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1 **Tree cover influences the soil C balance in Mediterranean cork oak-based silvopastoral systems**

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3 Antonio Pulina^{a*}, Sergio Campus^a, Chiara Cappai^a, Pier Paolo Roggero^a, Lorenzo Salis^b, Giovanna
4 Seddaiu^a

5

6 ^a Department of Agricultural Sciences and Desertification Research Centre (NRD), University of
7 Sassari, Sassari, Italy

8 ^b AGRIS Sardegna - Servizio Ricerca per la Zootecnia, Sassari, Italy

9

10 *Corresponding Author:

11 Antonio Pulina

12 Viale Italia 39A 07100 Sassari (IT)

13 Email: anpulina@uniss.it; ORCID 0000-0002-2220-0922

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15

16 **Abstract**

17 Mediterranean silvopastoral systems provide a wide range of ecosystem services, among which soil
18 C sequestration is one of the most relevant. Although the role of trees in enhancing soil C content of
19 silvopastoral systems is widely recognized, the impacts of the tree cover on the overall soil C balance
20 and hence the soil C sequestration rates are not clear enough to identify feasible options and effective
21 management pathways. This study aimed to assess the effect of trees on soil C balance in a
22 Mediterranean cork-oak silvopastoral system under contrasting cover classes. A three-year study was
23 conducted in a Long-Term Observatory in Sardinia (Italy). A hierarchical design was used to test the
24 tree effect (below and outside the tree crown projection) and the tree cover (high tree cover – from
25 39% to 42% – and regular tree distribution; low tree cover – from 15% to 36% – and irregular tree
26 distribution) on the soil C balance. The C balance was calculated as the difference between the C
27 input from the herbaceous and tree components of the ecosystem and the C output from soil
28 heterotrophic respiration. The position with respect to the tree stem influenced the C input, the C
29 output, and the overall C balance. The tree class influenced only the C output and C balance. The
30 annual C balance was higher (more gains) in low-covered areas below the tree crown projection
31 ($8.30 \pm 0.72 \text{ Mg ha}^{-1}$ of C) than in open pasture and high-covered areas. At the field level, the overall
32 weighted C balance was significantly influenced by tree cover; according to this last evidence, the
33 maximum C balance was reached at about 24% tree cover. The results highlight the importance of
34 management practices oriented to increase herbage production, tree cover, and regulating tree
35 distribution, which in turn can provide ecosystem services such as SOC sequestration.

36 **Keywords**

37 Agroforestry systems; C sequestration; Soil respiration; Grassland productivity; Litterfall

38 **1. Introduction**

39 Mediterranean silvopastoral activities on wooded grasslands play a crucial role in ecosystem services
40 provision, such as forage provisioning and C cycling regulation (Moreno et al., 2018) and hence
41 climate change mitigation potential (Lal, 2020). Mediterranean *Quercus*-based silvopastoral systems
42 are recognized as a priority by the “Habitats” Directive 92/43/EEC (type 6310 “Dehesas with
43 evergreen *Quercus* spp”). These agroforestry systems have been shaped by centuries of traditional
44 agro-silvopastoral activities (Eichhorn et al., 2006), as the typical scattered-trees vegetation structure
45 is generated by the combination of extensive livestock farming and forestry activities (Bagella et al.,
46 2013).

47 The role of trees in enhancing the soil C storage is recognized under a wide range of environmental
48 conditions (Haile et al., 2010; Takimoto et al., 2009), which also includes Mediterranean silvopastoral
49 systems (Cappai et al., 2017; Howlett et al., 2011; Seddaiu et al., 2018). Trees positively influence
50 the soil organic carbon (SOC) content under their crown projection due to continuous provisioning
51 to the soil of leaves, inflorescences, infructescences (e.g. acorns), and woody material such as
52 branches, usually referred to as litterfall, which contributes to the formation of organic matter with
53 different levels of quality and persistence (Cappai et al., 2017). The tree root systems influence the
54 SOC underneath the tree crown because of the fine roots rhizodeposition and turnover (Casals et al.,
55 2010; Moreno et al., 2005), which also influence soil fertility and the cycling of N and P (Nair et al.,
56 2019). The trees also contribute to establishing a microclimate under the crown projection in terms
57 of radiation, water balance, and temperature, resulting in differences in terms of plant communities
58 and biomass production (López-Sánchez et al., 2016; Seddaiu et al., 2018). Furthermore, the
59 interaction between biotic and abiotic factors results in differences in the microbiological activities
60 and hence in SOC losses as soil respiration (Lai et al., 2014; Uribe et al., 2015). In fact, it is widely
61 recognized that in these agroecosystems the main drivers of soil respiration are the soil water content
62 and temperature (Rey et al., 2002; Tang and Baldocchi, 2005). The soil water content constrains the

63 microbiological activity, and hence the soil respiration, under summer drought periods, thus resulting
64 the most important factor affecting SOC variations under Mediterranean climate (Oyonarte et al.,
65 2012; Pulina et al., 2018a). Also, the open areas within wooded grasslands significantly contribute to
66 the overall SOC content (Lal, 2004; Seddaiu et al., 2013). In these areas, the C input to soil is largely
67 affected by grassland above- and belowground primary productivity (Pausch and Kuzyakov, 2018).
68 Soil management can also be an important factor affecting the SOC content, e.g. through grazing
69 management, tillage (Uribe et al., 2015), fertilization (Fornara et al., 2016), oversowing with legume
70 species (Hernández-Esteban et al., 2018), and land use (Seddaiu et al., 2013).

71 The Mediterranean silvopastoral systems are threatened because of both trends of intensification, e.g.
72 causing a lack of tree regeneration (Rossetti and Bagella, 2014), and abandonment, e.g. leading to
73 shrubs encroachment, and increasing firewood risk (Moreno et al., 2018). The provision of
74 silvopastoral-associated ecosystem services, as SOC sequestration (Seddaiu et al., 2018), is
75 guaranteed over time by the anthropic presence and influence (Bugalho et al., 2011), without which
76 such habitats would tend to their natural potential vegetation (Bagella and Caria, 2011).

77 A lack of knowledge emerges from the literature on the effect of the tree cover in influencing SOC
78 sequestration and hence the climate change mitigation potential of these silvopastoral systems. As
79 these agroecosystems result from the close interaction between anthropic and environmental factors,
80 it becomes basic to understand the impact of management practices through the assessment of the
81 role of tree cover, as a proxy of the intensity of human activities, especially in terms of SOC balance.

82 This study aimed to assess the impacts of the position with respect to the tree crown projection within
83 different classes of tree cover on (i) seasonal dynamics of soil CO₂ emissions and their relationships
84 between environmental drivers, (ii) pasture and tree biomass production and contribution to soil C
85 input, and (iii) SOC balance, during a three-year experiment in a Mediterranean silvopastoral system.
86 The hypothesis was that in a Mediterranean silvopastoral agroforestry system the drivers regulating

87 the SOC balance are influenced not only by the position with respect to the tree, but also by the area
88 covered by trees within homogeneous farming units.

89

90 **2. Materials and Methods**

91 *2.1. Study site*

92 The study site was located in a subset of private farms inside the Long-Term Observatory of
93 Berchidda-Monti (NE Sardinia, Italy) (40° 49' N / 9° 17' - 9° 19' E; 287-325 m a.s.l.). The Observatory
94 is representative of the Sardinian wooded grassland-based semi-extensive livestock systems and,
95 overall, of the silvopastoral farming systems within the Mediterranean basin (Caballero et al., 2009).
96 Human activities have shaped the landscape for centuries as a mosaic of land uses such as cork oak
97 forests, grasslands, vineyards, and cork oak wooded grassland, which represents the largest land use
98 covering about the 30% of the Observatory (Seddaiu et al., 2018). The mean annual rainfall in the
99 Observatory is 632 mm, of which about 70% falls between October and May. The mean annual
100 temperature is 14.2 °C, and the aridity index (mean annual precipitation divided by mean annual
101 reference evapotranspiration) is 0.53. The dominant soil type is a Typic Dystrocherept (USDA, 2010),
102 derived from a granitic substratum (Carmignani et al., 2012) and characterized by a sandy loam
103 texture in the upper soil horizon. The SOC content at 0-40 cm depth ranges from 13.3 g kg⁻¹ in areas
104 outside the tree crown projection to 31.8 g kg⁻¹ in areas below the tree crown. The tree role in SOC
105 variation was already reported and discussed by Seddaiu et al. (2018). In this study, the SOC, the total
106 N content, and the C/N ratio measured in May 2014 are reported in Table 1 according to the study
107 layout below described. The potential vegetation is referable as *Violo dehnhardtii-Quercetum suberis*
108 association, mainly represented by *Quercus suber* L. forests (Bagella and Caria, 2011). During the
109 study period (2012-2015), the average temperature was 15.8 °C, the average maximum air
110 temperature occurred in August (32.9 °C), while the average minimum temperature occurred in
111 February (2.7 °C). The average yearly rainfall from 2012 to 2015 was 558 mm, and on average the

112 70% of total rainfall occurred from October to March. The average ET_0 (Allen et al., 1998) was 1022
113 mm, resulting in an average aridity index of 0.56.

114 2.2. *Study layout*

115 The study was carried out from autumn 2012 to spring 2015 at eight fields within silvopastoral farms.
116 Each field was a homogeneous and fenced wooded grassland. The fields during the observational
117 period had almost similar grazing management in terms of breed (dairy sheep, occasionally beef
118 cattle) and stocking rates, both according to farmer's management choices (Seddaiu et al., 2018).

119 The study layout was a two-factor hierarchical design, which was set as follows:

120 • Tree cover as the main factor, which was distinguished in two levels: i) high-covered fields
121 (High), with tree cover ranging from 42% to 39% of the field area, and ii) low-covered fields
122 (Low), with a tree cover ranging from 36% to 15%. The tree-covered area within each field
123 was measured by analyzing the available online orthophotos of the Sardinia Region
124 (<http://webgis.regione.sardegna.it/geoserver/ows?>). The distinction between High and Low
125 was carried out also taking into account the spatial distribution of trees. The spatial
126 distribution allowed to differentiate between them fields having similar cover but different
127 pattern of tree aggregation, i.e. identifying or not identifying large open grazing areas. The
128 spatial distribution pattern was defined according to the Uniform Angle Index (W) (Zhao et
129 al., 2014), which allows identifying regular, random, and cluster patterns of tree spatial
130 distribution. Each level was replicated in four different fields.

131 • Position with respect to the tree crown projection, which was distinguished in two levels: i)
132 areas below the crown projection of randomly selected trees (BT), and ii) areas outside the
133 tree crown projection (OT), far enough to trees to avoid any influence e.g. shading effects.

134 Field cover, W index values and patterns, and related classification are in detail reported in Figure 1.

135 All the variables were measured, unless otherwise specified as reported below in the text, in six

136 sampling points per field: three points BT, and three points OT. A list of the acronyms here and below
137 reported is provided in the Appendix (Table A.1).

138 2.3. Measurements

139 2.3.1. Soil Respiration, Water Content, and Temperature

140 The measurements of soil CO₂ fluxes were carried out from 1st August 2013 to 5th August 2014. The
141 total Soil Respiration (SR) and its heterotrophic fraction (Rh) were measured *in situ* at BT and OT
142 positions, using a portable, closed chamber (Smith et al., 2010), soil respiration system (EGM-4 with
143 SRC-1, PP-Systems, Hitchin, UK, <http://ppsystems.com>). Measurements of SR and Rh were carried
144 out between 8:30 and 13:00 (solar time) to collect data representative of daily means (Almagro et al.,
145 2009 and citations therein). Measurements of Rh were made in trenched plots (Hanson et al., 2000)
146 adjacent to SR sampling points, in which soil was isolated with a PVC cylinder (0.40 m diameter and
147 0.25 m height) open at both ends (Lai et al., 2012). The cylinder was inserted into the soil profile till
148 0.20 m depth, assuming this depth as sufficient to exclude the large part of active tree root biomass,
149 according to Unger et al. (2009, and citations therein). The soil within the cylinder was weeded using
150 10 mL L⁻¹ of glyphosate (ROUNDUP® POWER 2.0). The measurements started about six months
151 after trenching to avoid any influence of weeding on respiration (Lane et al., 2012) and disturbance
152 due to decomposition of decaying roots (Unger et al., 2009). The new seedlings emerging within the
153 cylinders over the observational period were immediately and carefully cut to ensure the absence of
154 any root activity.

155 At the same time and adjacent at the sampling point, soil temperature (SoilT, °C) (by using a
156 thermometer HD2101.2, Delta Ohm, Padua, Italy) and water content (SWC, %) (using a FieldScout
157 TDR 300 Soil Moisture Meter, Spectrum Technologies, Inc., Bridgend, UK) were measured at -10
158 cm and -7 cm depth, respectively.

159 Daily SWC and SoilT from February 2014 to May 2015 were measured at both BT and OT both at
160 one sampling point per plot using a WatchDog 1000 Series Micro Station (Spectrum Technologies,

161 Inc., IL, USA, <http://www.specmeters.com>), equipped with a WaterScout SM 100 Soil Moisture
162 Sensor and Soil Temperature Sensor for SoilT and SWC, respectively. The SoilT and SWC sensors
163 were placed in the soil at 10 cm and 7 cm depth, respectively. To calculate the soil C balance along
164 the whole period, from September 2012 to January 2014 daily SWC and SoilT at OT plots were
165 estimated using the PaSim - Pasture Simulation Model (Riedo et al., 1998), properly calibrated, and
166 validated for the study area (Pulina et al., 2018a). The monthly Rh at BT was estimated from monthly
167 OT/BT ratios observed from February 2014 to May 2015.

168 2.3.2. *Pasture production*

169 Pasture dry matter (DM) production and residues were measured at about monthly sampling intervals
170 between October 2012 to May 2015 using 1 m x 1 m movable grazing exclusion cages (Frame, 1981).
171 Cages were placed onto the ground in the sampling areas. Under BT, cages were placed fully below
172 the crown projection about 1.5 m away to the tree trunk in the North-East orientation. The North-East
173 orientation (mainly unshaded) was taken because the dominant wind in the study area is from the
174 South-West direction and the trees have typically flag-shape crowns. The pasture DM production
175 between two sampling dates (from day1 to day2) was calculated making the difference between the
176 DM biomass measured inside the cage at day2 and the DM biomass outside the cage at day1. The
177 intake was calculated as the production from day1 to day2 plus the difference of DM outside the cage
178 between day 1 and 2. Methodology, sampling intervals, and animal intake computation are in detail
179 described by Seddaiu et al. (2018).

180 2.3.3. *Stoking rates and animal faeces*

181 The stocking rates (LSU ha⁻¹) at each field were monitored over the whole period by weekly recording
182 the stocking size, the animal typology and condition, and the actual grazing hours. Monthly average
183 values of stoking rates were hence used to calculate the faeces amount produced per stock unit (see
184 below).

185 2.3.4. *Tree standing biomass*

186 The diameters of *Q. suber* L. trees were measured within fields in sixteen circular sampling plots –
187 three of which around the BT sampling plots above described –with variable size (radius ranging
188 from 9.8 m to 29.0 m) according to the local stand density and neighborhood competition. Each plot
189 was centered at one of the scattered cork oak trees inside the plot. If trees were close to each other,
190 the plot center was established to be coincident with their barycentre. The above- and belowground
191 stands biomass was determined using the allometric equations developed by the National Institute of
192 Agricultural Research and Technology and Food of Spain (Montero et al., 2005), whose parameters
193 are reported in the Appendix (Table A.2). The field-scale standing biomass was estimated through
194 linear regression models between the within-plot cover and both above- and belowground biomass.

195 2.3.5. *Tree litterfall*

196 Tree litterfall was sampled from September 2014 to September 2015 in three areas per field identified
197 by trees. Four traps (0.7 m x 0.7 m) were placed in each area 1.0 m upper the soil level. The trap was
198 a plastic net with meshes of 2 mm x 2 mm wide, aiming to guarantee water drainage without make
199 the litterfall pass. Three of the traps were placed starting from the trunk following the cardinal NE
200 direction according to spatial criteria: the first trap was placed within the space from the trunk to half
201 of the crown's radius length; the second trap was placed within the space of the second half of crown's
202 radius; the third one was placed in the orthogonal direction with respect to the first three ones, in the
203 homologous position of the first trap The average amount of litterfall collected within these first three
204 traps represented the amount of litterfall BT per sampling date. The fourth trap was placed outside
205 the crown in the same direction of the first and second trap, and keeping almost the same distances
206 between the first and the second trap. The distance from the edge of the crown of this last trap allowed
207 to avoid any influence of adjacent trees, especially in high-covered fields. . The litterfall collected in
208 the fourth trap represented the amount of litterfall OT per sampling date. The collected samples were
209 dried in a ventilated oven at 65 °C until achieving constant weight, after their separation in wood,
210 leaves, flowers, and acorns components.

211 2.4. Soil C balance

212 The soil C balance was calculated in a three-year timeframe (September 2012 to May 2015) as the
213 differences between the soil C losses as cumulated Rh in the considered period, and the soil C input
214 in the same timeframe, by following the framework proposed by Lai et al. (2018) and Pulina et al.
215 (2018b).

216 2.4.1. Soil C output

217 At each plot, soil C output was calculated as the result of the cumulative Rh (Mg ha^{-1} of C-CO₂),
218 obtained as the sum of the average cumulative daily values of Rh (kg ha^{-1} of C-CO₂). To estimate
219 daily cumulative Rh values within plots, a multiple regression model (Tang and Baldocchi, 2005)
220 was fitted as a function of both SWC and SoilT, as follows:

221
$$F = \beta_0 e^{\beta_1 T} e^{\beta_2 \theta + \beta_3 \theta^2} \quad (1)$$

222 where F ($\mu\text{mol m}^{-2} \text{s}^{-1}$ of CO₂) was the measured Rh, T ($^{\circ}\text{C}$) and θ ($\text{m}^3 \text{m}^{-3}$) were the contemporary
223 measured SoilT and SWC, respectively, and $\beta_0, \beta_1, \beta_2,$ and β_3 are the model parameters. The equation
224 was log-transformed to conduct the linear regression to estimate the parameters (Table 2), which were
225 hence used to estimate daily cumulative Rh by fitting the model with the daily average values of
226 measured SoilT and SWC.

227 2.4.2. Soil C input

228 The soil C input at the end of the yearly observation period (September 30th) was represented by the
229 C input from the herbaceous component of the pasture (C_{pas}), the C input from the grazing animal
230 faeces (C_{fae}), and the C input from trees (C_{tre}).

231 The C_{pas} was calculated as follows:

232
$$C_{pas} = C_{res} + C_{roo} + C_{rhi}$$

233 where C_{res} was the C input from pasture aboveground residues, C_{roo} was the C input from the
234 herbaceous decomposed root, and C_{rhi} was the C input from herbaceous species rhizodeposition. The
235 C_{res} was yearly estimated from pasture DM residues. The OT C_{roo} was calculated starting from the
236 Root biomass (R_b), which was estimated by multiplying the yearly aboveground DM biomass
237 production with a root:shoot ratio of 2.43, resulting from the average value between those proposed
238 by Mokany et al. (2006) for temperate grassland and savanna ecosystems. The R_b was then multiplied
239 by a fine roots turnover rate of 0.80 (Casals et al., 2010). The BT C_{roo} was calculated by multiplying
240 the OT value by 1.45, resulting in the average ratio between BT and OT root:shoot derived from what
241 was observed by Nair et al. (2019). The yearly C_{rhi} was estimated by multiplying the yearly R_b by
242 0.65 (Lai et al., 2017).

243 The C_{fae} was calculated starting from the amount of estimated monthly faeces from grazing animals.
244 The monthly amount of faeces was estimated according to Van Soest (1994) by following the
245 equation

$$246 \quad F_a = I - (D * I)$$

247 where F_a was daily faeces produced per animal, I was daily DM intake per animal, and D the DM
248 digestibility rate estimated at 0.7 (Van Soest, 1994).

249 The C_{tre} was calculated as a sum between the C input from the tree root system (C_{trs}), and the C
250 input from tree litterfall (C_{lit}).

251 The C_{trs} was calculated starting from the estimated tree belowground biomass as follows:

$$252 \quad C_{trs} = C_{fin} + C_{rhf}$$

253 where C_{fin} was the yearly C input from tree fine roots turnover and C_{rhf} was the yearly C input from
254 tree fine roots rhizodeposition. The belowground biomass C content was calculated by multiplying
255 by 0.50 (Merino et al., 2003; Montero et al., 2005) the estimated tree belowground biomass at field
256 scale.

257 The C_{fin} was estimated multiplying by 0.20 (Millikin and Bledsoe, 1999) the belowground stored C,
258 which was calculated dividing that estimated according to the allometric equations by 0.80, as only
259 the coarse roots (80% of total root biomass) were taken into account by the model, and then dividing
260 the obtained value by a fine roots turnover coefficient of 0.3 yr^{-1} (López et al., 2003). The Cr_{hf} was
261 hence estimated by multiplying the fine roots C by 0.50 (Pausch and Kuzyakov, 2018).

262 The Cl_{it} was calculated by first multiplying each measured litterfall component by their C
263 concentration, and then multiplying the litter C content by a yearly litterfall decomposition rate of
264 58% (Arosa et al., 2017). The C content in litterfall was measured twice (at autumn-winter and
265 summer-spring period, respectively) by an elemental analyzer (CHN628, LECO Corporation, MI,
266 USA).

267 2.5. Data Analysis

268 The effect of study factors on the relationships between both SoilT-SWC and CO_2 fluxes was
269 assessed. The significance of the linearized $\text{Flux} = a e^{b \text{SoilT}}$ (Rey et al., 2002) was assessed to test the
270 effect of SoilT on both SR and Rh. The model was assessed above the SWC threshold of 15.9%,
271 corresponding to a matric potential of -100 kPa (Saxton and Rawls, 2006), below which the limiting
272 water conditions overlap the temperature effects, according to Lado-Monserrat et al. (2014). Below
273 the same threshold, the effect of SoilT was tested through a simple linear model (lm). The effect of
274 SWC was tested by a second-degree polynomial model after SWC normalization with respect to a
275 fixed value of soil temperature ($25 \text{ }^\circ\text{C}$), according to Tang and Baldocchi (2005). The fitted models
276 were compared considering both SoilT and SWC as covariates. Contrasts between the levels of the
277 factors were performed testing the fitted ANCOVA models by multiple comparisons under general
278 linear hypothesis (glht).

279 The effect of the interaction between tree class and position and monitoring dates on SR, Rh, SWC,
280 SoilT, and tree litterfall was tested by generalized least squares (gls) models, through which models
281 with different correlation structures were tested (Onofri et al., 2016). The best model was identified

282 making an “*a posteriori*” selection based on the Akaike Information Criterion (AIC). The effect of
283 factors on pasture production and residues and the C balance items, as well as the field-scale C
284 balance, was tested by fitting a linear mixed model (lme) considering the random effect of the year
285 where necessary. Only the effect of the tree class was tested for C_{fae}, C_{fin}, C_{rhf}, and C_{trs}. If the
286 analysis of variance (anova) of the fitted model was significant, the least-square means (eemmeans)
287 were computed to compare means between levels. The parameters of the polynomial regression in
288 (Eq. 1) were estimated fitting a linearized model at each field for both BT and OT areas. The ANOVA
289 was computed on fitted models to assess their significance.

290 The C balance and their components (Mg ha⁻¹ of C) were also field-scaled by making the sum of the
291 weighted C values at both BT and OT according to the tree-covered (Tcov) and non-covered area of
292 each field, as follows:

$$293 \text{ Weighted } C = C_{BT} T_{COV} + C_{OT} (1 - T_{COV})$$

294 Linear and polynomial regressions were performed to assess the effects of the tree cover (%) on
295 weighted C_{pas}, C_{tre}, C_{input}, C_{output} and C_{balance}; the best models between linear and polynomial
296 are reported in the results. The maximum value of the second-order regression between C_{balance} and
297 cover was calculated through the first derivative of the regression function. The significance of the
298 Pearson’s correlation coefficient (cor.test) was tested to assess the relationship between weighted
299 SOC and C balance items. The significance of statistics was evaluated at P<0.05 unless otherwise
300 stated. The lm, anova and cor.test, glht (Hothorn et al., 2008), gls and lme (Pinheiro et al., 2018),
301 emmeans (Lenth, 2018) computations were performed using the RStudio application of R software
302 (version 3.5.1) (R Core Team, 2021).

303

304 **3. Results**

305 *3.1. Soil Respiration, Water Content, and Temperature*

306 The position significantly influenced the relationships between both SR and Rh and SoilT above the
307 SWC threshold of 15.9%, while no significant effects of SoilT on both SR and Rh were observed
308 above the same SWC critical value. Overall, under High-BT both SR and Rh showed a higher
309 sensitivity to SoilT increase than the other levels of position, as reported in Figure 2. The position
310 influenced the relationships between both normalized (with respect to a constant SoilT of 25 °C) SR
311 and Rh and SWC. Under High-BT the response of both SR and Rh to SWC variation was different
312 than OT positions, while differences between High-BT and High-OT were observed only for Rh, as
313 reported in Figure 3.

314 The SR and Rh were both significantly influenced by the interaction between the monitoring dates
315 and the position ($P < 0.0001$). Overall, both SR and Rh dynamics showed two peaks (autumn and
316 spring) profiles. Maximum fluxes of soil CO₂ were observed on May 20th in High-BT, when SR and
317 Rh were $12.03 \pm 2.02 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $8.06 \pm 0.58 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Minimum soil CO₂
318 emissions occurred on September 27th in High-OT areas, where SR and Rh were $0.73 \pm 0.12 \mu\text{mol m}^{-2} \text{s}^{-1}$
319 and $0.55 \pm 0.15 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Figure 4a,b). The SR was higher in High-BT than OT
320 in late spring and autumn (Aug 27th, Sep 11th, Oct 22nd, and Nov 8th), and at the beginning of summer
321 (June 24th), while no differences between treatments within dates were observed in winter and early
322 spring 2014 (Figure 4a). The Rh was higher in High-OT than the other treatments at the beginning of
323 autumn 2013 and from May 20th, 2014 to June 24th, 2014 (Figure 4b). No differences between areas
324 in both SR and Rh were observed in winter.

325 The SWC was significantly influenced by the interaction between the monitoring dates and the
326 position ($P < 0.0001$). Overall, the SWC showed an increasing dynamic from summer to spring, to
327 then decrease until the end of the experiment (Figure 4c). The maximum SWC was observed on
328 March 27th under Low-BT ($0.32 \pm 0.02 \text{ m}^3 \text{ m}^{-3}$), which was higher than High-OT ($0.27 \pm 0.02 \text{ m}^3 \text{ m}^{-3}$),
329 while no differences were observed with the other treatments ($0.29 \pm 0.01 \text{ m}^3 \text{ m}^{-3}$ on average in High-
330 BT and Low-OT, respectively). Minimum SWC was observed in High-OT at the beginning of the

331 monitoring period ($0.02 \pm 0.00 \text{ m}^3 \text{ m}^{-3}$), which was lower than Low-BT and Low-OT (0.05 ± 0.0 and
332 $0.06 \pm 0.01 \text{ m}^3 \text{ m}^{-3}$).

333 The SoilT dynamics were significantly influenced by the interaction between the date and the position
334 ($P < 0.0001$). The SoilT overall showed an opposite dynamic to SWC (Figure 4d). The maximum
335 values were observed on August 1st, 2013 in OT areas ($31.5 \pm 1.2 \text{ }^\circ\text{C}$ and $31.2 \pm 0.6 \text{ }^\circ\text{C}$ in High-OT and
336 Low-OT, respectively), as well as the minimum values, observed at November 29th, 2013 (5.0 ± 0.7
337 $^\circ\text{C}$ and $6.7 \pm 0.3 \text{ }^\circ\text{C}$ in High-OT and Low-OT, respectively). Overall, the OT SoilT was higher and
338 lower than the other treatments first during summer and spring and then in late autumn and winter,
339 respectively, as reported in Figure 4d.

340 3.2. Pasture production and residues

341 The pasture biomass production ($\text{Mg ha}^{-1} \text{ yr}^{-1}$ of DM) averaged for the three-year experiment was
342 significantly influenced ($P < 0.0001$) by both tree class and position. The biomass production on
343 average was higher under Low-OT ($3.8 \pm 0.3 \text{ Mg ha}^{-1}$ of DM) than Low-BT and High-OT (2.6 ± 0.3
344 Mg ha^{-1} and $2.5 \pm 0.3 \text{ Mg ha}^{-1}$ of DM, respectively), in which the biomass production was in turn
345 higher than High-BT ($1.4 \pm 0.3 \text{ Mg ha}^{-1}$). The average biomass residues at the end of the year (Mg ha^{-1}
346 yr^{-1} of DM) were significantly influenced ($P < 0.01$) by the tree class, while no significant effects of
347 the position were observed. The residues were on average higher under Low ($1.4 \pm 0.2 \text{ Mg ha}^{-1}$) than
348 High ($0.9 \pm 0.2 \text{ Mg ha}^{-1}$). The average pasture biomass production and residues along the experiment
349 are reported in Figure 5.

350 3.3. Tree and Litterfall Biomass

351 The tree above- and belowground biomass was higher ($P < 0.05$) in High (on average $53.7 \pm 1.5 \text{ Mg ha}^{-1}$
352 1 and $11.0 \pm 0.3 \text{ Mg ha}^{-1}$ above and below, respectively) than the Low class ($28.6 \pm 8.2 \text{ Mg ha}^{-1}$ and
353 $6.0 \pm 1.6 \text{ Mg ha}^{-1}$ above and below, respectively).

354 The tree litterfall biomass (Mg ha^{-1} of DM) was influenced by the interaction between the sampling
355 date (month) and the position ($P < 0.0001$). The tree litterfall dynamic had a bimodal profile, whose
356 two maximum values were observed in May and in October, respectively. In May 2014 the litterfall
357 was higher in High-BT ($2.7 \pm 0.4 \text{ Mg ha}^{-1}$) than Low-OT ($0.8 \pm 0.4 \text{ Mg ha}^{-1}$), while in October 2013 the
358 litterfall was higher in High-BT ($0.8 \pm 0.1 \text{ Mg ha}^{-1}$) than the other levels. The litterfall dynamics and
359 the differences between position/cover within the month are reported in Figure 6.

360 3.4. Soil C Balance

361 The position significantly influenced the Clit, the Ctre, the Cinput, the Coutput ($P < 0.0001$), and the
362 Cbalance ($P < 0.001$), while the Cres ($P < 0.01$), Croo, Crhi, Cpas ($P < 0.0001$) were significantly
363 influenced by the tree class. The Cpas and all the components determining it (Cres, Croo, Crhi) were
364 always higher in Low than High. The Clit, the Ctree, and the Coutput were higher in High-BT than
365 Low-BT, in which were higher than High-OT and Low-OT. The Cinput was higher in High-BT and
366 High-OT than Low-OT, in which in turn the Cinput was higher than High-OT. The overall Cbalance
367 was higher in Low-BT than High-BT, in which was in turn higher than High-OT and Low-OT. No
368 significant effects of factors were observed for Cfin, Crhf, Ctrs, and Cfae. Results are in detail
369 reported in Table 3. Table 3.

370 Considering the C balance items at field scale weighted according to the tree cover, the the tree cover
371 significantly influenced Cpas, Ctre, Coutput, and Cbalance at field scale, while a slight significant
372 effect on Cinput ($P = 0.023$) was observed (Figure 7). The overall C balance at field scale was higher
373 under Low ($4.36 \pm 0.34 \text{ Mg ha}^{-1}$ of C) than High ($2.43 \pm 0.34 \text{ Mg ha}^{-1}$ of C) fields, as reported in Figure
374 8. A significant Person's correlation coefficient (0.41, $P < 0.05$) between SOC and Cpas was observed,
375 while no significant correlations were observed between SOC and Ctre, Cinput, Coutput, and
376 Cbalance.

377

378 4. Discussion

379 *4.1. The effect of trees on SoilT, SWC, and CO₂ emissions*

380 The SoilT and SWC showed inverse dynamics over the monitoring period, as widely reported for
381 Mediterranean areas (e.g. González-Ubierna and Lai, 2019; Pulina et al., 2018a). Consequently, the
382 highest temperatures are associated with the driest periods, during which water scarcity strongly
383 affects the SOC cycle and hence SR and Rh (Almagro et al., 2009). The observed relationship
384 between SWC and normalized CO₂ fluxes confirmed this as the response of both SR and Rh to SWC
385 variation was directly proportional for SWC values below the critical threshold (15.9%). Above the
386 threshold, when water availability was not limiting for microbial and roots respiration, both SR and
387 Rh were mostly driven by SoilT variation. At the same time, under constant SoilT, the response of
388 CO₂ fluxes to SWC above the critical threshold tends to be inversely proportional, probably due to a
389 reduction of oxygen availability for respiration processes (Lado-Monserrat et al., 2014; Tang and
390 Baldocchi, 2005).

391 The higher sensitivity of both SR and Rh to SoilT and SWC variation observed in High-BT than OT
392 areas was associated with the higher accumulation of labile SOC and nutrients beneath trees than
393 outside the tree canopy projection (Cappai et al., 2017; García-Angulo et al., 2020; Seddaiu et al.,
394 2018; Uribe et al., 2015) which, in turns, was mainly related to trees litterfall (Cappai et al., 2017;
395 Rossetti et al., 2015b). Furthermore, the higher response of SR-Rh to increasing SoilT and SWC at
396 High-BT positions could be also led to an overall improvement of soil fertility and nutrients under
397 the tree canopy (Moreno and Obrador, 2007; Nair et al., 2019), that promotes physiological activities
398 of heterotrophic communities (Lai et al., 2014; Rossetti et al., 2015a) and that it is particularly marked
399 in high tree-covered areas (Bagella et al., 2020). Nevertheless, the absence of differences between the
400 responses of SR under High-BT and Low-BT to SWC variations could be explained as a result of
401 increased autotrophic activity due to the observed higher pasture biomass production beneath the tree
402 canopy under low-covered areas, which is below discussed.

403 *4.2. The effect of trees on biomass production of the herbaceous layer and litterfall*

404 On average, the three-year DM production of the herbaceous layer irrespectively of the cover class
405 was about two-fold higher outside the tree crown projection than beneath the tree. The detrimental
406 role of the trees on the biomass production of grasslands was already reported for Mediterranean sites
407 by Seddaiu et al. (2018) in the same study site and by other authors in the Spanish dehesa (López-
408 Carrasco et al., 2015), in the Portuguese montado (Serrano et al., 2018), and silvopastoral systems of
409 Northern Greece (Klossas et al., 2012). However, other authors highlighted a negligible impact of the
410 tree on pasture production (e.g. Carranca et al., 2015) or even a beneficial effect (e.g. Moreno, 2008)
411 depending mainly on soil water conditions that, in turn, were related to the annual rainfall amount. In
412 fact, under wetter conditions a much greater herbage yield of the pasture component underneath the
413 tree canopy was reported, while in drier ones differences were smoother or null or even the opposite
414 (Moreno, 2008; Seddaiu et al., 2018). In the Spanish dehesas, López-Carrasco et al. (2015) observed
415 a lower yield of the herbaceous layer under the tree than in open areas when annual rainfall was lower
416 than 600 mm. This water limiting effect was also reported by Lozano-Parra et al. (2018), who in
417 Spanish dehesas observed a more intense reduction of pasture growth below the tree canopies than
418 the open grassland during the driest years, as a result of more intense water deficit beneath the trees.
419 Other factors that may have influenced biomass production under the tree are the light and nutrients
420 competition between tree and herbaceous components (e.g. Orefice et al., 2019; Serrano et al., 2018)
421 and the different grazing behavior of the livestock. Grazing animals exploit less efficiently the
422 available pasture biomass beneath the tree than outside the tree canopy (Seddaiu et al., 2018) and this
423 can constrain pasture growth that can be promoted by grazing pressure if not too intense. Under
424 Mediterranean climate in sub-clover and ryegrass-based grasslands in Australia, Witschi and Michalk
425 (1979) observed a detrimental effect of both treading and high grazing pressure on late winter
426 production and pasture recovery. Areas beneath the tree canopies are submitted to intense treading
427 since animals rest below the tree crowns after grazing to restore during the hottest hours in spring and
428 summer (e.g. Bojkovski et al., 2014), and this could contribute to the lower forage production
429 underneath the trees (Seddaiu et al., 2018). Furthermore, the lower pasture growth below the tree

430 canopies could also be associated to the reduced PAR penetration to the ground (Marañón et al., 2009)
431 for the high amount of litterfall, up to nearly 90% higher than in the open areas, which can affect seed
432 germination and seedling establishment, especially of the more light demanding plant species
433 (Rossetti et al., 2015).

434 The strong influence of season in affecting the pattern of cork oak litterfall observed in this study
435 agrees with what was widely described under Mediterranean conditions (e.g. Andivia et al., 2018;
436 Andivia et al., 2015; Caritat et al., 2006; Zribi et al., 2015). The higher litterfall amount under High-
437 BT observed during almost the whole observation period was mainly interpreted as the result of
438 higher tree cover and higher tree vicinity. Under tropical forests with different tree species, Uriarte et
439 al. (2015) associated the observed litterfall increase to the effect of trees neighborhood, and in the
440 meanwhile of tree cover, in establishing favorable edaphic conditions in terms of nutrient (N, P, and
441 C:N) cycles. Therefore, the tree distribution patterns could play a positive role in nutrient cycling and
442 then can explain the increasing tree productivity and litterfall beneath trees in high tree-covered areas.

443 *4.3. The effect of trees on the annual soil C balance*

444 The higher C_{pas} under Low fields reflects what was observed and discussed above about pasture
445 productivity and residues, which led also to a higher contribution of root systems to the C input. The
446 contribution of trees to the C input was higher under High-BT due to a higher litterfall amount beneath
447 the trees, though the cover class did not influence the C_{tr}s, as a result of even-aged tree stands within
448 the study area (Rossetti and Bagella, 2014). The contribution of trees to the total C_{input} decreased
449 from about 81% in High-BT to 64% in Low-BT, while at the same conditions the pasture contribution
450 increased from 18% to 35%. Overall, the contrasting effect of cover classes on C_{pas} and C_{tr} resulted
451 in a slight significance of the effect of tree cover on field-weighted C input.

452 As above discussed, the higher C_{output} observed in High-BT could be attributable to both different
453 SOC contents and responses to environmental drivers of Rh, particularly SWC, as already observed
454 under Mediterranean conditions. This evidence confirms that drought periods are the most important

455 factor regulating SOC fluxes under semi-arid conditions (e.g. Reichstein et al., 2002), as highlighted
456 in these experimental conditions by the lack of significance of the effect of increasing SoilT above
457 the critical SWC threshold (about 16%). Water availability plays a crucial role in affecting the C
458 balance items and hence SOC stocks in the soil. In fact, despite faster soil drying in open areas under
459 increasing temperatures, resulting, as observed in 2014, in lower SWC in OT areas in spring, under
460 OT conditions the pasture vegetation could be advantaged from the absence of competition with trees
461 for water resources (Moreno et al., 2007). Moreover, since Ctre under BT conditions is mostly
462 represented by quickly mineralizable organic matter (Cappai et al., 2017), the higher water
463 availability for microbial activities may have promoted a stronger response to both SoilT and SWC
464 variation and then higher C-CO₂ losses in these conditions. However, it is necessary to point out the
465 uncertainty associated to the methodology of Rh measurement under BT. In fact, tranching may not
466 have completely excluded the deeper tree active roots, thus leading to an overestimation of the C
467 balance in that microhabitat.

468 In this study, areas beneath trees under low cover seemed the most favorable conditions to stock soil
469 C in wooded grasslands on a yearly timeslot. These findings highlight that trees are not the most
470 important factor which allows considering Mediterranean wooded grasslands as “winning”
471 agroecosystems in terms of C sequestration capacity as suggested by other authors (Howlett et al.,
472 2011; Lagomarsino et al., 2011; Mosquera-Losada et al., 2011; Seddaiu et al., 2018; Seddaiu et al.,
473 2013) but that a relevant role is played by pasture primary productivity. Despite a higher average
474 SOC in woodland than wooded grassland topsoils, in a study conducted in the same area, Bagella et
475 al. (2020) reported no significant differences between soil C within the same position with respect to
476 the trees along a land-use gradient from open grassland to woodland and no differences between SOC
477 under open areas in woodland and open grassland. Furthermore, Bagella et al. (2020) reported also
478 no differences in SOC between wooded grasslands and “patchy” scenarios combining woodland,
479 wooded and open grassland with the same overall tree cover. These findings support our results since
480 the relationship between tree cover and C balance identified a maximum value of C balance

481 corresponding to about 24% tree cover when tree distribution pattern become to be identified as
482 cluster. Beyond the tree cover value of 24%, the C balance showed a decreasing pattern until 42% of
483 tree cover, beyond which the balance was considered in a state of equilibrium. The results of this
484 study should however be interpreted with caution, as this evidence emerged from an apparent C
485 balance, whose absolute values can be misleading. These results and the lack of significant correlation
486 between the SOC content and the C balance suggested that the C balance did not take into account
487 the plausible levels of SOC saturation under these specific conditions, and the considered C balance
488 items do not explain the different recalcitrance levels of fractions composing the organic matter input
489 to soil (Cappai et al., 2017), as well as the rates of litterfall incorporation (e.g. Ma et al., 2014) and
490 rhizodeposition (e.g. Rees et al., 2005). As reported by Cappai et al. (2017), beneath the trees SOC is
491 near to saturation or oversaturated, and this could bias the correlation between the field-scaled C
492 balance and the SOC content. Therefore, the C_{input} and the resulting C_{balance} over years should not
493 be interpreted as an absolute rate of SOC sequestration or storage (Chenu et al., 2019), for whose
494 estimation a temporal upscaling is needed in analyzing both SOC contents and C_{balance} dynamics.
495 Finally, the resulting C balance may have been affected by the propagation of the errors from every
496 single items, due to adopted “budget-like” approach, which could limit the interpretation of the
497 observed differences. Nevertheless, our results put on evidence the high potential SOC sequestration
498 ability of the studied agro-silvopastoral systems. The socio-economic contexts that cause the
499 abandonment and intensification trends threatening Mediterranean wooded grassland (Camilli et al.,
500 2017; Moreno et al., 2018), generate the need for policies supporting the maintenance of such systems
501 (Mosquera-Losada et al., 2018) also considering their high potential in climate change mitigation.

502

503 **5. Conclusion**

504 The results emerging from this study highlight that in Mediterranean cork oak-based agro-
505 silvopastoral systems the pasture productivity plays a strong role in determining the C balance and

506 thus promoting SOC sequestration. Although an important role of trees in affecting C inputs and
507 outputs was confirmed, a good pasture production both outside and beneath the tree canopy was
508 reached under lower tree cover and/or with a tree distribution from random to tending to cluster.
509 Moreover, the environmental role of water deficit seemed to play a crucial role in regulating all the
510 complex interactions between factors influencing the soil C balance in Mediterranean agro-
511 silvopastoral systems.

512 The results highlight the decisive role of agronomic management in insuring SOC sequestration in
513 wooded grassland under Mediterranean conditions, as both grassland and livestock management good
514 practices could benefit pasture production and quality. The maintenance of such agroforestry systems
515 could be considered as a win-win strategy for enhancing ecosystem services provision, but also to
516 maintain the habitat *sensu* 92/43/EEC Directive, and their conservation is strongly dependent on the
517 human intervention on trees and management of open areas.

518

519

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526

Abbreviation	Meaning	Details	References
High	High covered areas		
Low	Low covered areas		
BT	Below the Tree crown projection		
OT	Outside the Tree crown projection		
SR	Total Soil Respiration	Measured from August 2014 to August 2015	
Rh	Heterotrophic soil respiration	Estimated from September 2012 to July 2014. Measured from August 2014 to August 2015	Tang and Baldocchi, 2005
SoilT	Soil Temperature at -10 cm depth	Measured from August 2014 to August 2015. Estimated through PaSim model from September 2012 to July 2014.	
SWC	Soil Water Content at -7 cm depth	Measured from August 2014 to August 2015. Estimated through PaSim model from September 2012 to July 2014.	
Cres	C input from pasture residues	Calculated from the DM residues yearly measured at the end of grazing seasons from 2013 to 2015 and multiplied by 0.45 (C content in DM herbaceous biomass)	Seddaiu et al., 2018
Rb	Root biomass of pasture	Estimated by multiplying the yearly aboveground DM biomass production with a root:shoot ratio of 2.4, and by 0.45 to estimate the C content	Mokany et al., 2006
Croo	C input from pasture roots decomposition	Under OT: estimated by multiplying Rb * 0.80 Under BT: Rb * 0.80 * 1.45	Casals et al., 2010; Nair et al., 2019
Crhi	C input from pasture rhizodeposition	Estimated multiplying Rb by 0.65	Lai et al., 2017
Cpas	C input from pasture	Cres + Croo + Crhi	
Clit	C input from tree litterfall	Measured from September 2014 to September 2015 and then multiplied by a decomposition rate of 58%	Arosa et al., 2017
Cfin	C input from tree fine roots decomposition	Estimated multiplying by 0.20 the belowground stored C, and then by 1/0.3 turnover coefficient	Millikin and Bledsoe, 1999; López et al., 2003
Crhf	C input from tree rhizodeposition	Estimated by multiplying the C in fine roots C by 0.50	Pausch and Kuzyakov, 2018
Ctrs	C input from tree root system	Cfin + Crhf	
Ctre	C input from trees	Ctrs + Clit	
Cfae	C input from animal faeces	Estimated from the average daily DM intake per animal	Van Soest, 1994
Cinput	Total C input	Cpas + Ctre + Cfae	
Coutput	Total C output	Cumulative Rh	
Cbalance	C balance	Cinput – Coutput	

531

532 Table A.2 Values of the parameters a and b for the function $Y = e^{SEE^2/2} \cdot e^a \cdot d^b$, the adjusted
533 coefficient of determination (R^2_{adj}), and the standard error of the estimation (SEE) for *Quercus suber*
534 L. and each fraction of the biomass.

Y	a	b	R²_{adj}	SEE
BT	-3.36627	2.60685	0.914	0.416653
BF	-3.01942	2.25213	0.893	0.405357
BR7	-6.43076	3.21136	0.87	0.574419
BR2-7	-3.3924	1.99526	0.796	0.525534
BR2	-5.33638	2.10315	0.676	0.754127
BH	-6.05826	2.14483	0.648	0.818511
Br	-2.81593	2.07774	0.924	0.356549

535 d: diameter at breast height under cork (cm); BT: total tree aboveground biomass; BF: biomass of the trunk; BR7: biomass
536 of branches with a diameter greater than 7 cm; BR2–7: biomass of the branches with diameter between 2 and 7 cm; BR2:
537 biomass of the branches of diameter less than 2 cm; BH: leaf biomass and Br: root biomass (source: Montero et al., 2005).

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774

775 **Tables**

776

777 Table 1. Soil Organic Carbon (SOC, Mg ha⁻¹), total N (N, Mg ha⁻¹), and C/N ratio at 0.40 m soil depth
778 (mean value ± standard error of the mean).

	SOC (Mg ha⁻¹)	N (Mg ha⁻¹)	C/N
High	125.5±12.9	9.3±1.1	13.8±0.6
Low	141.7±14.0	10.8±1.2	13.2±0.3
High-BT	152.0±8.2 a	10.9±1.1	14.2±0.8
High-OT	99.0±15.5 b	7.6±1.6	13.5±0.9
Low-BT	162.2±11.6 a	12.0±1.0	13.6±0.2
Low-OT	121.2±22.5 ab	9.6±2.1	12.8±0.5

779 Means followed by the same letters indicate no significant differences between levels of the factors according to the least-
780 square means test (P<0.05). High= high cover; Low= low cover; OT: outside tree canopy

781

782

783 Table 2. Parameters of the polynomial regression between Rh and both SoilT and SWC for each level
784 used to estimate the daily Rh, F statistic (model variance / residual variance), degrees of freedom of
785 residual variance (df residual), and P-value.

	β_0	β_1	β_2	β_3	F value	df residual	P value
High-BT	0.18634	0.1067	0.1537	-0.0034	11.65	56	<0.0001
High-OT	0.07279	0.0590	0.2651	-0.0075	12.39	55	<0.0001
Low-BT	1.28364	0.0041	0.1120	-0.0046	6.97	56	<0.001
Low-OT	0.21601	0.0382	0.1674	-0.0046	7.97	56	<0.001

786 High= high cover; Low= low cover; BT: below tree canopy; OT: outside tree canopy

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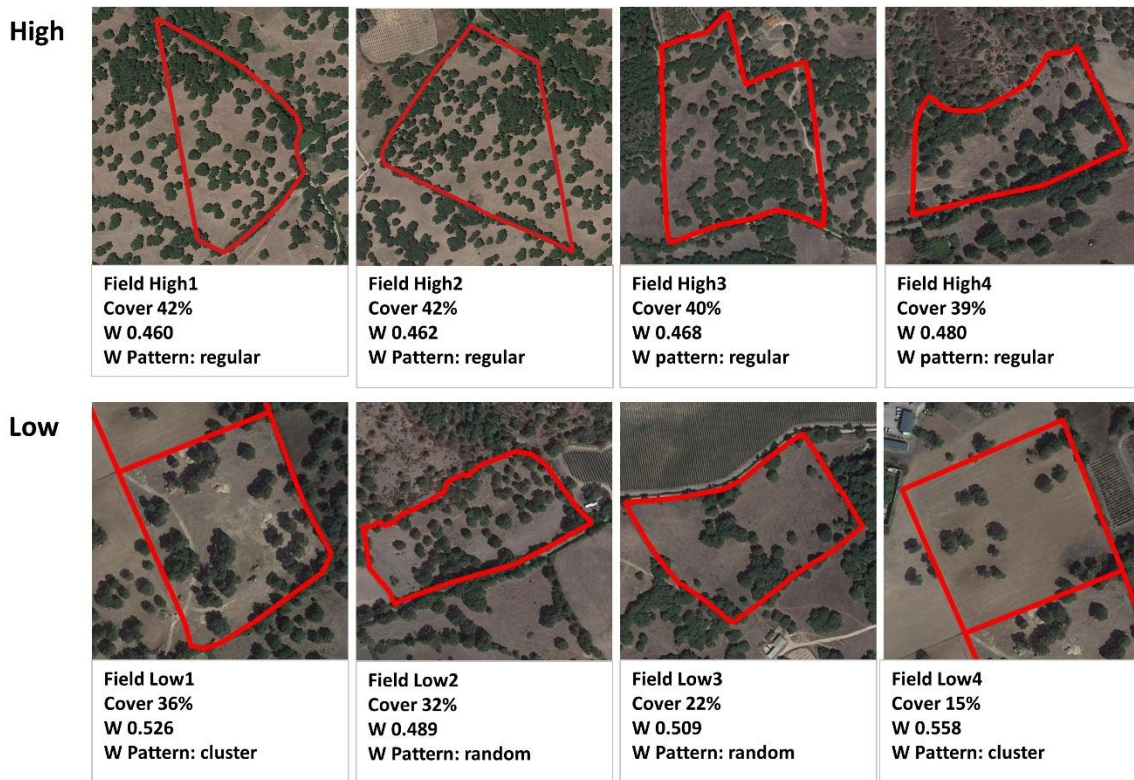
791

792 Table 3. Annual soil C balance items and overall balance (Mg ha⁻¹ yr⁻¹ of C, mean values ± standard errors of means) and P values associated with
 793 the study factors (ANOVA). Means followed by the same letters indicate no significant differences between levels according to the least-square means
 794 test (P<0.05). Values within brackets represent the range of the observations (min-max)

#	Item	Class			Position				
		P	High	Low	P	High-BT	High-OT	Low-BT	Low-OT
1	Cres	<0.01	0.41 ± 0.06 b	0.65 ± 0.06 a	NS	0.34 ± 0.04 (0.11-0.64)	0.47 ± 0.12 (0-1.31)	0.60 ± 0.04 (0.32-0.77)	0.69 ± 0.13 (0-1.38)
2	Croo	<0.0001	1.88 ± 0.19 b	3.27 ± 0.18 a	NS	1.71 ± 0.30 (0.56-3.28)	2.06 ± 0.25 (0.59-2.92)	3.25 ± 0.30 (1.58-4.62)	3.30 ± 0.20 (2.03-3.82)
3	Crhi	<0.0001	1.53 ± 0.16 b	2.66 ± 0.14 a	NS	1.39 ± 0.24 (0.69-4.03)	1.67 ± 0.20 (0.72-3.59)	2.64 ± 0.24 (1.95-5.69)	2.68 ± 0.16 (2.5-4.71)
4=1+2+3	Cpas	<0.0001	3.82 ± 0.40 b	6.58 ± 0.35 a	NS	3.43 ± 0.57 (1.44-7.74)	4.21 ± 0.56 (1.32-7.82)	6.49 ± 0.56 (3.85-10.88)	6.67 ± 0.45 (4.59-9.73)
5	Clit	<0.05	2.27 ± 0.55 a	1.57 ± 0.24 b	<0.0001	3.59 ± 0.46 a (2.88-4.93)	0.95 ± 0.25 c (0.39-1.600)	2.16 ± 0.17 b (1.86-2.47)	0.99 ± 0.11 c (0.74-1.28)
6	Cfin*	NS	11.45 ± 0.07 (11.28-11.56)	9.14 ± 1.11 (6.19-11)	-	-	-	-	-
7	Crhf*	NS	1.72 ± 0.01 (1.69-1.73)	1.37 ± 0.17 (0.93-1.65)	-	-	-	-	-
8=6+7	Ctrs*	NS	13.17 ± 0.08 (12.98-13.3)	10.51 ± 1.27 (7.11-12.65)	-	-	-	-	-
9=5+8	Ctre	<0.05	8.85 ± 3.00 a	6.83 ± 2.28 b	<0.0001	16.76 ± 0.51 a (15.86-18.23)	0.95 ± 0.25 c (0.39-1.60)	12.66 ± 1.23 b (9.54-14.73)	0.99 ± 0.11 c (0.74-1.28)
10	Cfae	NS	0.13 ± 0.02 (0.06-0.29)	0.18 ± 0.01 (0.1-0.24)	-	-	-	-	-
11=4+9+10	Cinput	NS	12.81 ± 1.62	13.58 ± 1.27	<0.0001	20.32 ± 0.5 a (18.22-24.53)	5.29 ± 0.63 c (2.38-9.5)	19.33 ± 0.72 a (15.78-22.92)	7.84 ± 0.42 b (5.97-10.7)
12	Coutput	<0.01	9.83 ± 1.06 a	7.65 ± 0.70 b	<0.0001	14.24 ± 0.90 a (9.81-21.45)	5.41 ± 0.55 c (3.14-9.49)	10.12 ± 0.94 b (4.3-13.46)	5.19 ± 0.21 c (4.09-6.56)
13=11-12	Cbalance	<0.001	2.98 ± 0.96 b	5.93 ± 0.78 a	<0.0001	6.08 ± 1.13 b (-1.72-12.79)	-0.12 ± 0.91 c (-4.91-4.3)	9.21 ± 0.73 a (4.98-12.39)	2.65 ± 0.29 c (0.99-4.33)

795 *Cfin, Crhf, Ctrs refer to average values within BT areas

796 High: high cover; Low: low cover; BT: below tree canopy; OT: outside tree canopy; Cres: C input from pasture residues; Croo: C input from pasture roots decomposition; Crhi: C
 797 input from pasture rhizodeposition; Cpas: C input from pasture; Clit: C input from tree litterfall; Cfin: C input from tree fine roots decomposition; Crhf: C input from tree
 798 rhizodeposition; Ctrs: C input from tree root system; Ctre: C input from trees; Cfae: C input from animal faeces

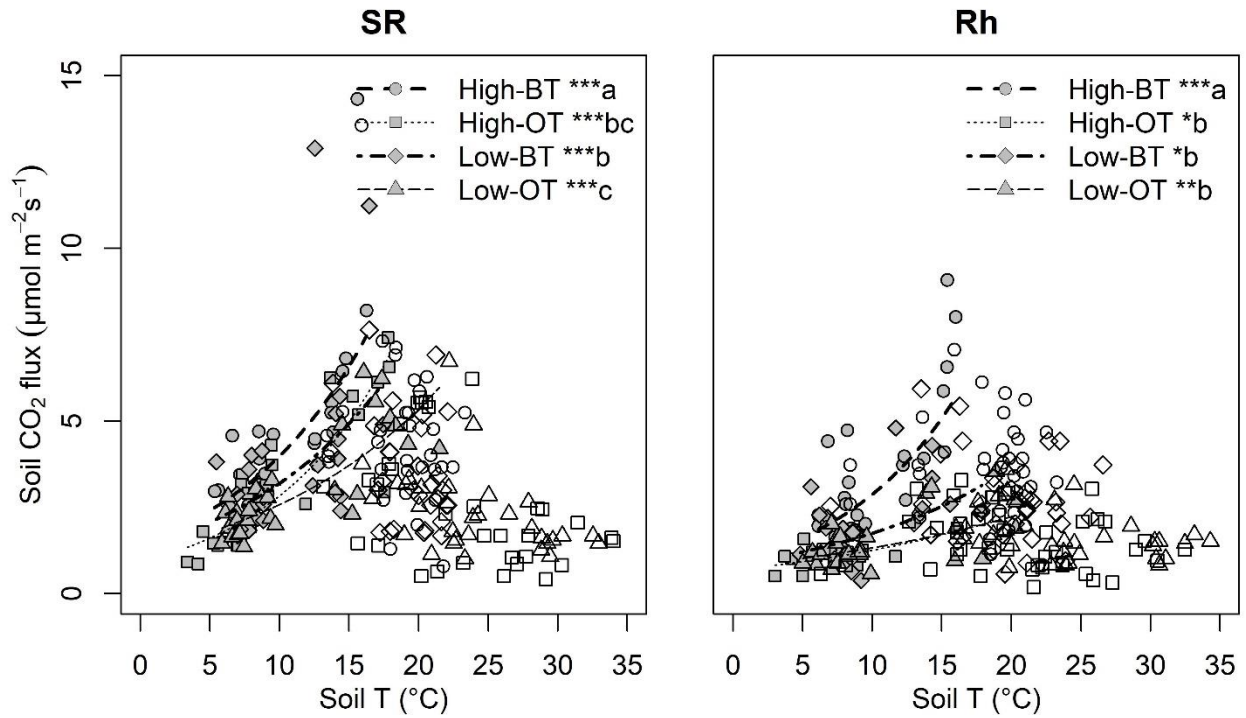


800

801 Figure 1. Study fields classification. The W pattern is defined, according to Hu et al. (2014), as
 802 random when W is within the 95% confidence interval of a reference mean of 0.500 (0.485÷0.515),
 803 regular when W is lower than the lower limit, and cluster when W is higher than the upper limit. High
 804 = high cover; Low = low cover

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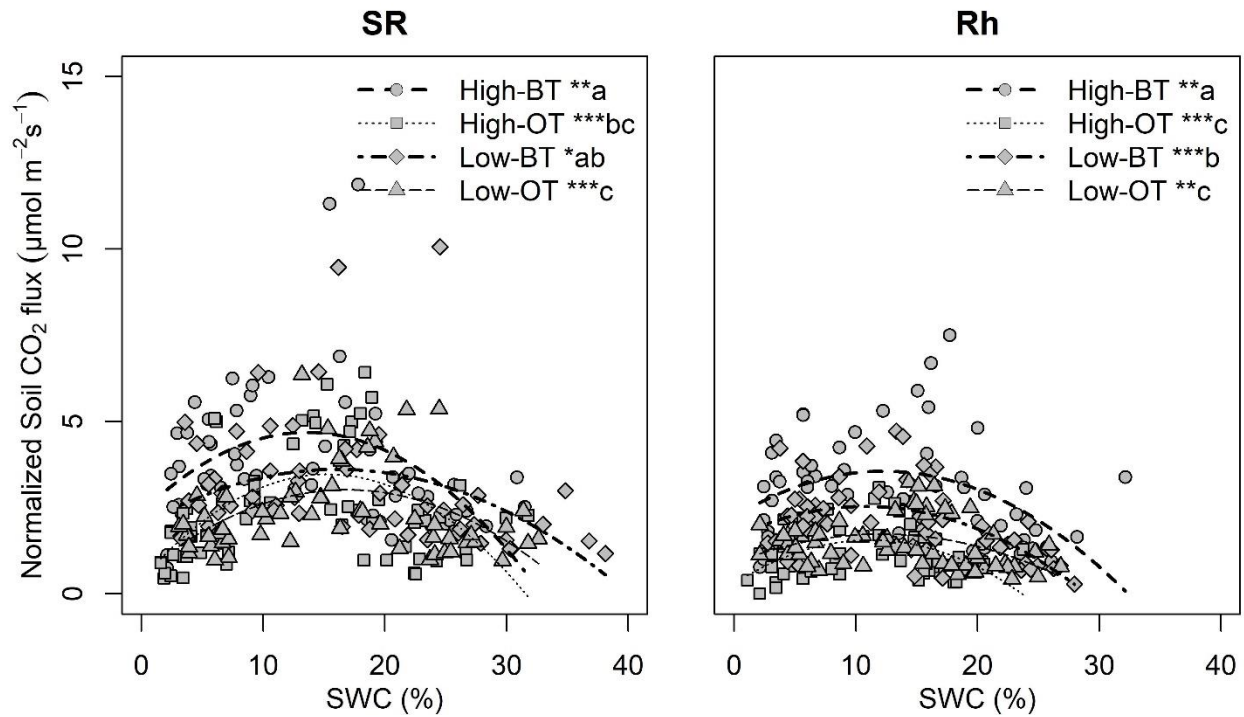
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809 Figure 2. Relationships between total Soil Respiration (SR, $\mu\text{mol m}^{-2} \text{s}^{-1}$), heterotrophic Respiration
 810 (Rh, $\mu\text{mol m}^{-2} \text{s}^{-1}$), and Soil Temperature ($^{\circ}\text{C}$) above (full bullets) and below (blank bullets) the soil
 811 water content critical threshold. Different letters indicate different models according to ANCOVA
 812 multiple comparisons. High = high cover; Low = low cover; BT: below tree canopy; OT: outside tree
 813 canopy. (***: $P < 0.0001$; **: $P < 0.001$; *: $P < 0.01$)

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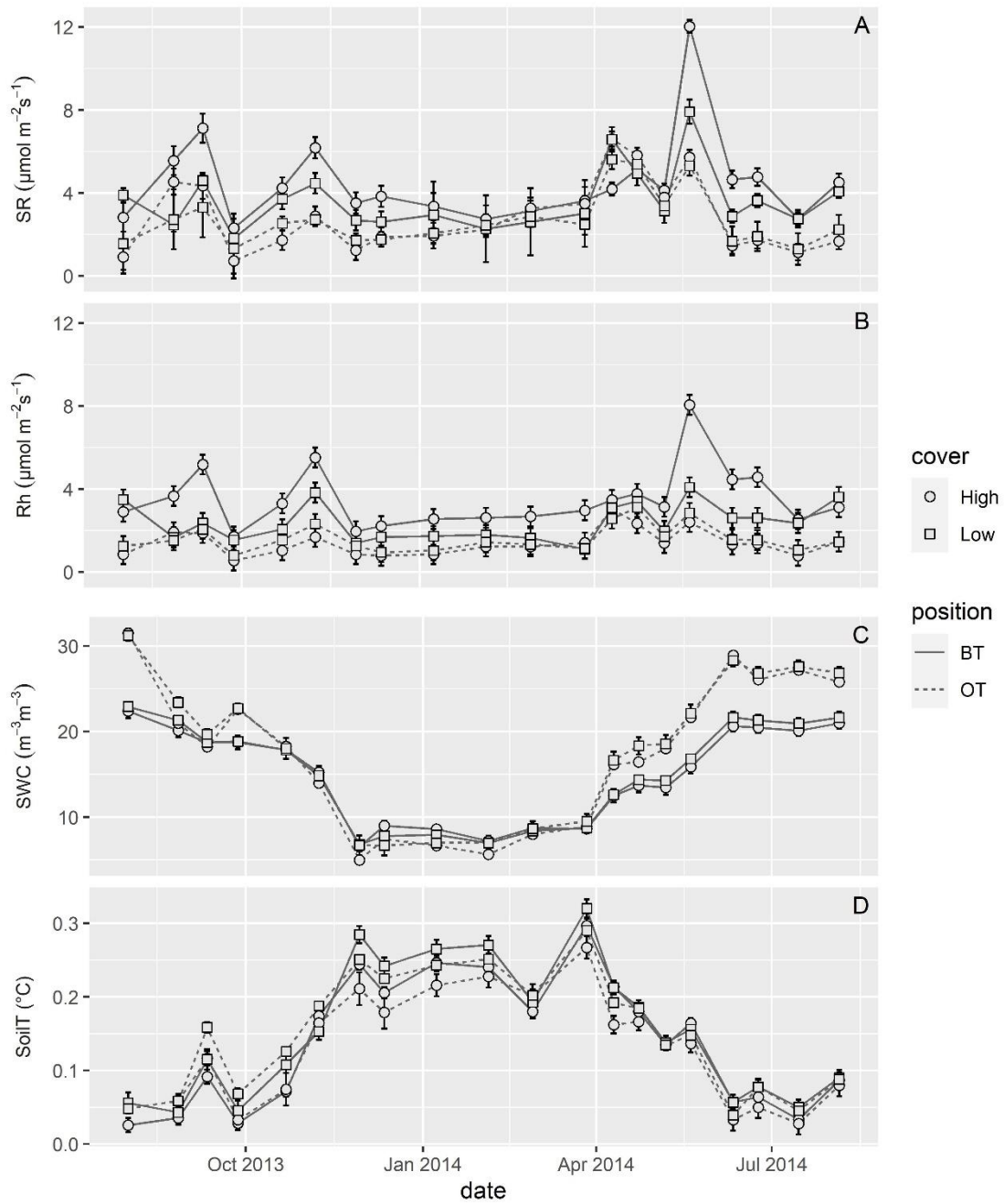
817

818 Figure 3. Relationships between normalized total Soil Respiration (SR, $\mu\text{mol m}^{-2} \text{s}^{-1}$), normalized
 819 heterotrophic Respiration (Rh, $\mu\text{mol m}^{-2} \text{s}^{-1}$), and Soil Water Content (SWC, %). Different letters
 820 indicate different models according to ANCOVA multiple comparisons. High = high cover; Low =
 821 BT: below tree canopy; OT: outside tree canopy. (***: $P < 0.0001$; **: $P < 0.001$; *: $P < 0.01$)

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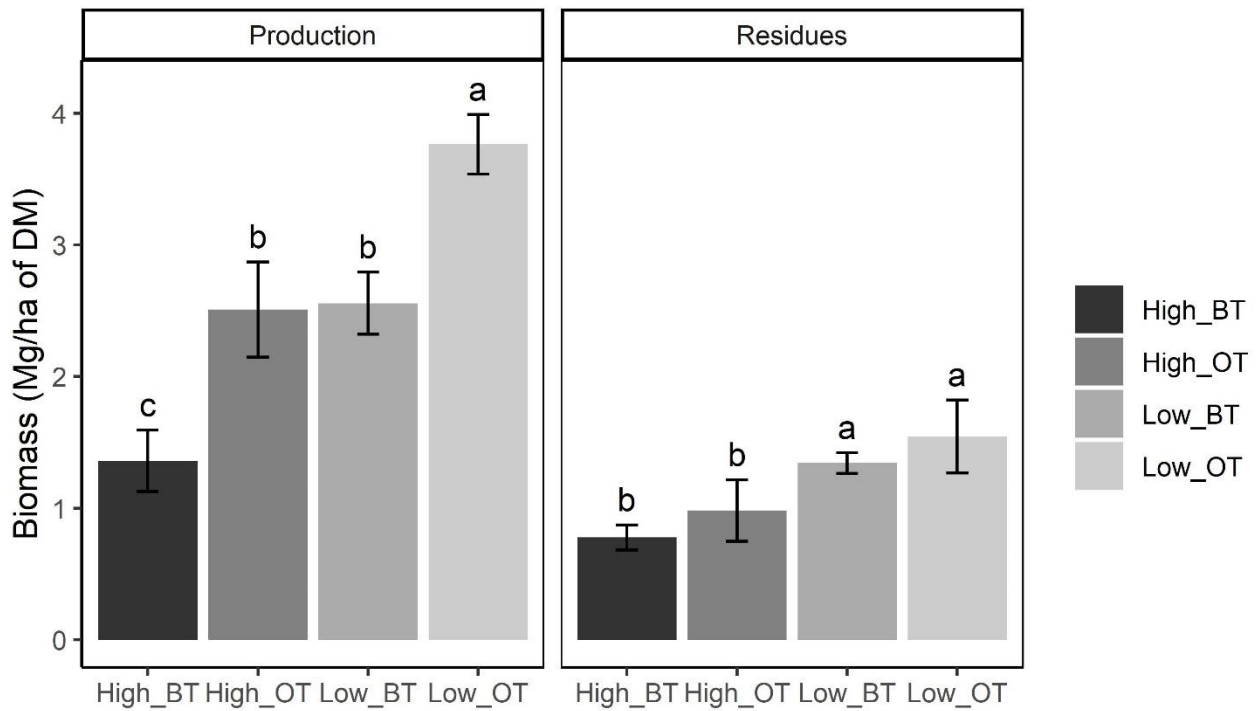


826

827 Figure 4. Dynamics of total Soil Respiration (SR, A), its heterotrophic fraction (Rh, B), Soil Water
 828 Content at -7 cm depth (SWC, C), and Soil Temperature at -10 cm depth (T – 10 cm, D) from August
 829 2013 to August 2014. Vertical bars represent the within-date standard error of the least square means
 830 (n=4). High = high cover; Low = low cover; BT = below the tree crown; OT = outside the tree crown
 831 projection

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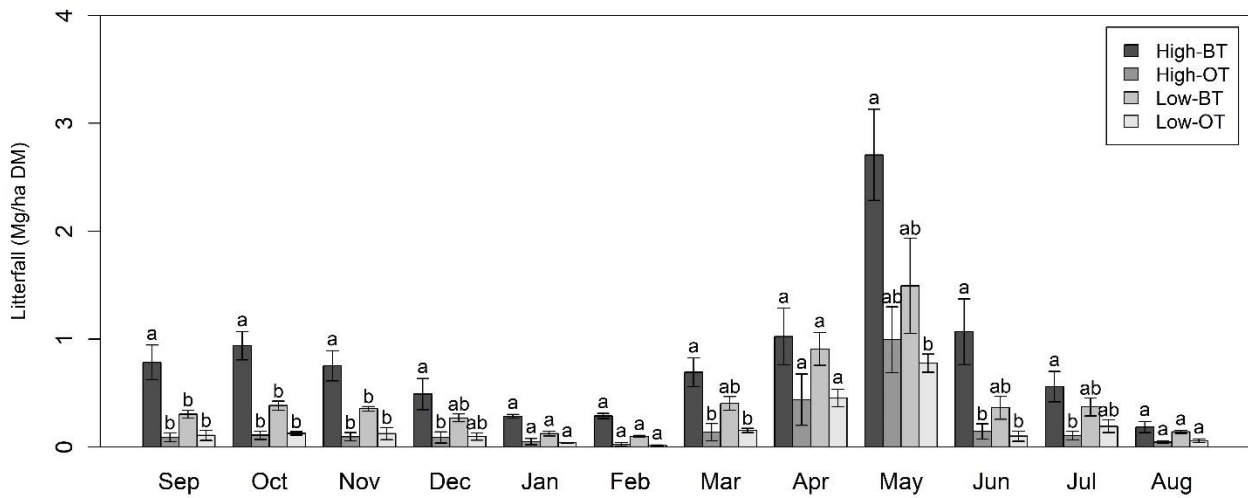


835

836 Figure 5. Mean values (from 2012-13 to 2014-15) of Pasture Production (left box) and Pasture
 837 Residues (right box) (Mg ha⁻¹ of DM). Bars overtopped by the same letter represent means not
 838 significantly different according to the least square means test (P<0.05). Bars represent the standard
 839 errors of means (n=4). High = high cover; Low = low cover; BT = below the tree crown; OT = outside
 840 the tree crown projection

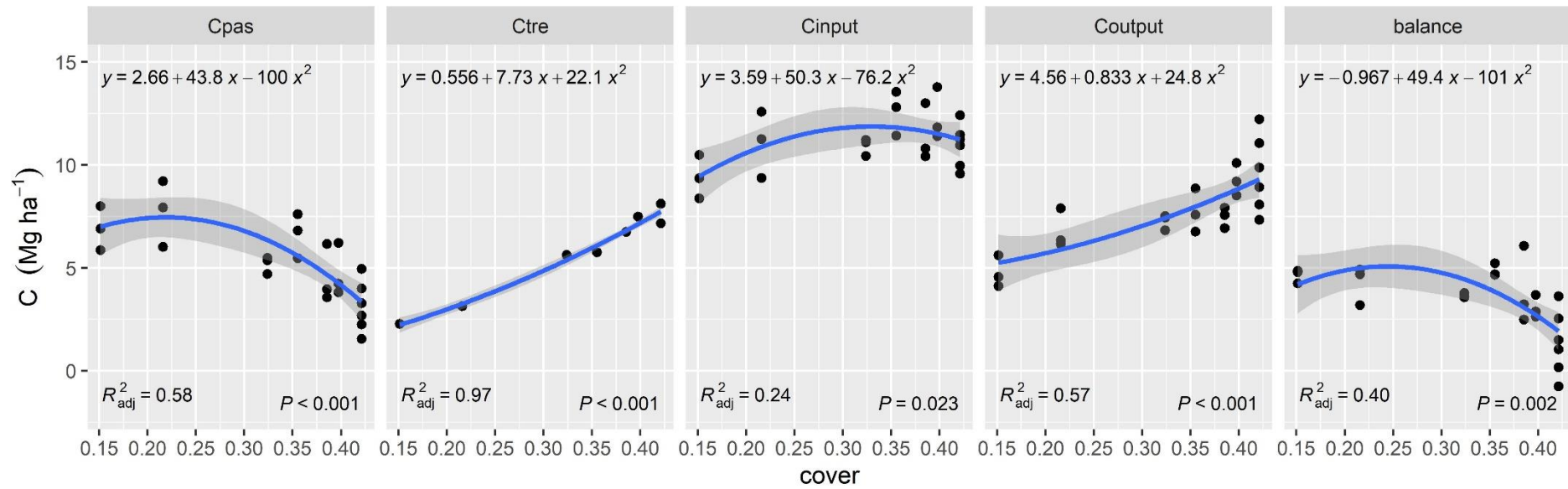
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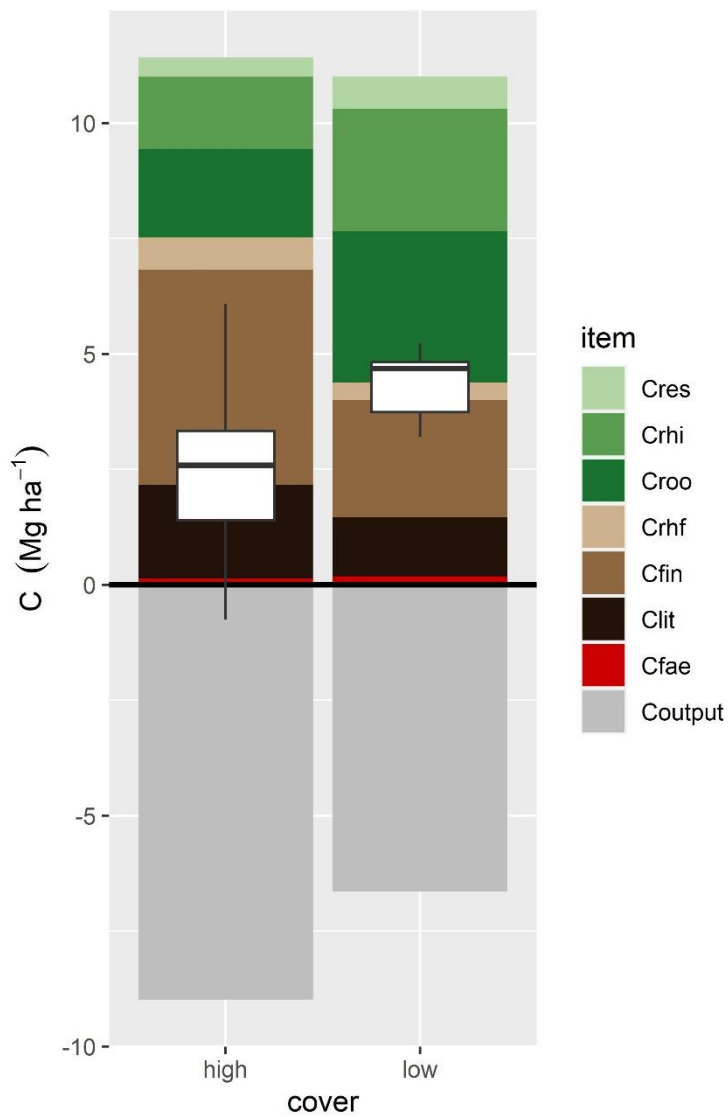
845 Figure 6. Tree litterfall (Mg ha⁻¹ of DM) monthly dynamics (September 2014 – August 2015). Bars
 846 overtopped by the same letter represent means not significantly different according to the least-square
 847 means test ($P < 0.05$). Bars represent the standard errors of means ($n=4$). High = high cover; Low =
 848 low cover; BT = below the tree crown; OT = outside the tree crown projection



850

851 Figure 7. Relationships between the field-scale weighted C input from pasture (Cpas), C input from trees (Ctre), total C input (Cinput), C output
 852 (Coutput), and C balance (balance), and the tree cover (cover)

853



854

855 Figure 8. Field-weighted C balance items (Mg ha^{-1} of C) under High and Low tree cover. Green-scale
 856 bars represent the balance item contributing to the C input from pasture (C_{pas}), brown-scale bars
 857 represent the C input from trees (C_{tre}), red bar represents the C input from faeces (C_{fae}), grey bar
 858 represents the C output. The boxplot within bars represent the field-weighted C balance (box's edges
 859 represent the interquartile range, vertical lines are the 95% confidence interval, lines within boxes
 860 represent are median values)

High



Field High1
Cover 42%
W 0.460
W Pattern: regular



Field High2
Cover 42%
W 0.462
W Pattern: regular



Field High3
Cover 40%
W 0.468
W pattern: regular



Field High4
Cover 39%
W 0.480
W pattern: regular

Low



Field Low1
Cover 36%
W 0.526
W Pattern: cluster



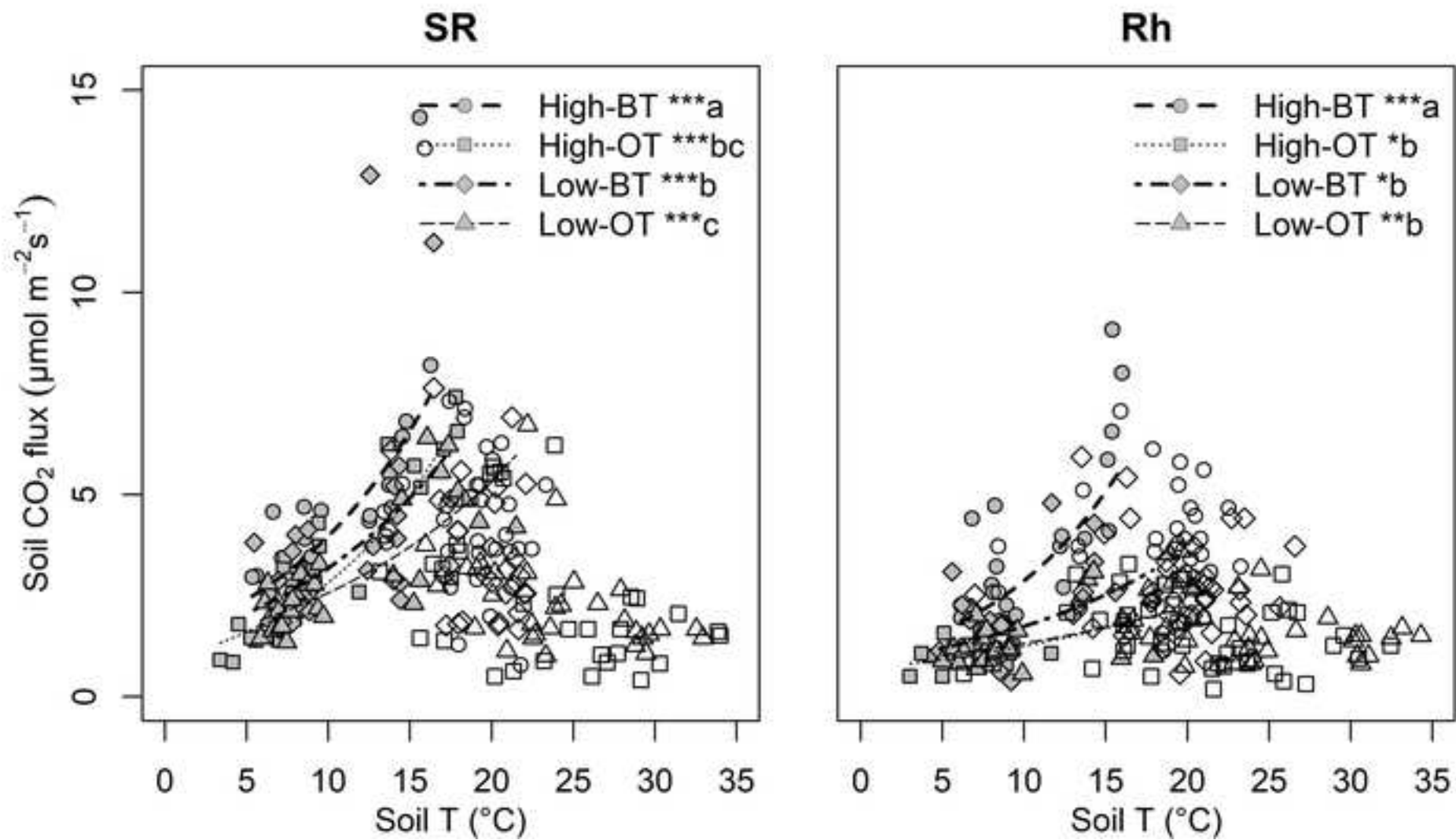
Field Low2
Cover 32%
W 0.489
W Pattern: random

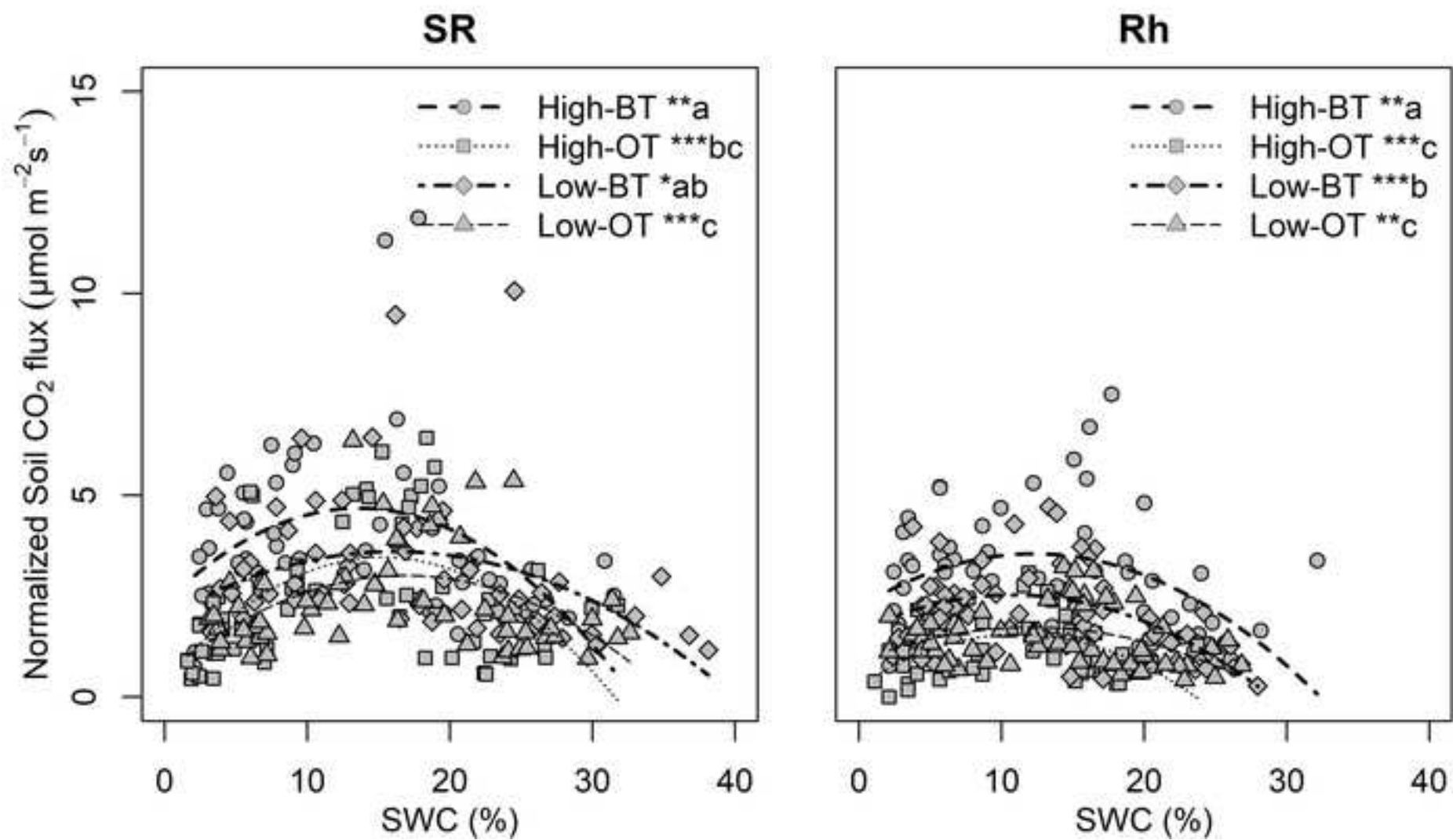


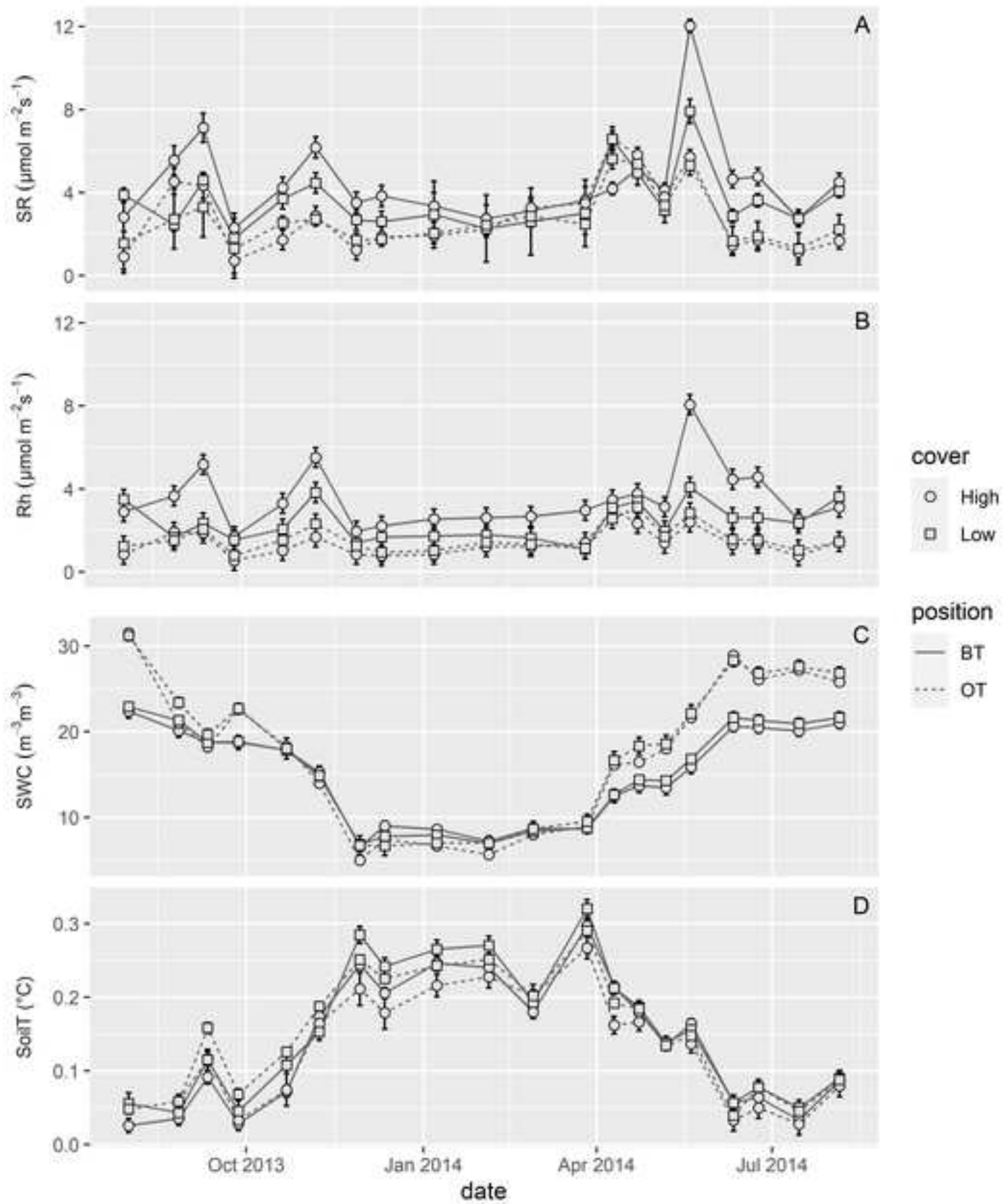
Field Low3
Cover 22%
W 0.509
W Pattern: random

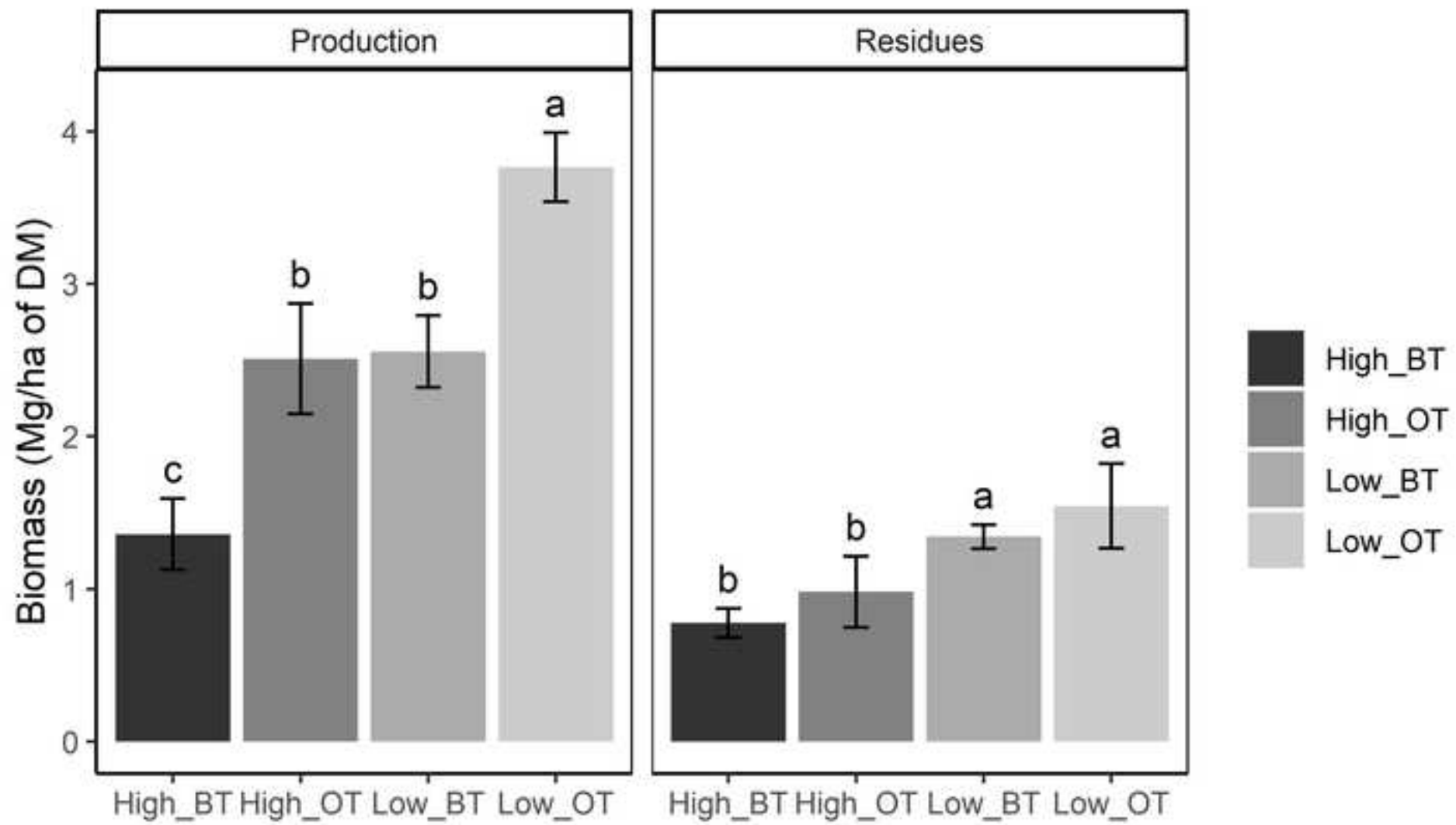


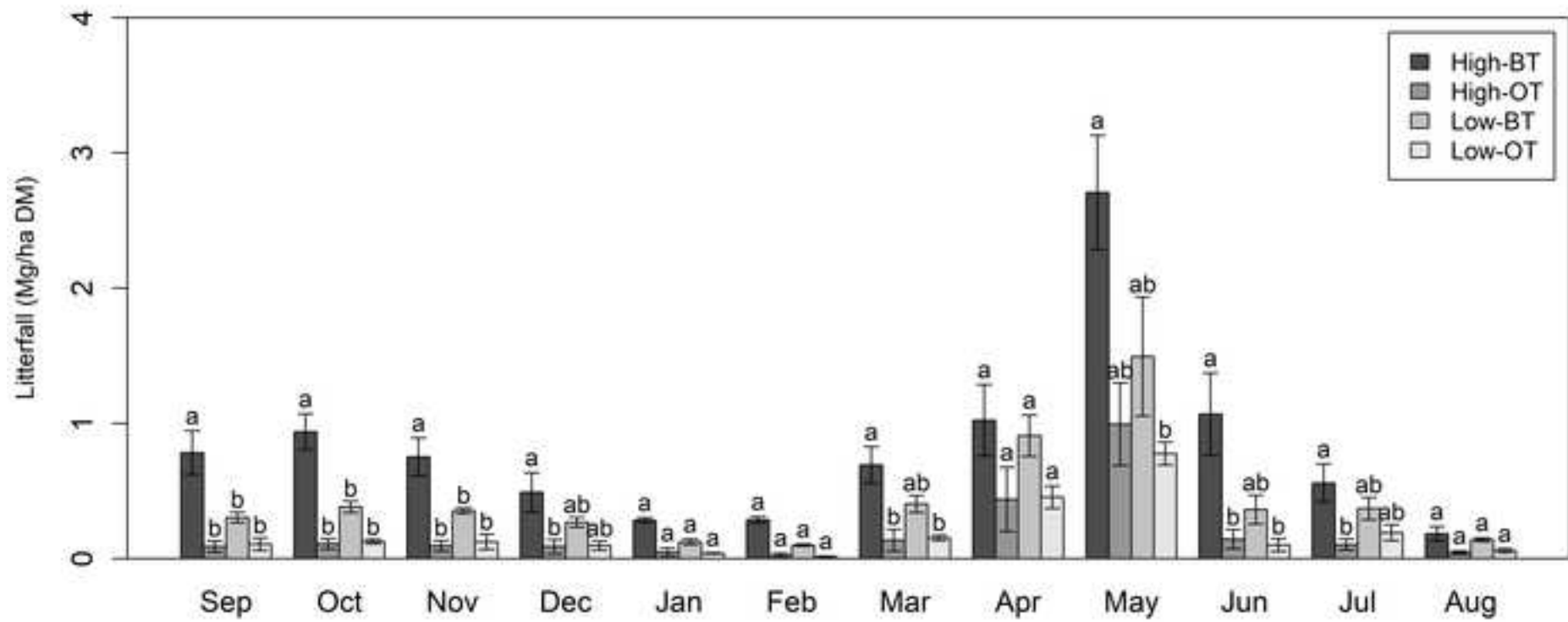
Field Low4
Cover 15%
W 0.558
W Pattern: cluster

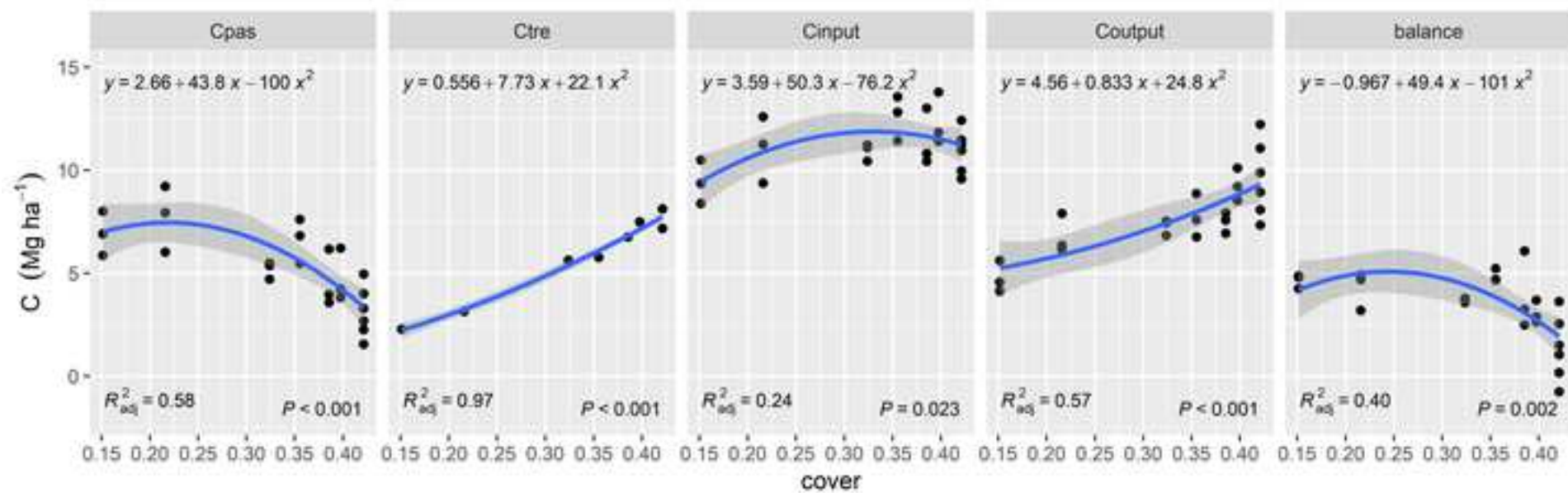












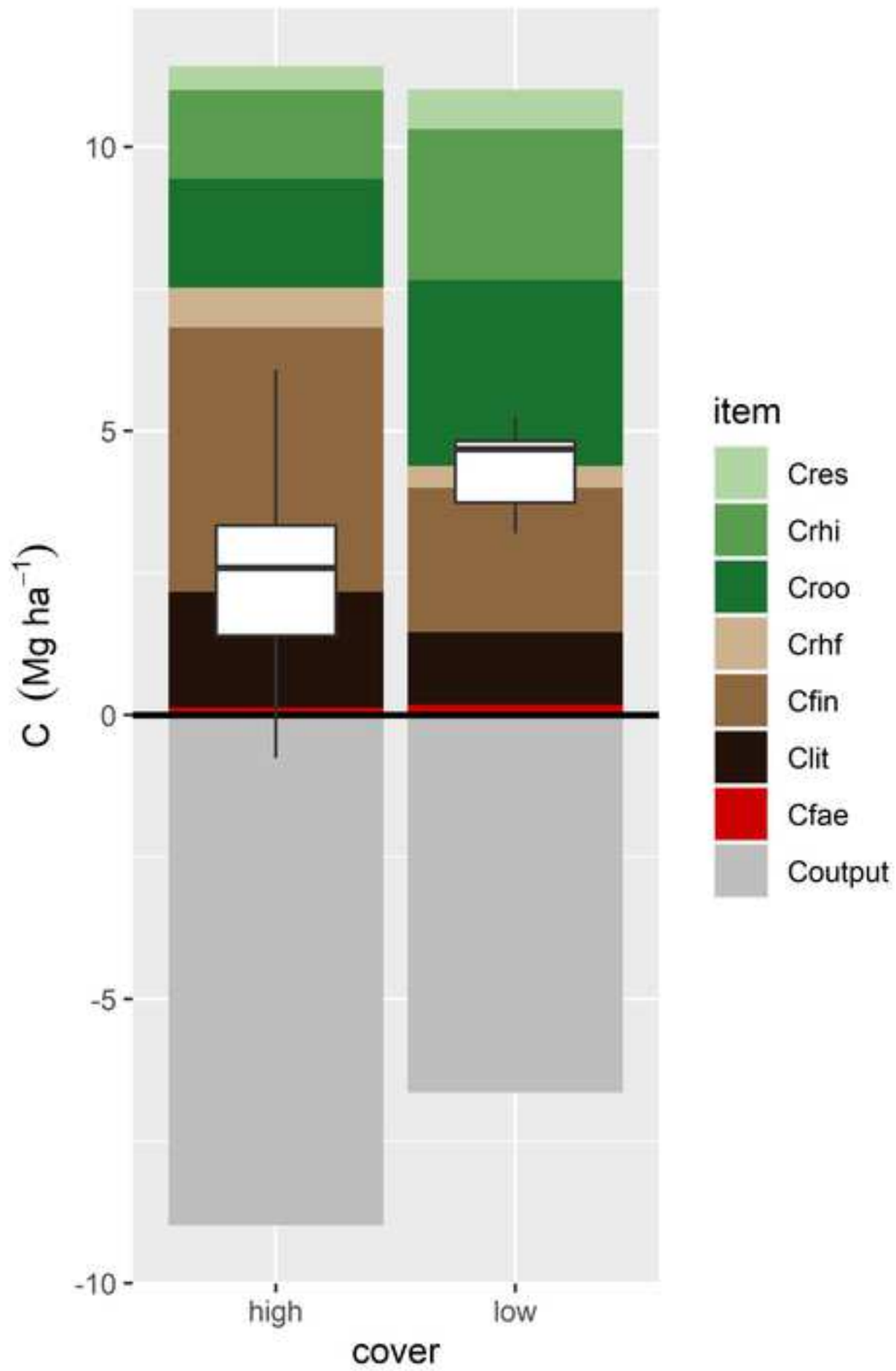


Table 1. Soil Organic Carbon (SOC, Mg ha⁻¹), total N (N, Mg ha⁻¹), and C/N ratio at 0.40 m soil depth (mean value ± standard error of the mean).

	SOC (Mg ha⁻¹)	N (Mg ha⁻¹)	C/N
High	125.5±12.9	9.3±1.1	13.8±0.6
Low	141.7±14.0	10.8±1.2	13.2±0.3
High-BT	152.0±8.2 a	10.9±1.1	14.2±0.8
High-OT	99.0±15.5 b	7.6±1.6	13.5±0.9
Low-BT	162.2±11.6 a	12.0±1.0	13.6±0.2
Low-OT	121.2±22.5 ab	9.6±2.1	12.8±0.5

Means followed by the same letters indicate no significant differences between levels of the factors according to the least-square means test (P<0.05). High= high cover; Low= low cover; OT: outside tree canopy

Table 1. Parameters of the polynomial regression between Rh and both SoilT and SWC for each level used to estimate the daily Rh, F statistic (model variance / residual variance), degrees of freedom of residual variance (df residual), and P-value.

	β_0	β_1	β_2	β_3	F value	df residual	P value
High-BT	0.18634	0.1067	0.1537	-0.0034	11.65	56	<0.0001
High-OT	0.07279	0.0590	0.2651	-0.0075	12.39	55	<0.0001
Low-BT	1.28364	0.0041	0.1120	-0.0046	6.97	56	<0.001
Low-OT	0.21601	0.0382	0.1674	-0.0046	7.97	56	<0.001

High= high cover; Low= low cover; BT: below tree canopy; OT: outside tree canopy

Table 1. Annual soil C balance items and overall balance ($\text{Mg ha}^{-1} \text{ yr}^{-1}$ of C, mean values \pm standard errors of means) and P values associated with the study factors (ANOVA). Means followed by the same letters indicate no significant differences between levels according to the least-square means test ($P < 0.05$). Values within brackets represent the range of the observations (min-max)

#	Item	Class			Position				
		P	High	Low	P	High-BT	High-OT	Low-BT	Low-OT
1	Cres	<0.01	0.41 \pm 0.06 b	0.65 \pm 0.06 a	NS	0.34 \pm 0.04 (0.11-0.64)	0.47 \pm 0.12 (0-1.31)	0.60 \pm 0.04 (0.32-0.77)	0.69 \pm 0.13 (0-1.38)
2	Croo	<0.0001	1.88 \pm 0.19 b	3.27 \pm 0.18 a	NS	1.71 \pm 0.30 (0.56-3.28)	2.06 \pm 0.25 (0.59-2.92)	3.25 \pm 0.30 (1.58-4.62)	3.30 \pm 0.20 (2.03-3.82)
3	Crhi	<0.0001	1.53 \pm 0.16 b	2.66 \pm 0.14 a	NS	1.39 \pm 0.24 (0.69-4.03)	1.67 \pm 0.20 (0.72-3.59)	2.64 \pm 0.24 (1.95-5.69)	2.68 \pm 0.16 (2.5-4.71)
4=1+2+3	Cpas	<0.0001	3.82 \pm 0.40 b	6.58 \pm 0.35 a	NS	3.43 \pm 0.57 (1.44-7.74)	4.21 \pm 0.56 (1.32-7.82)	6.49 \pm 0.56 (3.85-10.88)	6.67 \pm 0.45 (4.59-9.73)
5	Clit	<0.05	2.27 \pm 0.55 a	1.57 \pm 0.24 b	<0.0001	3.59 \pm 0.46 a (2.88-4.93)	0.95 \pm 0.25 c (0.39-1.600)	2.16 \pm 0.17 b (1.86-2.47)	0.99 \pm 0.11 c (0.74-1.28)
6	Cfin*	NS	11.45 \pm 0.07 (11.28-11.56)	9.14 \pm 1.11 (6.19-11)	-	-	-	-	-
7	Crhf*	NS	1.72 \pm 0.01 (1.69-1.73)	1.37 \pm 0.17 (0.93-1.65)	-	-	-	-	-
8=6+7	Ctrs*	NS	13.17 \pm 0.08 (12.98-13.3)	10.51 \pm 1.27 (7.11-12.65)	-	-	-	-	-
9=5+8	Ctre	<0.05	8.85 \pm 3.00 a	6.83 \pm 2.28 b	<0.0001	16.76 \pm 0.51 a (15.86-18.23)	0.95 \pm 0.25 c (0.39-1.60)	12.66 \pm 1.23 b (9.54-14.73)	0.99 \pm 0.11 c (0.74-1.28)
10	Cfae	NS	0.13 \pm 0.02 (0.06-0.29)	0.18 \pm 0.01 (0.1-0.24)	-	-	-	-	-
11=4+9+10	Cinput	NS	12.81 \pm 1.62	13.58 \pm 1.27	<0.0001	20.32 \pm 0.5 a (18.22-24.53)	5.29 \pm 0.63 c (2.38-9.5)	19.33 \pm 0.72 a (15.78-22.92)	7.84 \pm 0.42 b (5.97-10.7)
12	Coutput	<0.01	9.83 \pm 1.06 a	7.65 \pm 0.70 b	<0.0001	14.24 \pm 0.90 a (9.81-21.45)	5.41 \pm 0.55 c (3.14-9.49)	10.12 \pm 0.94 b (4.3-13.46)	5.19 \pm 0.21 c (4.09-6.56)
13=11-12	Cbalance	<0.001	2.98 \pm 0.96 b	5.93 \pm 0.78 a	<0.0001	6.08 \pm 1.13 b (-1.72-12.79)	-0.12 \pm 0.91 c (-4.91-4.3)	9.21 \pm 0.73 a (4.98-12.39)	2.65 \pm 0.29 c (0.99-4.33)

*Cfin, Crhf, Ctrs refer to average values within BT areas

High: high cover; Low: low cover; BT: below tree canopy; OT: outside tree canopy; Cres: C input from pasture residues; Croo: C input from pasture roots decomposition; Crhi: C input from pasture rhizodeposition; Cpas: C input from pasture; Clit: C input from tree litterfall; Cfin: C input from tree fine roots decomposition; Crhf: C input from tree rhizodeposition; Ctrs: C input from tree root system; Ctre: C input from trees; Cfae: C input from animal faeces

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: