wide, and 5 cm in

165 thickness. A layer of gravel approximately 1.5 cm deep was placed at the bottom to ensure proper

166 drainage, followed by commercially supplied well-homogenised loamy sand topsoil (bulk soil)

167 sieved to 8 mm, filling the rhizotrons up to 10 cm from the top. The initial soil solution pH was 7.3

168 ± 0.032 SD, with soil texture estimated by Bouyoucos method consisted of 86.1% sand, 5.7% clay,

169 and 8.2% silt (Bouyoucos, 1962).

170 Thirty rhizotrons were assigned to one of six treatment groups, with each treatment replicated five

171 times. Spring barley plants (*Hordeum vulgare* L., var. Westminster) were transplanted into the

172 rhizotrons on 11th July 2019, 17 days after plant emergence, placed one plant in the middle of each

173 rhizotron, ensuring a distance of 15 cm from each side. Throughout the experiment, the rhizotrons

174 were kept at a 70° angle, with the transparent side facing downward to encourage extensive root

175 development on that side for improved visual observation. An automated irrigation system was

176 installed with two drippers per rhizotron, maintaining water content at 55-60% of water holding

177 capacity (Harding & Ross, 1964). The growth rate of the barley plants was monitored every 1 to 2

178 weeks.

179 On 11th June 2019, five biomass residue treatments were established as mulch on the soil surface

180 of the rhizotrons 30 days prior to the transplantation of barley plants. A Control treatment (C)

181 without residues was also established as reference. The biomass residue treatments, referred to as

182 Types, were established as follows: Perennial Ryegrass (*$P_{(1)}$*) 1 plant species (*Lolium perenne* L.),

183 Smart Grass^{TM} (*$S_{(6)}$*) 6 species (Perennial Ryegrass (*Lolium perenne* L.), Timothy (*Phleum pratense*

184 L.), Red Clover (*Trifolium pratense* L.), White Clover (*Trifolium repens* L.), Plantain (*Plantago*

185 *lanceolata* L.), Chicory (*Cichorium intybus* L.)), Biomix^{TM} (*$B_{(12)}$*) 12 species (those in Smart Grass as

186   well as Cocksfoot (*Dactylis glomerata* L.), Festulolium, Meadow Fescue (*Festuca pratensis* Huds.),

187   Aslike Clover (*Trifolium hybridum* L.), Black Medick (*Medicago lupulina* L.), and Lucerne (*Medicago*

188   *sativa* L.)), Herbal™ (*H(17)*) 17 species (those in Smart Grass as well as Cocksfoot (*Dactylis glomerata*

189   L.), Festulolium, Tall Fescue (*Festuca arundinacea* Schreb.), Meadow Fescue (*Festuca pratensis*

190   Huds.), Aslike Clover (*Trifolium hybridum* L.), Sweet Clover (*Melilotus* spp.), Sainfoin (*Onobrychis*

191   *spp.*), Birdsfoot Trefoil (*Lotus corniculatus* L.), Yarrow (*Achillea millefolium* L.), Burnet (*Sanguisorba*

192   *minor* Scop.), and Sheep's Parsley (*Petroselenium crispum* Mill.), and Wood chips (*W*) (Table S1).

193   No threatened species were utilised in this research (IUCN, 2000), and all experimental activities,

194   including the collection of plant material, were conducted in compliance with relevant institutional,

195   national, and international guidelines and legislation. The plant material was sourced from

196   DiverseForages grassland experiment at the University of Reading farm.

197   The residue types were selected to address our hypothesis encompassing residue mixtures with

198   distinct characteristics, including residue diversity and functional traits. Residue diversity refers to

199   the composition or richness of species within a residue mixture. Functional traits pertain to the

200   chemical composition, which determines residue quality, as well as the morphological features of

201   the residues (Santonja et al., 2018; Hattenschwiler et al., 2011). Residues of higher quality are those

202   characterised by lower C:N ratio, higher N content and lower recalcitrance, while the opposite is

203   true for residues of lower quality (Frasier et al., 2016; Fog, 1988). The wood chips were obtained

204   by shredding tree residues, including branches, twigs, and leaves, collected from the Harris Garden

205   on the University of Reading campus on 9th June 2019. Fresh plant residues of all other types were

206   collected from field plots planted with the aforementioned forage mixtures on 4th June 2019. All

207   plant material was stored at 4°C until application.

208   All residues, except for wood chips, were cut to approximately 30 cm in length to ensure

209   consistency. Wood chips were shredded to pieces from 1 to 5 cm. The residues, representing 23 g

210   of dry mass, were then placed onto the soil in the rhizotrons as fresh mulch. No additional

211   fertilisation was applied during the experiment.

212   *Sampling protocol*

213   Soil samples were collected from the rhizotrons at two different time points using a tube of 5 or 10

214   cm diameter, as appropriate, which was inserted vertically at the desired depth of the soil profile

215   after removing the glass side panel of the rhizotrons. The first sampling occurred 69 days after the

216   application of mulch (day 69), during barley growing stage (GS) 59-60, when most plants had

217   completed the emergence of inflorescence and were beginning to flower (Tottman et al., 1986). At

218   this time point, three soil samples were collected from the left side of each rhizotron, facing the

219   glass, at depths of 0-5 cm, 20-25 cm, and 50-55 cm (Fig. 3). The second sampling took place 195

220   days after the mulch application (day 195), after the completion of grain ripening in barley. Soil

221   samples were collected from 0-5 cm and 50-55 cm depths, this time from the right side of the

222   rhizotrons. Sampling at a depth of 20-25 cm was not repeated during the second period because

223   the initial measurements showed that the fluctuations of nutrient elements, except for N, at this

224   depth were not significant. All soil samples were air-dried and sieved to 2 mm. Soil samples

225   collected at 0-5 cm depth during both time points were divided into two parts. One part was air-

226   dried and then sieved to 2 mm, as mentioned before, while the other part was sieved to 2 mm

227   immediately after extraction, freeze-dried, and stored at -20°C for subsequent phospholipid fatty

228   acid (PLFA) analysis. The plant residues (total amount) were collected from the soil surface of the

229   rhizotrons on day 195. They were carefully sieved through a 1 mm mesh to remove any residual

230   soil dust and then dried at 80°C for 48 hours until a constant weight was achieved to determine the

231   dry mass of the residues. At the same time (day 195), barley plants, and additional soil samples

232   were collected for root sampling at depths of 0-10 cm, 35-45 cm, and 75-85 cm. The roots were

233   extracted by submerging the soil samples in tap water over a 1 mm sieve and collecting the floating

234   roots. Barley seeds were collected after the ripening stage GS99 (Tottman et al., 1986). All plant

235   materials were dried at 80°C for 48 to 72 hours until a constant weight was achieved.

236   **Fig. 3**

237   *Soil and plant material analysis*

238   Total C, N, protein content of seeds and plant residues, C:N ratio of plant residues, and total C and

239   N of soil samples were estimated by combustion using the LECO CHN 628 analyser (LECO

240   Corporation, 2018). Dry mass of plant residues and barley plants was estimated by drying them at

241   80°C for 48 to 72 hours, and the soil samples were dried at 105°C for 24 hours. The ANKOM 2000

242   Fiber Analyzer was used to measure recalcitrant substances in plant residues (ANKOM Technology,

243   2018). Samples were first dried at 80°C to a constant weight and ground to <1 mm using a Fritsch

244   grinder by Glen Creston Ltd. Plant residue fibre analysis consisted of three successive processes to

245   determine the percentages of Neutral Detergent Fibre (NDF; hemicellulose, cellulose, and lignin),

246   Acid Detergent Fibre (ADF; cellulose and lignin), and Acid Detergent Lignin (ADL; lignin). Individual

247   component contents were calculated by sequential subtraction. Cellulose = ADF − ADL and

248   hemicellulose = NDF − ADF. Neutral detergent solution (NDS), alpha-amylase, and anhydrous

249   sodium sulfite were used as reagents for NDF analysis, acid detergent solution for ADF analysis, and

250   72% by weight sulfuric acid for ADL analysis.

251   Soil solution pH was measured using a calibrated pH meter. Sieved and air-dried soil samples

252   weighing 10 g were placed in centrifuge tubes with 25 ml of ultra-pure water. The tubes were

253   shaken for 15 minutes on an end-over-end shaker before measurement (Blakemore et al., 1981).

254   Available soil N was estimated by KCl extraction according to the standard protocol (Great Britain.

255   M.A.F.F., 1986), using a San Continuous Flow Injection Analyzer (SKALAR Instruments, 2018). This

256   analysis used 40 g of air-dried soil in a 200 ml 1M KCl solution. The concentrations of available P, K,

257   Mg, Mn, Fe, Cu, and Zn nutrients for plant uptake in the soil samples were evaluated using the

258   Mehlich 3 method with a Perkin Elmer − Optima 7300 DV ICP-OES analyser (AgroEcoLab, 2018;

259   PerkinElmer, Inc., 2018). This analysis used 2 g of air-dried soil samples in 20 ml of Mehlich 3

260   extracting solution (Mehlich, 1984; Pierzynski, 2000).

261   Soil samples for microbial analysis were freeze-dried in an Edwards Super Modulo freeze dryer

262   (Edwards Company, 2019) immediately after collection and stored to -20℃ until analysis. Soil

263   microbial biomass of gram+ bacteria, gram- bacteria, fungi, protozoa, and cyanobacteria, as well as

264   the fungi-to-bacteria ratio, were estimated using PLFA analysis of freeze-dried soil samples

265   weighing 3 ± 0.1 g following the procedure described by Sizmur et al. (2011). The PLFA analysis

266   involved 24 compounds to evaluate the biomass of the decomposer microbial groups (nmol g^{-1} of

267   freeze-dried soil). The groups were represented as follows: Gram+ bacteria by iC15:0, a-C15:0,

268   C15:0, i-C16:0, i-C17:0, C18:0, and C20:0, Gram- bacteria by 2-OH-C12:0, C14:1, C15:1, 2-OH-C14:0,

269   and 3-OH-C14:0, Fungi by C18:3ω6, C18:2ω6c, C18:2ω6t, C18:1ω9c, C18:3ω3, and C20:1ω9,

270   Protozoa by C20:4ω6 and C20:3ω6, and Cyanobacteria by C20:5ω3, although in previous studies

271   C18:2ω6c has also been detected in Cyanobacteria, and C18:1ω9c and C18:3ω3 in higher plants

272   and green algae (Quideau et al., 2016; Buyer & Sasser, 2012; Amir et al., 2010; Zelles, 1999;

273   Frostegård & Bååth, 1996; Vestal & White, 1989). The samples were analysed using an Agilent

274   6890N Network GC System Gas Chromatographer (Agilent Technologies, 2019). Methyl

275   tetradecanoate (C14:0) internal standard, methyl nonadecanoate C19:0 internal standard,

276   Bacterial Acid Methyl Ester (BAME) Mix Quantitative standard, and Supelco 37 Component (FAME)

277   Mix Quantitative standard were utilised. The chromatograms were processed using Agilent

278   ChemStation – G2190BA – B04. The Retention Time Window was set to 0.10 min and 1.5%, with

279   Methyl tetradecanoate (C14:0) and Methyl nonadecanoate (C19:0) internal standards as reference

280   compounds.

281   Air-dried soil samples were used to estimate soil texture with a hydrometer by adding 50 ml sodium

282   hexametaphosphate solution (SHMP) at 50 g/l to 40 g soil subsamples (Bouyoucos, 1962). Soil

283   water holding capacity (WHC) was estimated gravimetrically using 50 g fresh soil samples with the

284 method described by Harding and Ross (1964). The height of the main stem of barley plants from

285 the soil surface to the base of the flag leaf was used as a proxy for growth rate (cm/d). The grain

286 yield was estimated by the total dry grain mass per rhizotron after the ripening stage GS99

287 (Tottman et al., 1986). The number of ears per plant and the number of tillers per plant were also

288 used as additional factors for estimating plant productivity.

289 *Statistical analysis:*

290 All statistical analyses were performed using Minitab 19 (Minitab, LLC, 2017), except for Principal

291 Component Analysis, which was conducted in R-Studio (RStudio, PBC, 2017), with the rhizotron

292 serving as the unit of replication for this study (n=5). A significance level of $\alpha = 0.05$ was used. One-

293 way ANOVA was employed to detect differences among treatments or residue types.

294 Measurements taken at different time points (day 69 and day 195) or soil depths were analysed

295 using a Mixed Effects Model, with rhizotrons as random factors and treatments, residue types,

296 time, and soil depth as fixed factors. Before conducting variance analyses, normality and

297 homogeneity were assessed using the Darling-Anderson and Levene tests, respectively. In cases

298 where ANOVA assumptions were not met, the Kruskal-Wallis test was applied for one-way ANOVA,

299 and data transformation using $\log_{10}$ or Box-Cox transformation with optimal or rounded λ was

300 performed. Tukey post-hoc comparisons were conducted when significant differences were found.

301 Dunnett test was used to compare mulch against the Control treatment. Data were separated and

302 analysed for specific sampling times or depths when normality or equality of variances were not

303 met, even after data transformation. Descriptive statistics, including means and standard

304 deviations, were reported instead of standard errors unless otherwise stated.

305 Regression analysis was carried out to examine the significance and degree of the effect of a single

306 independent variable on a response variable in cases where an apparent influence was observed

307 (e.g., residue dry mass loss vs. initial residue NDF or N content, soil K vs. initial residue C:N ratio).

308 Homogeneity of variance was tested using the Levene test, and normality of data distribution was

309   assessed using the Anderson-Darling test. Spearman and Pearson correlations were used to

310   evaluate significant correlations between variables. The Spearman correlation was also employed

311   when normality or equality of data variances in the regression analysis was not met.

312   Multivariate analysis was conducted using Principal Component Analysis (PCA) to simultaneously

313   assess differences among treatments in multiple variables and examine the relationships between

314   treatments and barley plant parameters.

315

316   **Results**

317   *Initial residue quality and residue dry mass loss*

318   The initial C:N ratio was significantly different among residues of different diversity (n = 5, F =

319   12143.32, p-value < 0.001, $\log_{10}$ transformation of data). All types were significantly different

320   except $S_{(6)}$ and $B_{(12)}$ (Fig. S1, and Tables 1, and S3). Generally, residues with higher species number

321   had higher C:N ratio except W which had the highest. There were significant differences among

322   residue types in initial recalcitrance (Fig. S2, and Table S4). All types were significantly different in

323   initial hemicellulose except $P_{(1)}$ and $H_{(17)}$, which had the highest values (N = 5, F = 109.48, p-value <

324   0.001) while $S_{(6)}$ and $B_{(12)}$ had the lowest. The initial NDF content of residues was significantly

325   different among residue types (Kruskal-Wallis test, N = 5, H-value = 22.46, p-value < 0.001) with the

326   highest values in W and $H_{(17)}$ treatments. W type had a significantly higher value of initial cellulose

327   from all other types (N = 5, F = 41.46, p-value < 0.001). The initial ADF content of residues was

328   significantly different among all types except between $S_{(6)}$ and $P_{(1)}$, which had the lowest values (F

329   = 1743.45, p-value < 0.05, N = 5) while W and $B_{(12)}$ had the highest values. W and $B_{(12)}$ types had the

330   highest values of initial ADL (lignin) content of residues significantly different from all other types

331   (N = 5, F = 10.33, p-value < 0.001). Moreover, residue dry mass loss was significantly negatively

332   correlated with NDF, ADF, lignin, and cellulose, but not hemicellulose, considering all treatments

333   together (Table S5). Initial residue N content could significantly (N = 5, F = 34.29, p-value < 0.001)

334   explain 59.85 % of the residue mass loss as residue dry mass loss = -5.016 +55.76 N − 8.534 N^2 (n =

335   5, r^2 = 88.7 %, F = 55.87, p-value < 0.001, Fig. 4).

336   **Fig.4**

337   The dry mass loss was significantly different among residues of different diversity (types) (n = 5, F

338   = 72.14, p-value < 0.001, Fig. 5, and Table S2), with the $S_{(6)}$ decomposing the most quickly (95%)

339   and wood chips achieving only half this rate.

340   **Fig. 5**

341   **Table 1**

342   *Effect on soil solution pH and on soil nutrients*

343   Soil solution pH, measured at 0-5 cm depth, was not significantly different between treatments in

344   samples taken on day 69 or day 195 (Table S6). In both timepoints, soil solution pH was significantly

345   increased in all treatments when compared to the initial bulk soil value (N = 5, F = 68.33, p-value <

346   0.001 for day 69, and F = 61.59, p-value < 0.001 for day 195). Moreover, the pH was significantly

347   increased (N = 30, F = 50.96, p-value < 0.001) on day 195 (7.72 ±0.15) than on day 69 (7.46 ±0.12).

348   At 0-5 cm depth, total soil C did not differ among treatments or between sampling timepoints (day

349   69 and day 195). Total soil N was significantly lower in Control (0.16 ±0.011 %) than in $P_{(1)}$ (0.17

350   ±0.01) treatment, considering the entire set of samples from both day 69 and day 195 together (N

351   = 10, T-value = 3.12, p-value = 0.034). On day 69 total soil N was statistically significant lower in $S_{(6)}$

352   treatment than in the bulk soil prior to its use in the experiment (T-value = -3.44, adjusted p-value

353   = 0.027). On day 195 $H_{(17)}$, W, and Control had the lowest values in total soil N in descending order

354   (Table S7).

355   Both on day 69 and day 195 in samples from 50-55 cm depth, available N was detected only as NH_4^+

356   and not as NO_2^- and NO_3^- which were probably leached. Ammonium availability was significantly

357   higher on day 69 (2.49 ±0.62 mg kg^{-1}) than on day 195 (1.50 ±0.31 mg kg^{-1}) considering all

358   treatments together (N = 30, T-value = -8.32, p-value < 0.001). There were significant differences

359   among treatments in interaction with time (Table S8).

360   Soil content of available P, K, Mg, Fe, Mn, Zn, and Cu was measured from 0-5 and 20-25 cm depth

361   on day 69. All elements were significantly affected by depth except P (Table S9). Potassium was the

362   only element where significant differences had been detected among treatments in combined

363   samples (N = 10, F = 3.74, p-value = 0.012). More specifically, soil K in Control (62.19 ±14.03 mg kg^{-1}

364   ) was significantly lower than in $H_{(17)}$ (78.65 ±21.24 mg kg^{-1}, T-value = 3.97, p-value = 0.01) and in

365    $P_{(1)}$ (75.78 ±15.06 mg kg^{-1}, T-value = 3.28, p-value = 0.033) treatments (Fig. S3). At 0-5 cm depth soil

366   K in Control was significantly lower than from all other treatments (N = 5, F = 3.65, p-value = 0.013),

367   while at 20-25 cm depth soil K in Control was significantly lower only with $H_{(17)}$ treatment (N = 5, F

368   = 2.27, p-value = 0.08, T-value = 3.07, adjusted p-value = 0.022) (Table S10, Fig. 6). There were no

369   significant differences among treatments in the other elements.

370   **Fig. 6**

371   Considering all samples from 0-5 and 20-25 cm depth together, bulk soil (N = 4) had significantly

372   higher values for both soil K, and soil Mg than other treatments (N = 10), and significantly lower Fe

373   value than the $H_{(17)}$ treatment (T-value = 2.88, adjusted p-value = 0.022, Table S11). Generally, bulk

374   soil had higher values in macronutrients K, and Mg, and lower in micronutrients Fe, Zn, and Cu.

375   *Effect on soil microbes*

376   There were no statistically significant differences among treatments in the different microbial

377   groups or in total microbial biomass on both day 69 and day 195 (Tables 2, 3, and S12).

378   **Table 2**

379   **Table 3**

380   However, the treatments followed similar patterns of PLFA values which show a clear trend in

381   microbial group fluctuations (Tables 2, and 3). Treatments of higher quality residues ($S_{(6)}$, $P_{(1)}$ and

382    $B_{(12)}$) had higher values on day 69 and lower on day 195 comparing to those of lower quality residues

383   (W and $H_{(17)}$), while Control had the lowest or of the lowest values on both days (Tables 2, 3, and

384   S12). The pattern $S_{(6)}$>$P_{(1)}$>$B_{(12)}$>$H_{(17)}$>W>C in descending order was the prevalent one on day 69 for

385   all microbial groups, with slight alterations. It is exactly matched to that of dry mass loss and is the

386   reverse of recalcitrance measures (Tables 2, S2, and Fig. 7).

387   **Fig. 7**

388   Spearman correlation showed a statistically significant positive correlation (N = 25, ρ = 0.405, p-

389   value = 0.045) between total microbial biomass on day 69 and total microbial biomass on day 195.

390   F:B ratio was significantly negatively correlated with both total microbial biomass and initial %C

391   content of residues but only on day 69, where also total microbial biomass was positively related

392   with %C content. Moreover, F:B ratio was significantly negatively related with initial % cellulose

393   content of residues on day 195 (Table S13). The fungi: bacteria ratio on day 69 was 0.255, taken

394   Gram+ and Gram- as total bacteria biomass (nmol of compounds per g of freeze-dried soil), and

395   considering all treatments together, while the mean values of treatments in descending order was

396   C>$S_{(6)}$>W>$H_{(17)}$>$P_{(1)}$>$B_{(12)}$. On day 195, the fungi: bacteria ratio was raised to 0.436, and the order of

397   the mean values of treatments was $P_{(1)}$>W>$B_{(12)}$>$H_{(17)}$>$S_{(6)}$>C, while the difference was slight in

398   Control treatment (Fig. 7, and Table S14).

399   *Effect on barley plants*

400    $H_{(17)}$ treatment had the highest value of barley root dry mass at 0-10 cm depth (0.545 ±0.117),

401   significantly different from W (0.331 ±0.088) (T-value = -3.23, p-value = 0.037, Table S15).

402   Differences among treatments in yield, evaluated either by the seeds' dry mass per plant by the

403   number of ears per plant or by the number of tillers per plant, were not statistically significant.

404 However, in all these criteria used to evaluate yield, $H_{(17)}$ treatment had the highest value while

405 Control had the lowest. Likewise, differences in barley plant dry mass (without seeds and roots), in

406 seed biomass, and in seed protein content were not significant, but there were significant

407 differences in C content of seeds (N = 5, F = 3.64, p-value = 0.014). $B_{(12)}$ treatment (44.568 ±0.133)

408 had significantly higher value than Control (44.157 ±0.232, T-value = -3.50, p-value = 0.020) and

409 $H_{(17)}$ (44.192 ±0.247, T-value = -3.20, p-value = 0.039) (Table S16).

410 Main stem height (cm) of barley plants seems to follow a double S curve at all treatments from day

411 32 to day 116 after plant emergence (Fig. S4) (Boyle, 2017). Total main stem elongation rate (cm/d)

412 of the different treatments between each timepoint and day 32 after plant emergence, growing

413 stage GS31 (Tottman et al., 1986), is depicted in Fig. 8.

414 **Fig. 8**

415 Total growth rate, represented by the total main stem elongation rate, was initially rising, reaching

416 its peak 46 days after plant emergence (GS39) and steadily declining after that. Control peaked in

417 the main stem elongation rate a little later than the rest of the treatments (on day 53 after plant

418 emergence).

419 Spearman correlation was significantly negative between yield and seed protein content (ρ = -

420 0.709, p-value < 0.001), and between seed C and initial residue C:N ratio (ρ = -0.441, p-value =

421 0.027), and significantly positive between seed C and seed protein content (ρ = 0.417, p-value =

422 0.038). In contrast, there were no significant Spearman correlations between main stem elongation

423 rate (at any timepoint) and yield or seed protein or seed C or initial residue chemical composition

424 (C:N ratio, C, N, NDF content) (p-values > 0.05).

425 Principal Component Analysis (PCA) was conducted to detect differences among treatments

426 considering many variables. The included variables were: v1 = total barley dry mass (g), v2 = root

427 dry mass (g) in soil samples from 0-10, 35-45, and 75-85 cm depth, v3 = final number of tillers, v4 =

428   yield (seed dry mass per plant), v5 = % seed protein, and v6 = soil NH_4^+ (mg/kg oven dry soil) at

429   harvest time (Fig. 9).

430   **Fig. 9**

431   Fig. 9 shows that all treatments were overlapped but $H_{(17)}$ treatment was distinctly different than

432   all the others. $H_{(17)}$ was clearly positively related with barley root dry mass (v2) more than any other

433   treatment, and $S_{(6)}$ treatment was positively related with seed protein and soil NH_4^+ content (v5

434   and v6). *W* treatment and Control were negatively related with total barley dry mass, final number

435   of tillers, and yield (v1, v3, and v4).

436

437   **Discussion**

438   Various factors have been shown to constrain plant residue dry mass loss, including residue C:N

439   ratio, N:P ratio, lignin, cellulose, polyphenol content, and combinations thereof (Zhonglu et al.,

440   2015; Xu et al., 2017; Vahdat et al., 2011). This research confirms the significant influence of initial

441   residue hemicellulose, cellulose, and lignin content (NDF - rather than ADF or ADL) and N content

442   in residue dry mass loss, as found in previous research using similar material (Gaitanis et al., 2023).

443   NDF content was negatively correlated with mass loss, while N content showed a positive

444   correlation up to a threshold value of about 3.2% beyond which it turned negative (Fig. 4). This

445   finding aligns with previous studies showing that, while N is important at the start of the

446   decomposition process, very soon the readily available C may be the limiting factor and therefore,

447   adding N can reduce residue decomposition due to microbial competition (Fog, 1988). Moreover,

448   the negative correlation between f:b and initial C content of residues, and between f:b and total

449   microbial biomass on day 69 is further evidence that the readily available C was the limiting factor

450   of decomposition, at least until day 69, favouring bacterial growth (Table S13).

451   Previous research has indicated that direct N mineralisation is typically observed when the C:N ratio

452   of decomposed material of plant residues is 24 or less. Otherwise, the released N from organic

453   matter decomposition is immobilised by soil decomposer microorganisms for the needs of their

454   metabolic activity. This happens because the C:N ratio of soil microbes is approximately 8, the

455   community typically assimilates about a third of the released C (Brust, 2019). However, the C:N

456   threshold for direct N mineralisation can rise when soil microbes and fauna with a C:N exceeding 8

457   are involved in decomposition (Frouz et al., 2015). Vigil & Kissel (1991) reported that the break-

458   even C:N point differentiating between N mineralisation and immobilisation can be as high as 40.

459   Therefore, in our experiment, direct net N mineralisation was expected to take place in all residue

460   addition treatments (C:N between 10.7 to 16.8), except in the W treatment (C:N of 50.1), where

461   initial N immobilisation was anticipated.

462   In addition to C:N ratios, NDF is a useful measure of recalcitrance as it encompasses hemicellulose,

463   cellulose, and lignin. An intermediate decomposition rate characterises hemicellulose and

464   cellulose, while lignin decomposes more slowly (Hadas et al., 2004). In this study, the dry mass loss

465   of residues was negatively correlated with NDF, cellulose and lignin, but not with hemicellulose.

466   This finding is consistent with a study on N mineralisation of plant residues that found a relationship

467   between lignin and cellulose, but not hemicellulose (Zhonglu et al., 2015). Additionally, Jensen et

468   al. (2005) reported that holocellulose (hemicellulose and cellulose combined) explained the C

469   mineralisation rate of above-ground residues more effectively than lignin. Therefore, in line with

470   Gaitanis et al. (2023), NDF can be used as measure of recalcitrance and all three factors (NDF, N,

471   and C) should be considered in residue dry mass loss investigations rather than relying solely on

472   the C:N ratio.

473   Adding plant litter with a low C:N ratio typically leads to increased respiration activity of soil

474   microbial biomass (Tahir & Marschner, 2017; Swift et al., 1979). In our experiment, residues of

475   higher quality, based on the initial C:N ratio and % NDF content ($B_{(12)}$, $P_{(1)}$, and $S_{(6)}$) stimulated higher

476   microbial biomass activity on day 69, resulting in higher residue dry mass loss at this stage than

477   residues of lower quality (W and H$_{(17)}$). The high-quality residues provided sufficient N and other

478   nutrients for microbial decomposers during that period, unlike the treatments with lower-quality

479   residues (Tahir & Marschner, 2017; Campbell et al., 1993). Conversely, lower-quality residues

480   enhanced microbial biomass on day 195 relative to higher-quality residues. Lower-quality residues

481   still resulted in lower mass loss, maintaining their initial fertilisation capacity longer than higher-

482   quality residues, as predicted by our hypothesis. Adding residues of lower quality at first reduced

483   N availability and decreased the activity of soil microbial decomposers. These results indicate that

484   higher-quality residues underwent faster initial decomposition than lower-quality residues, only

485   for the decomposition rate of lower-quality residues to catch up later.

486   Soil microbial diversity is typical for its temporal, spatial, and hierarchical variability, nutrient flux

487   above-ground plant detritus is one of the factors driving it (Lukac, 2017). Indeed, a decrease in

488   biomass of Gram+, Protozoa, and Cyanobacteria was observed in higher-quality residues ($B_{(12)}$, $P_{(1)}$,

489   and $S_{(6)}$) from day 69 to day 195. In contrast, the biomass of the same microbial groups increased

490   in lower-quality residues (W and H$_{(17)}$) as well as in the Control (Table S17). These three microbial

491   groups are favoured during the early stages of decomposition of high-quality residues and the later

492   stages of lower-quality residue decomposition. These findings are consistent with previous

493   research (Liang et al., 2017; Nottingham et al. 2009). Fungi were likely favoured by increased

494   recalcitrance on day 195, and both fungi and Gram- bacteria benefited from feeding on the

495   increased bacterial necromass resulting from the decomposition of easily decomposable material

496   during the earlier stages (Nottingham et al., 2009). Furthermore, Gram- bacteria had a competitive

497   advantage as they were favoured by relatively higher moisture content on the soil surface (Chen et

498   al., 2020), particularly under lower-quality residues, which remained more intact with larger

499   particle sizes due to a slower decomposition rate than higher-quality residues. The suppression of

500   microbial activity in no-residue Control on both day 69 and day 195 was consistent with previous

501   research (Calderón et al., 2016).

502 The soil chemistry reflected the effect of residue diversity and quality on soil microbial populations.

503 All treatments maintained total soil N content on day 69 close to that of the bulk soil before its use

504 in the experiment, except $S_{(6)}$ treatment which had statistically significant lower value than the bulk

505 soil (Table S7). Nitrogen mineralisation thus compensated for the amount taken up by barley plants.

506 On day 195, treatments with high-quality residues ($S_{(6)}$, $P_{(1)}$, and $B_{(12)}$) still maintained total soil N

507 content close to that of the initial bulk soil. Treatments with residues of lower quality ($H_{(17)}$, and W)

508 had significantly less total N and Control had the least. The amount of N released by the residues

509 of lower quality up to at least day 69 was comparable with that of the residues of higher quality.

510 By day 195, however, the higher-quality residues released more N than the lower-quality residues.

511 This shows that the residues of higher quality are more effective than those of lower quality in their

512 ability to supply N to the crop at least up to the end of the current growing season, although the

513 opposite trend is evident at the later stages of decomposition (Table S7). A similar conclusion was

514 drawn by Gaitanis et al. (2023) where in addition a reverse trend in residue decomposition was

515 observed not only between residues of higher and lower quality but also between residues of short

516 and long size. Furthermore, the N release dynamics observed suggest that the slow release of

517 nutrients from plant-based mulch may reduce N loss by leaching, in contrast to the homogeneous

518 incorporation of shredded residues into soil (Ambus et al., 2001). However, mulching could increase

519 N losses in the form of NH_3 by volatilisation (Singh & Khind, 1992), although this can be mitigated

520 (Cassim et al., 2024).

521 The cultivation of barley led to a decrease in availability of soil macronutrients K and Mg, and an

522 increase in micronutrients Fe, Zn, and Cu. Furthermore, the Control treatment exhibited

523 significantly lower available soil K levels than any other treatment. Among the nutrients analysed,

524 K was the only nutrient significantly affected by residue treatments apart from N. These results are

525 consistent in great extent with Gaitanis et al. (2023). In our case soil K content was higher in the

526 $H_{(17)}$ treatment (Fig. S3 and Table S10). Higher species richness may have activated a more diverse

527 soil microbial population, resulting in increased mineralisation of specific nutrients (Santonja et al.,

528   2017). In Gaitanis et al. (2023) residue species richness was suspected to increase Arbuscular

529   Mycorrhizal Fungi root colonisation. However, contrary to our hypothesis, the diversity of plant

530   residues, measured as the number of plant species, did not show a significant overall effect on soil

531   microbial biomass. Therefore, it is more likely that the plant residue diversity may have a stronger

532   effect on microbial species diversity within specific microbial groups than on total microbial

533   biomass as it appears in previous research (Shu et al., 2021). Diverse residue mixtures have been

534   proposed as an alternative to crop diversity to provide nutritional benefits in cases where

535   monocultures cannot be avoided (Struijk et al., 2020). Additionally, mulch composed of mixtures

536   with varying recalcitrance has been found to increase soil macronutrient content (Wang et al.,

537   2021).

538   According to their influence on barley plants, our treatments can be classified in three categories.

539   The first category are treatments characterised by low initial residue C:N ratio, high residue N

540   content, and low recalcitrance ($B_{(12)}$, $P_{(1)}$, and $S_{(6)}$ treatments), resulting in high seed protein content,

541   high barley plant biomass, but low yield. In the second category are treatments characterised by

542   low initial residue C:N ratio, low residue N content, and high recalcitrance ($H_{(17)}$ treatment),

543   resulting in low seed protein content, high barley plant biomass, and high yield. In the third

544   category are treatments characterised by high initial residue C:N ratio, low residue N content, and

545   high recalcitrance (W treatment), resulting in low seed protein content, low barley plant biomass,

546   and low yield. Control treatment resulted in a similar response to those in the third category. These

547   assumptions were further confirmed by the fact that initial residue C:N ratio was negatively

548   correlated with both seed C and seed protein content.

549   The results of this study illustrate the influence of residue chemical composition on nutrient

550   dynamics, nutrient plant uptake and, consequently, crop growth. The main stem elongation rate of

551   barley plants in Control was delayed compared to the other treatments, an effect reflected in lower

552   yield. This highlights the beneficial effect of mulch on crop performance. Plant-based mulch

553   increased seed protein content and crop yield compared to no mulch treatment (Awopegba et al.,

554   2017). The differences in plant growth and yield due to different treatments in this experiment

555   were not significant, either because the main effect of mulching overshadowed these or because

556   the residues did not have sufficient time to decompose and fully affect the soil. Regular mulch

557   application and adaptation of appropriate field management systems may be necessary for the

558   long-term benefits to become apparent. This experiment utilised rhizotrons; therefore, results

559   should be considered cautiously and verified in field conditions. However, rhizotrons that allow

560   non-destructive soil sampling during the cultivation period can be proved valuable for evaluating

561   the effects of organic mulch in future research.

562   **Conclusions**

563   Our results indicate that lower-quality residues can maintain their fertilisation capacity over a

564   longer time horizon than higher-quality residues, as the latter initially tend to decompose faster

565   although the decomposition rate is reversed at later stages of decomposition influencing soil

566   microbial community. Residue dry mass loss was highly influenced by the initial residue NDF, C, and

567   N content. An increase in soil microbial biomass in residues of lower quality was observed at later

568   stage of decomposition than those of higher quality. Residue diversity, indicated by the number of

569   plant species, was generally not found to significantly influence soil microbial biomass. The fungal-

570   to-bacterial ratio in the soil increased with the duration of the decomposition progress in all residue

571   treatments. Residue chemical composition has a more immediate impact on crop quality than on

572   crop yield.

573   The use of diverse mulch can contribute to the enrichment of soil nutrients, potentially affect seed

574   crop quality, and may impact soil microbial populations during later stages of the cultivation period.

575   However, the influence of residue diversity or quality on nutrient dynamics, microbial biomass,

576   seed quality, and crop yield is gradual and not always easily detectable. Further research is needed

577   to investigate the effects of continuous application of diverse long size residue mulch in no-tillage

578   or conservation tillage practices on chemical and biological soil properties, crop yield, and crop

579   quality.

580

581   **Statements and Declarations**

588   **Conflicts of interest/Competing interests:** Mark Tibbett is an Editor of Soil Research. To mitigate

589   this potential conflict of interest they were blinded from the review process. The other authors

590   have no relevant financial or non-financial interests to disclose.

591   **Availability of data and material:** Not applicable.

592   **Code availability:** Not applicable.

593   **Authors' contributions:** Conceptualisation, D.G.; Experimental design, D.G., and M.L.; Experimental

594   work, D.G.; Laboratory analyses, D.G.; Data analysis, D.G.; Data curation, D.G., M.L., and M.T.;

595   Supervision, M.L., and M.T.; Writing – original draft, D.G.; Writing – review and editing, D.G., M.L.,

596   and M.T.

597   **Ethics approval:** Not applicable.

598

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874

875 **Summary Text for the Table of Contents**

876 The use of plant-based mulch can potentially increase soil fertility reducing the need of artificial

877 fertilisers which has a positive environmental impact in urgent issues such as global warming. Our

878 research demonstrates the value of not only the easily decomposable but also of the more

879 recalcitrant mulches on soil nutrient availability and their influence on soil microbial communities

880 at later stages of decomposition. These findings can help in the application and management of the

881 appropriate mixtures of mulches.

882

883 **Table 1** Classification of mulching material types in descending order of initial or final mean values

884 of selected properties. Mean values and standard deviations are shown (n = 5). Mulching materials

885 were: perennial ryegrass (**P**, 1 plant species), Smart Grass (**S**, 6 species), Biomix (**B**, 12 species),

886 Herbal (**H**, 17 species), and wood chips (**W**). NDF is Neutral Detergent Fiber (hemicellulose, cellulose

887 and lignin), ADF is Acid Detergent Fiber (cellulose and lignin), and ADL = Acid Detergent Lignin

888 (lignin)

| Properties	Residue Types	Mean ±StDev
Initial C:N ratio	$W^a > H^b > S^c > B^c > P^d$	50.87 ±1.32, 16.78 ±0.12, 14.88 ±0.11, 14.87 ±0.11, 10.72 ±0.07
Initial % C	$W^a > P^b > H^c > S^c > B^d$	47.58 ±0.18, 43.24 ±0.07, 42.72 ±0.06, 42.67 ±0.07, 38.79 ±0.34
Initial % N	$P^a > S^b > B^c > H^d > W^e$	4.03 ±0.03, 2.87 ±0.02, 2.61 ±0.04, 2.55 ±0.01, 0.94 ±0.02
Species richness	$H > B > S > W > P$	
% initial NDF	$W^a > H^b > B^{bc} > P^c > S^d$	59.37 ±0.98, 53.50 ±0.25, 41.78 ±0.55, 40.48 ±1.16, 34.62 ±1.22
% initial ADF	$W^a > B^b > H^c > S^d > P^d$	42.33 ±0.62, 26.20 ±0.41, 23.92 ±0.16, 20.01 ±0.75, 19.87 ±0.31
% initial ADL	$W^a > B^a > H^b > P^b > S^b$	15.09 ±0.74, 14.30 ±1.35, 8.25 ±3.76, 9.57 ±1.86, 6.97 ±2.98
% dry mass loss	$S^a > P^b > B^b > H^b > W^c$	92.09 ±4.48, 80.11 ±5.52, 79.42 ±2.94, 77.60 ±4.78, 40.20 ±7.23

889 Letters in superscript indicate difference at p<0.05

890   **Table 2** Classification of treatments (*B,* Control *(C), H, P, S,* and *W*) in descending order, according

891   to their values in microbial biomass for the different microbial groups as well as in total microbial

892   biomass on day 69 after mulch application. Mean values and standard deviations are shown (n =

893   5). The values are in nmoles of phospholipid fatty acid compounds g^{-1} of freeze dry soil. The residue

894   types were Perennial ryegrass *(P)* (1 plant species), Smart Grass *(S)* (6 species), Biomix*(B)* (12

895   species), Herbal *(H)* (17 species), and wood chips *(W)*

Microbial groups	Residue types	Mean ±StDev
Gram+	S>P>B>H>W>C	1046.50 ±676.72, 931.11 ±533.93, 846.32 ±817.17, 624.05 ±512.01, 621.52 ±334.46, 558.05 ±341.55
Gram-	S>P>B>W>H>C	115.40 ±60.33, 108.98 ±44.51, 100.53 ±56.47, 75.07 ±18.18, 74.19 ±27.41, 68.35 ±20.47
Fungi	S>P>B>H>W>C	285.08 ±168.84, 233.86 ±119.48, 210.87 ±189.57, 172.31 ±129.66, 166.55 ±74.95, 150.60 ±84.75
Protozoa	B>S>P>H>C>W	54.88 ±41.37, 46.86 ±26.96, 42.51 ±20.81, 37.08 ±19.17, 23.25 ±8.28, 22.77 ±4.65
Cyanobacteria	P>S>B>H>W>C	15.72 ±10.59, 14.66 ±6.23, 14.24 ±7.79, 11.26 ±5.00, 6.90 ±1.22, 6.59 ±2.14

896

897   **Table 3** Classification of treatments (*B,* Control *(C), H, P, S,* and *W*) in descending order, according

898   to their values in microbial biomass for the different microbial groups as well as in total microbial

899   biomass on day 195 after mulch application. Mean values and standard deviations are shown (n =

900   5). The values are in nmoles of phospholipid fatty acid compounds g^{-1} of freeze dry soil. The residue

901   types were Perennial ryegrass *(P)* (1 plant species), Smart Grass *(S)* (6 species), Biomix*(B)* (12

902   species), Herbal *(H)* (17 species), and wood chips *(W)*

Microbial groups	Residue types	Mean ±StDev
Gram+	S>H>W>C>P>B	875.60 ±375.62, 866.34 ±145.85, 715.64 ±306.31, 697.79 ±585.89, 581.41 ±340.59, 552.37 ±377.95
Gram-	S>H>W>C>P>B	172.87 ±82.50, 168.71 ±39.37, 141.80 ±59.53, 131.07 ±94.60, 122.49 ±43.30, 108.36 ±62.48
Fungi	H>S>W>P>B>C	446.09 ±135.66, 433.93 ±173.23, 360.99 ±180.22, 357.96 ±211.69, 294.71 ±168.70, 266.69 ±128.44
Protozoa	S>H>W>P>C>B	49.26 ±18.49, 45.73 ±9.23, 38.74 ±8.91, 34.45 ±19.24, 31.78 ±17.91, 28.91 ±14.47
Cyanobacteria	S>W>H>C>B>P	17.63 ±9.25, 17.06 ±12.31, 16.22 ±4.79, 11.45 ±3.51, 10.97 ±4.81, 10.64 ±5.06

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Fig. 1

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Fig. 2

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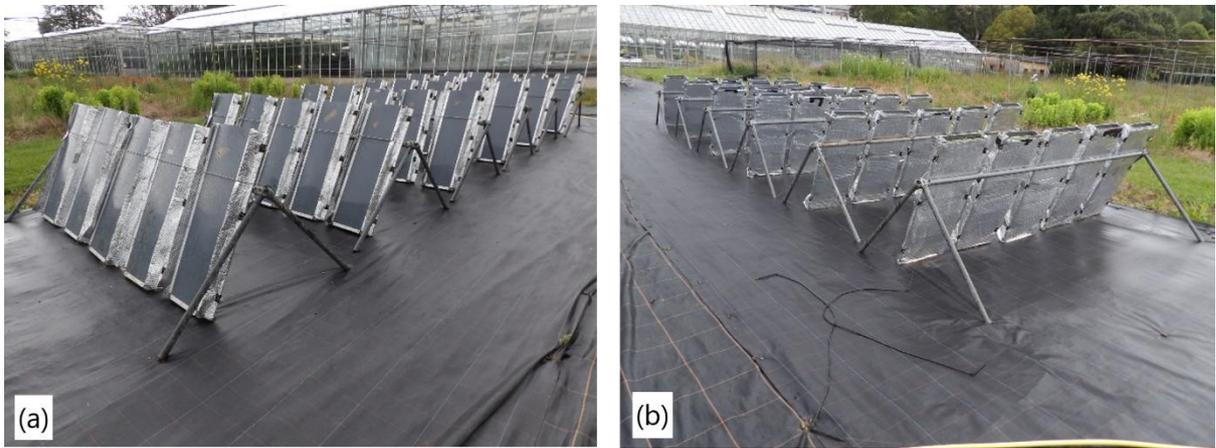
Plant-based mulches and soil fertility
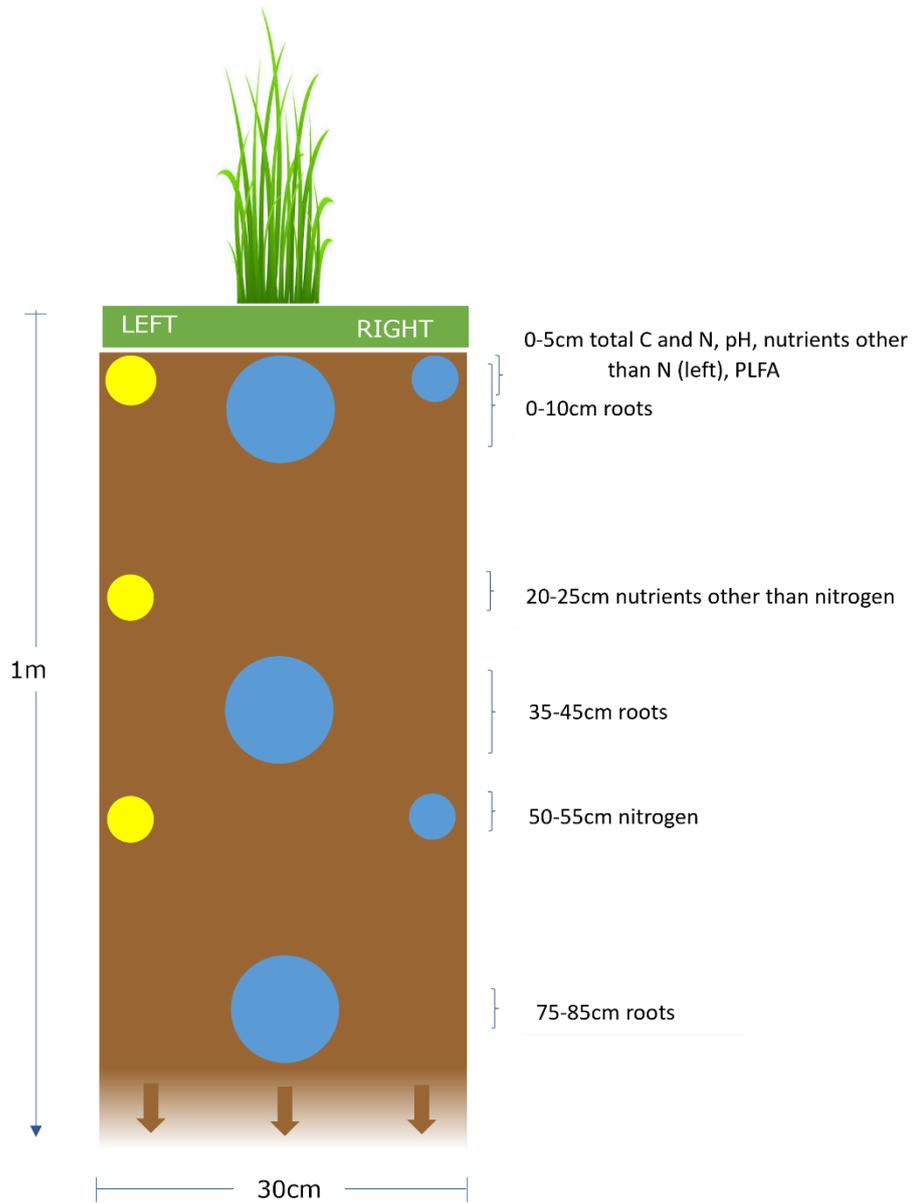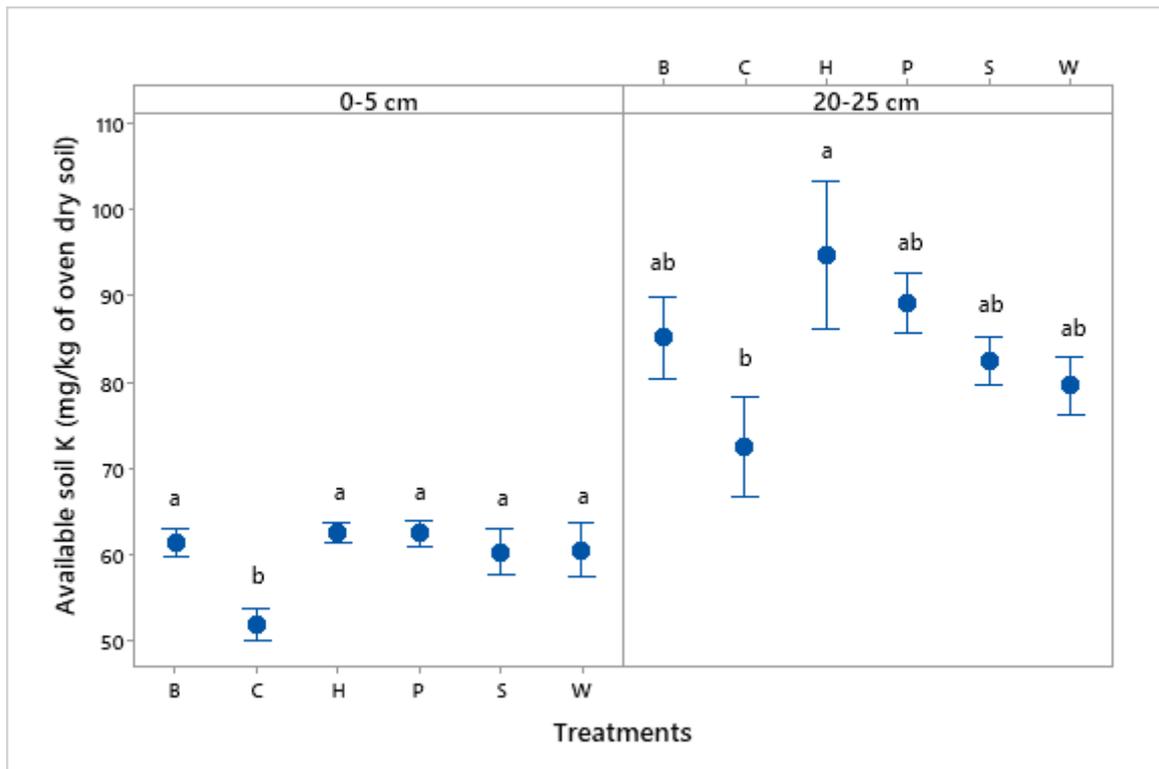

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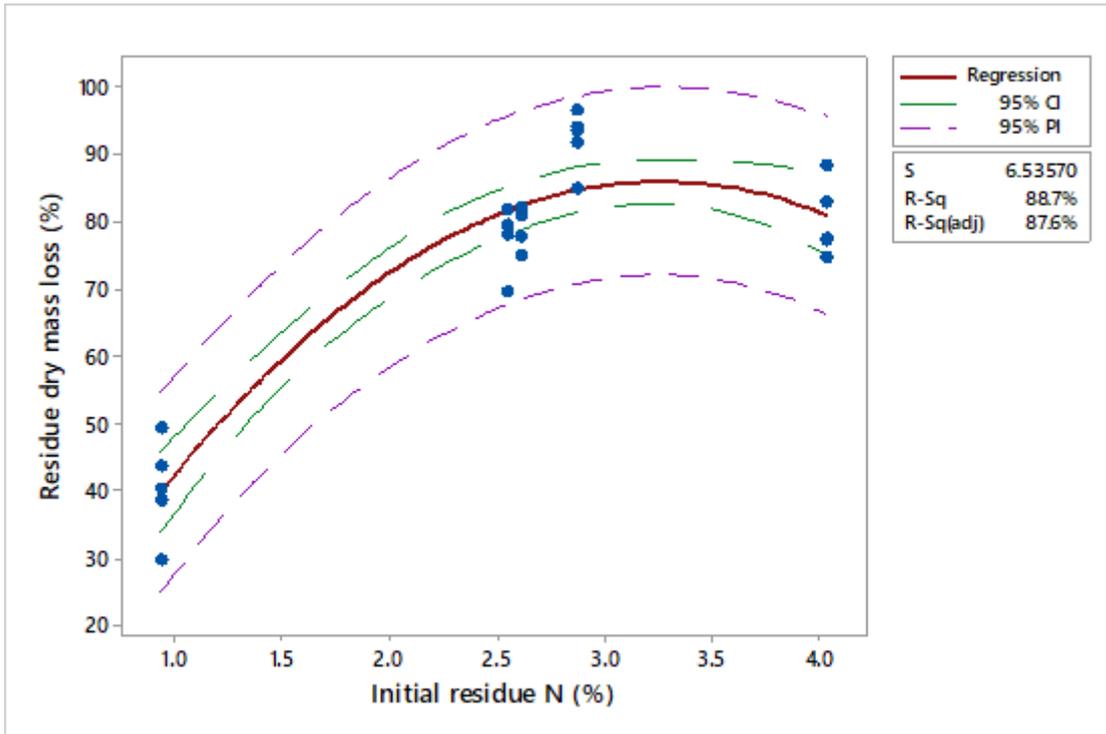
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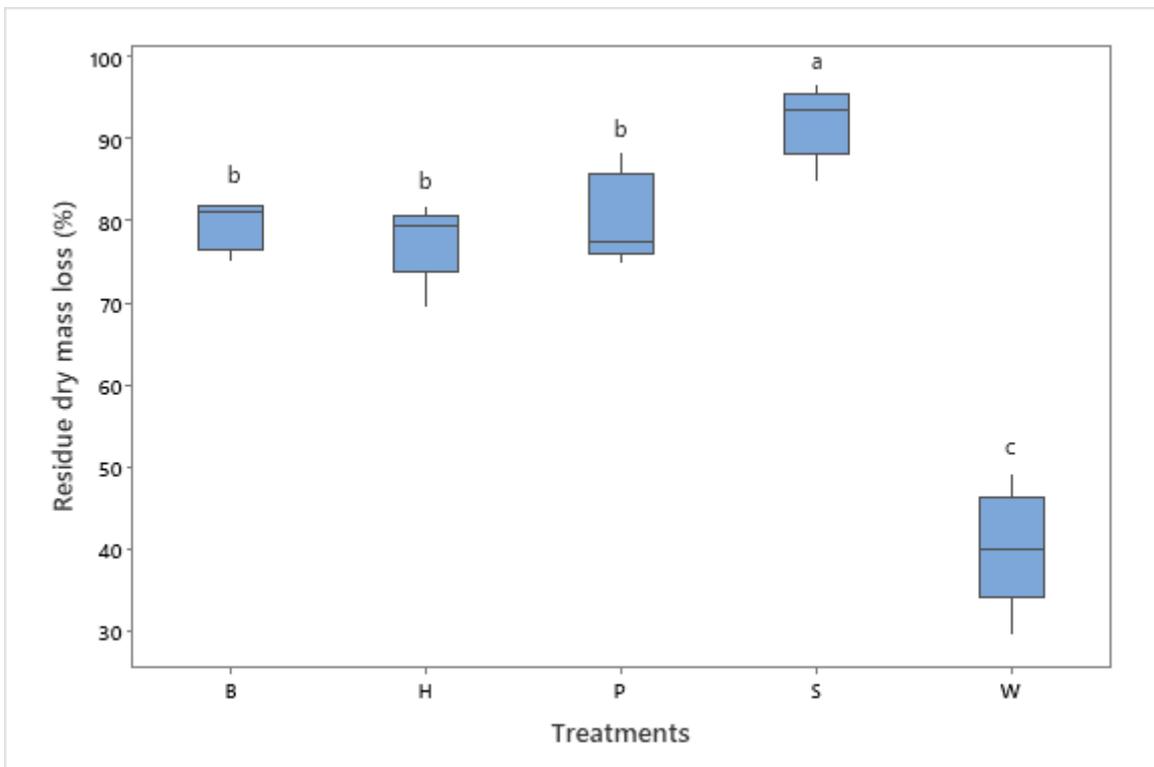
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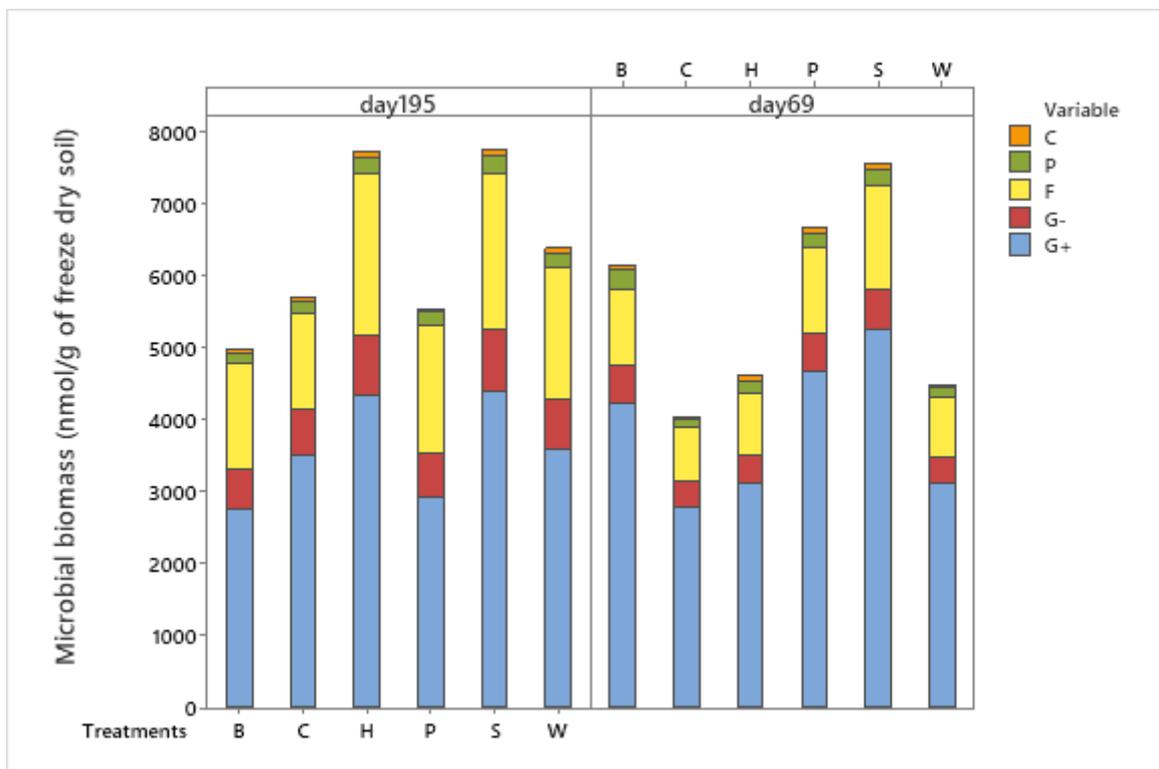914 **Fig. 5**

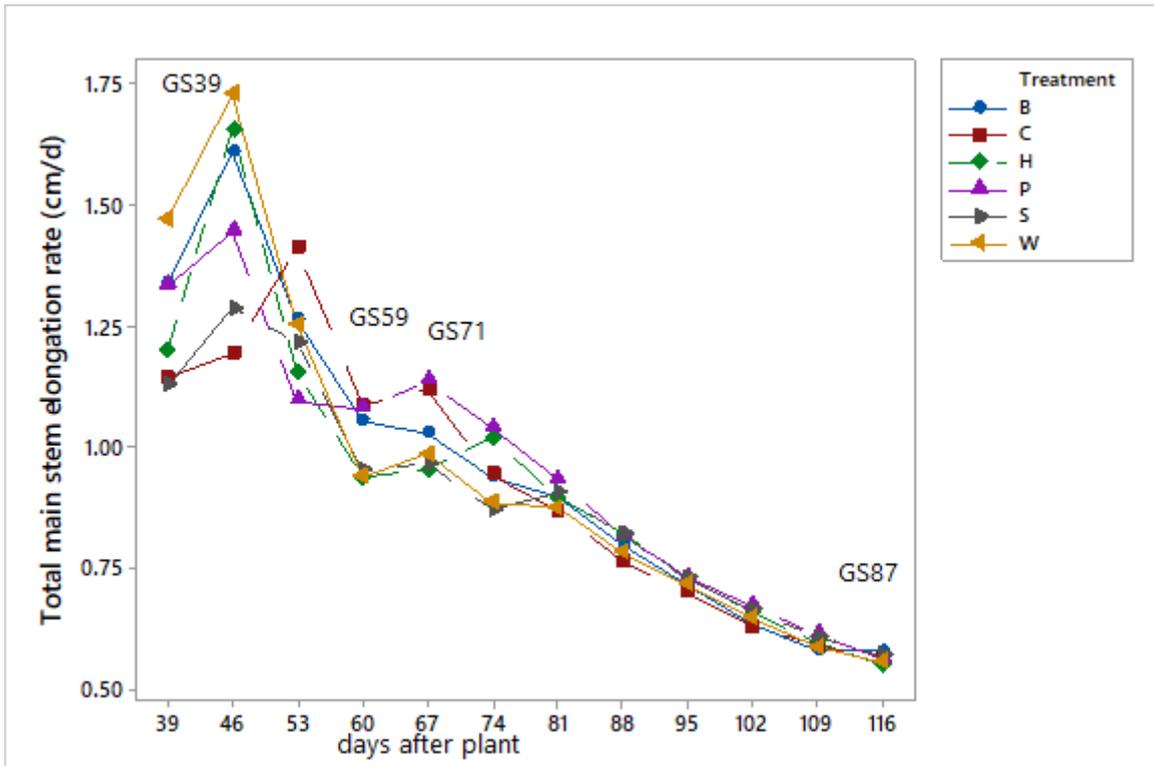Plant-based mulches and soil fertility
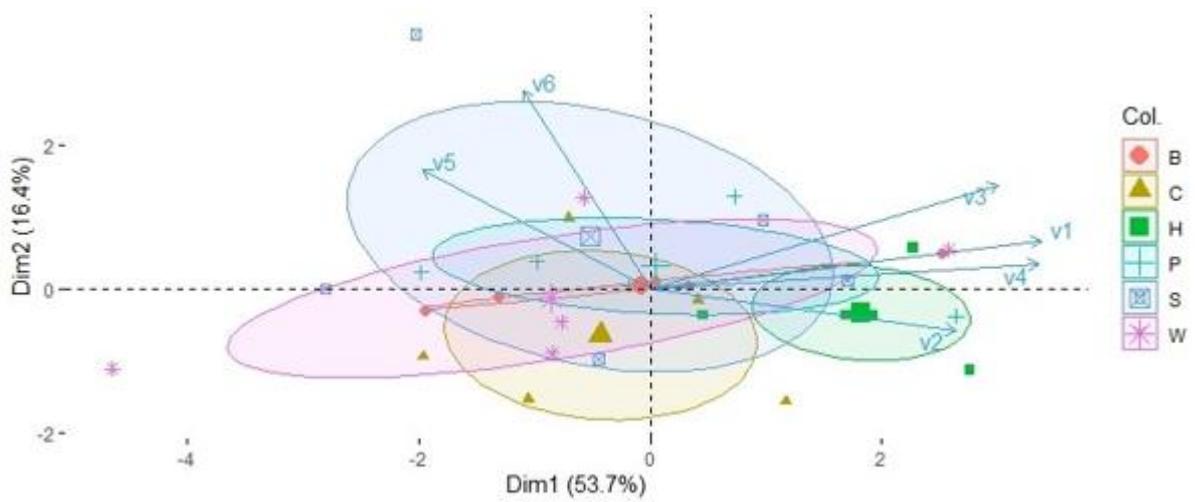

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916 **Fig. 6**

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919   **Fig. 7**

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921   **Fig. 8**

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923   **Fig. 9**

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925 **Fig. 1** Rhizotrons set at the outdoor. From left to right a) back side, b) front side with the glass

926 covered with alluminum foil

927 **Fig. 2** From left to right a) easily removable plexy glass on rhizotrons, b) wood chips (shredded

928 branches and twigs with their leaves), c) mulch application, d) root development at the glass side

929 **Fig. 3** Rhizotron and soil sampling design. Yellow highlighted sites indicate soil samples taken 69,

930 and blue highlighted sites were sampled 195 days after mulch application. Large blue circles in the

931 middle indicate root sampling on day 195

932 **Fig. 4** Fitted line plot of polynomial (quadratic) regression analysis of residue dry mass loss (%) with

933 initial residue N content (%) (red line). Confidence interval is enclosed by green dashed lines, and

934 prediction interval by purple dashed lines. Confidence level = 95.0. Regression equation: residue

935 dry mass loss = -5.016 +55.76 N – 8.534 N^2

936 **Fig. 5** Dry mass loss of mulching material after 195 days (N = 5). Letters represent the following

937 plant materials: perennial ryegrass (**P**, 1 plant species), Smart Grass (**S**, 6 species), Biomix (**B**, 12

938 species), Herbal (**H**, 17 species), and wood chips (**W**). Letters next to box plots indicate difference

939 at p<0.05

940 **Fig. 6** Available soil K content (mg/kg of oven-dried soil) for the different treatments (*B, C, H, P, S,*

941 and *W*), on day 69 after mulch application at 0-5 and 20-25 cm depths (N = 5). Means and bars of

942 one standard error from the mean are depicted. The residue types were Perennial ryegrass *(P)* (1

943 plant species), Smart Grass *(S)* (6 species), Biomix *(B)* (12 species), Herbal *(H)* (17 species), and wood

944 chips *(W)*. Control *(C)* treatment was with no residues. Treatments that do not share a common

945 letter are significantly different (p-value < 0.05)

946 **Fig. 7** Mass of different microbial groups (G+ = gram positive bacteria, G- = gram negative bacteria,

947 F = fungi, P = protozoa, and C = Cyanobacteria), expressed in nmol g^{-1} of freeze-dried soil, for the

948 different treatments (*B, C, H, P, S,* and *W*) from soil samples at 0-5 cm depth, on day 69 and on day

949   195 after mulch application (N = 5). The residue types were Perennial ryegrass *(P)* (1 plant species),

950   Smart Grass *(S)* (6 species), Biomix*(B)* (12 species), Herbal *(H)* (17 species), and wood chips *(W)*

951   **Fig. 8** Total main stem elongation rate (cm/d) of the different treatments (*B,* Control *(C), H, P, S,*

952   and *W*) measured from 26th July to 1st November 2019 (rates among each timepoint and 26th July).

953   Values derived from damaged stems were omitted and the next highest stem was considered

954   instead. GS = Barley growing stage (Tottman et al. 1986). The residue types were: Perennial ryegrass

955   *(P)* (1 plant species), Smart Grass *(S)* (6 species), Biomix*(B)* (12 species), Herbal *(H)* (17 species), and

956   wood chips *(W)*

957   **Fig. 9** PCA biplot of variables v1 = total barley dry mass (g), v2 = root dry mass (g) in soil samples

958   from 0-10, 35-45, and 75-85 cm depth, v3 = final number of tillers, v4 = yield (seed dry mass per

959   plant), v5 = % seed protein, and v6 = soil NH_4^+ (mg/kg oven dry soil) on day 195 after mulch

960   application, for the treatments *B,* Control *(C), H, P, S,* and *W*. The residue types were: Perennial

961   ryegrass *(P)* (1 plant species), Smart Grass *(S)* (6 species), Biomix *(B)* (12 species), Herbal *(H)* (17

962   species), and wood chips *(W)*. Large points depict mean values, while ellipses depict confidence

963   intervals (α = 0.05) of mean values for each treatment. The percentages represent data variation

964   explained by the two first Principal Components (Dim1 and Dim2), bottom axis represents Dim1

965   normalised score, and left axis represents Dim2 normalised score