1   **Factors controlling the buildup of humus and particulate organic matter in European beech**

2   **and Scots pine stands at their southernmost distribution limits (Moncayo Massif, Spain)**

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15   **ABSTRACT**

16   Forests of European beech (*Fagus sylvatica*) and Scots pine (*Pinus sylvestris*) in the Iberian

17   Peninsula contain some of the southernmost localities of these species and thus may display

18   highly sensitive responses to environmental variations as a result of subjection to particular

19   climate stresses. In this study, we investigated the effects of elevation and dominant tree species

20   (beech vs. pine) on the buildup of organic matter (OM) and nutrients within the humus (O layers)

21   and particulate organic matter (POM) of the topsoil (Ah layer) in centennial Scots pine forests

22   compared to that in natural beech forests along an altitudinal gradient from 1,100 to 1,600 m.a.s.l.

23   in the Moncayo Massif (northern Spain). The contents of nonstructural, hemicellulose, cellulose,

24   lignin-like, soluble ash and free lipid fractions and of C and major nutrients (N, P, S, K, and Ca)

25   were analyzed and related to the buildup of OM within individual layers, which we used as an

26   inverse surrogate for decomposition rates. The stocks of the studied variables in the humus and

27   the POM were examined in relation to elevation and tree species. The highest storage values were

28   found in the Scots pine sites at higher elevations in the form of thick layers of strongly

29 decomposed organic matter (OH layers) and in the POM. Climate, as it related to elevation, was

30 found to be the major driver of the early and intermediate stages of decomposition (represented

31 by humus) but showed little effect on POM, which was more strongly affected by the tree species.

32 The buildup of OM has its largest correlation with the content of Ca (which was higher under

33 beech vegetation and decreased with increasing elevation), suggesting an effect of Ca in

34 improving habitat conditions for biological activity.

35 **KEYWORDS** litter decomposition, organic layers, land use change, climate gradient, elevation,

36 calcium

37 **1. INTRODUCTION**

38 Organic matter (OM) is a vital component of forest soils and is important for nutrient cycling,

39 hydrological regulation, site productivity and sustaining the global carbon (C) budget (Seely et

40 al., 2010). In forest ecosystems, most of the OM enters the soil through the forest floor in the form

41 of leaf litter (Cotrufo et al., 2000; Berg, 2018). Soils of temperate and cold regions usually show

42 one or several organic (O) layers on their surface, often referred to as humus layers, mainly

43 consisting of fresh and decaying plant debris (Prescott et al., 2000; Berg, 2018), In the forest floor,

44 the soluble cellular components of OM are rapidly oxidized or leached into the soil, whereas the

45 structural components persist on the soil surface for some time while being gradually fragmented

46 and mixed with mineral soil by physical processes and soil fauna (Eijsackers & Zehnder, 1990;

47 Cotrufo et al., 2000; Berg & McClaugherty, 2014). Once incorporated into the mineral soil, the

48 plant fragments form the light or particulate fraction of OM (particulate organic matter, here

49 onwards POM), which is characterized by its low density and coarse (sand, 50–2,000 μm) size

50 (Gregorich & Ellert, 1993; Zeller & Dambrine, 2011) and has been observed to be the major

51 contributor to OM in the upper centimeters of forest soils (Cao et al., 2020).

52 The climate and quality of organic substrates are considered to be the main limiting factors of

53 OM decomposition (Swift et al., 1979; Aerts, 1997). In general, litter quality is recognized as the

54 most important driver, although the effect of climate becomes more critical under unfavorable

55 climatic conditions (Zhang et al., 2008; Djukic et al., 2018). If decomposition is slow or

56 incomplete, OM accumulates in the humus layers (Prescott et al., 2000; Berg 2018; Kõlli 2018a)

and POM fraction (Gabarrón–Galeote et al., 2015; Campo & Merino, 2016; Soucémarianadin et al., 2019). Because of their close relationship to litter inputs and decomposition rates, both the humus layers and the POM are indicators of changes in OM accumulation and decomposition in response to changes in land use (Six et al., 2002; DeGryze et al., 2004; Seeber & Seeber, 2005; Gamper et al., 2007; Poeplau & Don, 2013; Kõlli 2018b).

The conversion of natural or semi-natural broadleaved forests into conifer forests has been a major land use change in Europe over the past two centuries (Ellenberg, 1988). In particular, the extensive woodlands of European beech (*Fagus sylvatica* L.) were transformed into monocultures of Scots pine (*Pinus sylvestris* L.) (Paule, 1995; Bartsch & Röhrig, 2009), although, in recent decades, beech has been underplanted and mixed stands are now frequent (Rumberger et al., 2004; Knoke et al., 2008). The impact of such changes has been examined in a number of studies reporting variations in the thickness and type of humus layers (Prietzel, 2004; Rumberger et al., 2004; Bens et al., 2006) and in the C and nitrogen (N) reservoirs in the forest floor (Schulp et al., 2008; Leuschner et al., 2013; Simková et al., 2014). These variations have been mainly attributed to the higher quality of deciduous litter compared to conifer litter in terms of nutrients and the presence of easily decomposable components. In contrast, little information is available on the impact of European beech-Scot pine replacements on the quality and amount of the POM fraction. Conversely, the amount and characteristics of the humus in beech and Scots pine woodlands vary considerably within their distribution boundaries, which is determined by climate (Lalanne et al., 2010; Meier & Leuschner, 2010; Bringmark et al., 2011). In the north of the Iberian Peninsula, beech and Scots pine woodlands reach to the southernmost boundary of their distributions near their tolerance limits; as a result, their ecosystems show more distinctive functional responses to changes (Weber et al., 2013; Martínez del Castillo et al., 2016). Furthermore, in southern and central Europe, a shift towards a warmer and drier climate is forecasted, so the conditions at the distribution boundaries of this ecosystem are considered to be representative of those that will dominate more northerly Europe in the present century (Martínez-Vilalta et al., 2008; Tegel et al., 2014).

In this study, we researched variations in the stocks of C and several major nutrients [nitrogen (N), phosphorus (P), sulfur (S), potassium (K), and calcium (Ca)] in the humus layers and POM fraction of the topsoil as a result of the replacement of beech forests with Scots pine a century ago along an altitudinal climate gradient at Sierra del Moncayo, which is one of the southernmost locations of both species in Europe. Our objective was to assess the contributions of litter quality and climate to the accumulation of decaying OM under beech and Scots pine vegetation subjected to particular climate stress conditions. We hypothesized that near the climate boundaries of both beech and Scots pine, climate would have a more dominating effect than that of the tree species on the OM and nutrient buildup in the humus layers and POM fraction.

2. METHODOLOGY

2.1. Study area and field work

The study was carried out in the Moncayo Natural Park on the northern face of the Moncayo Massif (Iberian Range, NE Spain, 41°48′31″N, 1°49′10″ W) (Figure 1). The experimental area covered the natural altitudinal distribution range of *F. sylvatica*, which ranges from approximately 1,100 to 1,700 m.a.s.l. and encompasses a climate gradient where the mean annual temperature decreases from 10.4 to 7.2°C and annual rainfall increases from 549 to 1,413 mm almost linearly (Figure 2). The lithology of the bedrock is siliceous and is dominated by Early Triassic quarzitic sandstone. Soils are generally stony, loamy or sandy-loam textured with a high cation exchange capacity but are highly leached and very acidic (Girona-García et al., 2018). The soil types range from Dystric Cambisols occurring at lowest elevations to Albic Umbric Podzols occurring at the highest elevations (Badía et al., 2016). Part of the area is occupied by Scots pine forests that date back to the end of the XIXth century and the beginning of the XXth century. These afforestations were aimed to control soil erosion resulting from uncontrolled logging for charcoal production, and no signs of erosion are evident at present. The age of the afforestation meets the minimum period of 100 years required for the organic layers to reach equilibrium after beech-Scots pine replacements (Bens et al., 2006). However, the former beech vegetation can still be traced in the soils of these pine forests by specific beech biomarkers (Girona-García et al., 2019).

111   Three natural stands dominated by beech and three forested stands dominated by Scots pine in

112   their highest, middle and lowest elevations represented in the study area were selected (Figures 1

113   and 2). Table 1 shows the main plant species present in each stand. All the stands were exposed

114   to the northeast and had an approximate slope of 20%. Within each stand, three sampling sites

115   were located at approximately the same altitude at a distance of approximately 25 m from each

116   other. In each site, a representative 30 x 30 cm square area was selected for describing and

117   sampling the humus profile, recording the occurrence and characteristics of the following layers

118   if present, as defined by Zanella et al. (2018): (i) nOL, formed by new, mostly untransformed

119   plant litter; (ii) vOL, formed by older plant debris, altered since being shed to soil, but still very

120   low-fragmented (< 10% fine organic material); (iii) rzoOF, consisting of zoogenically-

121   transformed litter fragments with a low proportion (10-30%) of fine material; (iv) mzoOF,

122   containing similar amounts (30-70%) of fragmented leaf residues and disordered masses of

123   organic material; (v) OH, with a predominance of unidentifiable plant residues (> 70% fine

124   material); and (vi) meAh, with mixed organic and mineral material and a mesostructure of organic

125   origin. The material of each humus layer within the sampling area was collected for composition

126   quantification and analysis. Samples from the underlying topsoil (meAh layer) up to a 10 cm

127   depth were also taken using metal cores of a known volume. In total, 77 samples of the humus

128   and topsoil layers were analyzed of which 40 were from beech-vegetated sites and 37 were from

129   Scots pine forested sites (Table 2).

130   **2.2. Laboratory procedures**

131   The analyses aimed to assess the biochemical quality of the humus and POM, as determined by

132   the levels and ratios of potentially limiting elements and recalcitrant substances whose abundance

133   can limit the rate and extent of decomposition (Zhang et al., 2008; Berg & McClaugherty, 2014).

134   The stoichiometric ratios also provide information on the ease with which the contained nutrients

135   can be mobilized during decomposition, either increasing, decreasing or even halting mobility if

136   these ratios approached certain critical values (Prescott, 2005; Berg and McClaugherty, 2014). If

137   the ratio of a component A to a component B (e.g. C to N) is above the critical ratio, then the

138   element B is in limiting concentrations relative to A and will be selectively retained over A during

139 decomposition, whereas if the ratio falls below the critical ratio, then B is in surplus relative to A

140 and will be preferentially released.

141 The humus samples were dried at 60°C in an oven until a constant weight was achieved and were

142 then ground using a cutting mill (1 mm) (Retsch Mühle, Haan, Germany). The topsoil samples

143 were air dried and sieved at <2 mm. The sand-sized fraction was separated by dispersion with

144 sodium hexametaphosphate and sieved through a 50 μm sieve. The light POM was then separated

145 from the heavier mineral sand by flotation in a saturated sodium chloride solution (density ≈ 1.2

146 g cm^{-3}) (Wolf et al., 1994), oven dried at 60°C and ground with a cutting mill. Representative

147 subsamples of the humus, topsoil and POM were oven dried to determine the dry mass at 105°C,

148 and the bulk density of the topsoil was obtained by dividing the 105°C dry mass of the soil sample

149 by the known volume of the sampling core.

150 The total organic matter content of the humus, topsoil and POM was analyzed by the loss-on-

151 ignition method at 550°C in a muffle furnace. The total contents of C and N were determined

152 using a CN Vario Max elemental analyzer (Elementar, Hanau, Germany). The rest of the nutrients

153 in the humus and POM samples were determined from the ashes obtained by incineration with

154 the addition of magnesium nitrate (Kalra & Maynard, 1991) in a muffle oven at 550°C. The ash

155 obtained was dissolved in hydrochloric acid and analyzed for P using the molybdate blue method,

156 for S by turbidimetry with barium chloride, for K by flame spectrophotometry and for Ca by

157 EDTA-complexometric titration.

158 The organic composition of the humus and POM was assessed by the procedure outlined by Van

159 Soest et al. (1991) using an Ankom 200 Fiber Analyzer (Ankom Technol., Fairport NY, USA).

160 In this procedure, the samples are treated with a neutral detergent, an acid detergent and a 72%

161 sulfuric acid solution to sequentially extract the nonstructural (cell content, CC), hemicellulose

162 (HEM) and cellulose (CEL) components of the OM. The acid-unhydrolyzable residue (AUR),

163 which is composed of lignin, cutin, suberin and waxes (Preston et al., 2009), remains after

164 treatment in addition to a specified amount of acid-unhydrolyzable minerals (i.e., silica), whose

165 content was determined by ashing at 550°C and subtracting from that the AUR content. The silica

166 matter was also subtracted from the total ash content to assess the acid-soluble ash (ASA) fraction.

167   We considered ASA to be a more reliable measure than total ash since it is free of silica

168   contamination resulting from dust and mineral soil particles. The levels of free lipids were also

169   analyzed from the humus and topsoil samples using a 12-h Soxhlet extraction with petroleum

170   ether.

171   **2.3. Data processing and statistical analysis**

172   The results of the analyses were expressed in concentration units per oven dried ash-free mass of

173   OM. From these results, mass ratios were calculated between the concentrations of C, N, P, S, K

174   and Ca to CEL and AUR to C, N, P, S, K and Ca; the lignocellulosic index (LCI) was calculated

175   according to the following expression: LCI=AUR/(hemicellulose+cellulose+AUR).

176   In addition, the concentration values were combined with the mass of the humus layers per unit

177   area and the bulk density of the topsoil to obtain the stocks per unit area (Mg ha^{-1}) in the humus

178   (per layer and total), POM and, for the free lipids, topsoil layer.

179   The concentrations of the main fractions [CC, HEM, CEL, AUR, ash (ASA) and free lipids] and

180   major elements (C, N, P, S, K and Ca) of the OM were analyzed by principal component analyses

181   (PCA) to explore their variations across the different layers between the two forest types. The

182   concentrations of the OM components in the nOL layers and the POM were compared between

183   beech and Scots pine sites using t–tests. The relationship between the amount of OM stored in the

184   individual layers and the levels and ratios of the various OM components was investigated using

185   Pearson's correlation analysis. This analysis was performed jointly and separately for the humus

186   and POM samples. Finally, the stocks of OM and OM constituents were analyzed by analysis of

187   covariance (ANCOVA), controlling for the effect of elevation as a covariate and forest type

188   (beech, Scot pine) as a fixed factor separately for the whole humus and POM stocks. The study

189   variables were transformed as necessary using cubic, square, log, power, square root, natural

190   logarithm, inverse or inverse square root functions to fulfill the normality and homoscedasticity

191   requirements of the statistical tests. All statistical tests were performed using SPSS software (IBM

192   Corporation, Armonk NY, USA). For ease of interpretation, the mean and other descriptive

193   statistics given in the results are based on the nontransformed data.

194   **3. RESULTS**

3.1. Humus profile and mass of the layers

We found the thickest and most complex humus profiles in the Scots pine locations at the highest elevations (approximately 1,600 m.a.s.l.), where nOL/rzoOF/mzoOF/OH/meAh layer sequences were observed (Table 2), which were described as Humimor. At lower elevations (1,120 and 1,430 m.a.s.l.), the Scots pine locations exhibited simpler sequences, which were classified as Dysmulls (nOL/rzoOF/meAh) or Hemimoders (nOL/rzoOF/mzoOF/meAh). All beech locations, ranging between 1,185 and 1,320 m.a.s.l., showed nOL/(vOL)/rzoOF/(mzoOF)/meAh sequences which were classified as Hemimoder.

The largest OM stock for an individual layer (137 Mg ha^{-1}) was found in the POM of a beech location at mid-elevation, followed by the OH layers of some of the highest elevation Scots pine locations (111 and 60 Mg ha^{-1}) and the POM of Scots pine locations at all elevations (mostly >20 Mg ha^{-1}) (Table 3). In turn, the lowest stocks (<3 Mg ha^{-1}) were recorded in the vOL and mzoOF layers under beech vegetation. The POM represented an important part of the OM in the meAh layer at 0-10 cm depth, averaging $33 \pm 11\%$ ($\pm$ standard deviation) of the total OM, $40 \pm 12\%$ of the total C, and $29 \pm 12\%$ of the total N in the soils of beech sites and $43 \pm 9\%$ of the total OM, $44 \pm 12\%$ of the total C and $37 \pm 15\%$ of the total N in the soils of Scots pine sites.

3.2. Humus and POM composition

The average concentrations of the various components of the humus layers and POM for the two forest types and the distinct altitudes are presented in the Appendices (Figures A.1 and A.2). Among the humus layers, nOL has the most similar composition to that of recently shed litter, whereas the material recovered as POM from the topsoil depth (0-10 cm) can be assumed to be largely derived from aboveground litter. Hence, in this study, the nOL layer and the POM were regarded as approximations to the early and late stages of decay.

In Figure 3, the PCA graph summarizes the variations in the major OM fractions during decomposition in beech and Scots pine sites. The first and most explanatory axis of this PCA was conspicuously related to the degree of decay, and the samples were distributed along this axis with decreasing levels of nonstructural OM (CC) and increasing levels of nonlabile polymers (CEL, AUR) (Figure 3), with the nOL and POM samples exhibiting the lowest (on the left) and

223 highest (on the right) scores, respectively. The humus samples from the Scots pine sites showed

224 similarly low scores along this axis (on the left), whereas the POM samples showed much higher

225 scores (on the right), highlighting that the humus material underwent little alterations while being

226 fragmented and transferred across the distinct humus layers but was abruptly transformed during

227 its incorporation into POM. In contrast, the humus in the beech sites showed a gradual

228 compositional shift until being eventually incorporated into the POM. The second axis largely

229 reflected the differences between the beech (Figure 3, on top) and Scots pine (on bottom) sites.

230 These included (see Tables 4 and 5) the differences in AUR and free lipids that arose during

231 decay, the difference in CC that was present initially and persisted during decay, and the

232 difference in HEM that lessened with decay.

233 Figure 4 presents the results of the PCA of the elemental composition. Again, the first axis

234 represents the variations among the humus layers, whereas the second axis represents the

235 variations between the beech and Scots pine sites. The first axis is associated with increasing

236 levels of N, P and S from the nOL layer (Figure 4, on the left) to the POM of the Scot pine sites

237 and the mzoOF layer and POM of the beech sites (on the right), with larger increases for beech

238 sites than for Scots pine sites. The second axis is positively correlated with the Ca levels and

239 negatively correlated with the C levels, thus separating the samples taken under beech (Figure 4,

240 top), which are higher in Ca, from those taken under Scots pine (bottom), which are higher in C.

241 The second axis also likewise separated the mzoOF samples from the POM of beech sites,

242 suggesting a strong Ca release and a selective C preservation coinciding with incorporation into

243 POM under beech vegetation.

244 In both PCAs, elevation plotted inversely to the levels of free lipids (Figure 3) and Ca (Figure 4),

245 which suggested a negative correlation of these variables with altitude. However, the second axis

246 was positively correlated with elevation in both plots, which mirrors the fact that the beech

247 locations were selected at lower altitudes compared to the Scots pine locations. This makes it

248 difficult to separate the effects of altitude and forest type on the OM composition along this axis.

249 Further insight into the decomposition process can be obtained through examining the

250 composition of the nOL layers and POM. Table 4 shows the compositional values of the nOL

251   layer for beech and Scots pine sites compared to the typical composition of litter for these species

252   according to Berg & McClaugherty (2014). Overall, the nOL layers of the beech sites were richer

253   in minerals (as assessed by ASA), certain nutrients (S, and especially K, and Ca) and labile

254   polymers (HEM) than the nOL layer under the Scots pine sites, which were conversely higher in

255   C and nonstructural OM (CC). No differences were observed between beech and Scots pine sites

256   for the levels of N, P, CEL, AUR and free lipids. Table 4 also presents the average values of

257   certain stoichiometric ratios in the nOL layer in comparison with the critical values reported for

258   foliage litter. The nOL layer showed high C:N ratios but was still within the highly variable range

259   of critical C:N values reported for foliage litter (Prescott, 2005). The values of the C:P, C:S, N:P,

260   AUR:N and AUR:P ratios of the nOL layer were clearly above their respective critical values.

261   The C:P, C:S, N:P, and AUR:P ratios were close to being significantly higher ($P < 0.10$) for pine

262   sites than for beech sites.

263   Table 5 shows the average levels of the components of POM under beech and Scots pine

264   vegetation. The CC, Ca, and S concentrations remained significantly or nearly significantly ($P <$

265   0.10) different in the POM, and new variations emerged in the levels of AUR of the POM and of

266   free lipids of the meAh layer, which were significantly or nearly significantly larger in beech sites

267   than in Scots pine sites. The AUR:N ratios of the POM remained above their critical values, which

268   means that the release of N from POM was still limited by AUR degradation. The release of C

269   and nutrients during decomposition can be seen in Figure 5, which shows the ratios of their

270   concentrations in the POM to the concentrations in the nOL layer. According to this Figure,

271   elements were immobilized during decomposition according to the following decreasing

272   sequence: S (pine) ≈ K (pine) > S (beech) ≈ P (both) > N (both) > K (beech) ≈ Ca (both) ≈ C

273   (both). The enrichment ratios found for C and Ca in beech and Scots pine sites and for K in the

274   beech sites were close to 1, indicating no immobilization of these elements occurred, i.e., they

275   were released in rates that were proportional to the total OM decay.

276   **3.3. Relationships between the buildup and composition of the individual layers**

277   Table 6 shows the Pearson's correlation coefficients between the OM composition and the storage

278   of OM and C within the individual humus layers and within the POM in the meAh layer.

279   Regarding the organic components, the OM and C stored in the set of humus layers and the POM

280   were significantly ($P < 0.05$, Pearson's test) positively correlated with the AUR level and

281   negatively correlated with the levels of HEM and free lipids. However, when only the humus

282   layers were considered, the OM and C stocks were not correlated with AUR and were instead

283   positively correlated with CC, negatively correlated with CEL and slightly correlated with HEM

284   and free lipids. Conversely, when only the POM samples were considered, no correlation was

285   found between the OM and C stocks and the organic composition of the POM.

286   In relation to the elemental concentrations, the buildup of OM and C across the humus layers and

287   the POM showed the most significant correlation to the Ca concentration, to which they displayed

288   a very significant negative exponential relationship (Figure 6), and positively to P when jointly

289   considering the humus and POM. When only the humus layers were considered, the OM and C

290   stocks also showed significant negative correlations with the S and K concentrations, while for

291   POM alone, apart from Ca, a very significant ($P < 0.01$) correlation was observed with the N

292   levels.

293   Regarding the quantitative ratios between components, grouping the humus layers and the POM,

294   the OM and C stocks of the individual layers showed very significant ($P < 0.001$) correlations

295   with the ratios of Ca to other elements (C:Ca, N:Ca, P:Ca, S:Ca, and K:Ca) and (positively) with

296   the CEL:N ratio and significant ($P < 0.05$) positive correlations with the CEL:P ratio and the LCI.

297   Considering only the humus layers, the OM and C stocks were correlated with the ratios of Ca to

298   most other elements (C:Ca, N:Ca, P:Ca, and S:Ca), with the ratios of K to other elements (C:K,

299   N:K, P:K, and S:K, with the exception of K:Ca), with the CEL:C (negatively), and with the

300   CEL:N, AUR:C and AUR:Ca ratios (positively). When only the POM was considered, the OM

301   and C storage showed correlations with the Ca level and ratios, the N concentration and the C:N

302   ratio.

303   **3.4. Total humus and POM stocks**

304   The total stocks of OM within the whole humus profile significantly increased ($P < 0.05$,

305   ANCOVA) (Table 7) with altitude, as did the stocks of most OM components (with the only

306   exception of CC), as shown in Figures 7 and 8. In turn, the effect of forest type was found to be

307 significant ($P < 0.05$) for the humus stocks of total OM, CC, AUR and C, which were higher

308 under Scots pine, and for the ASA, which was higher under beech, but was not significant for

309 HEM, lipids, N, P, S, K and Ca. In contrast, the stocks of the total OM and of the various OM

310 components in the POM were mostly found to be larger under Scots pine than under beech

311 vegetation (Table 7, Figures 7 and 8) but were not significantly affected by altitude, with the

312 exception of Ca, which significantly decreased with increasing elevation.

313 **4. DISCUSSION**

314 **4.1. Humus profile**

315 The nOL, vOL, rzoOF, mzoOF, OH, and POM series comprises a sequence of organic pools with

316 well-defined characteristics along which the OM is successively transferred as it becomes more

317 decomposed. High decomposition rates lead to the rapid transfer of OM, resulting in the thinning

318 or even the disappearance of certain layers, whereas low decay rates lead to the buildup of OM.

319 Therefore, in absence of significant soil erosion, the buildup of OM within the individual layers

320 can be used as an inverse proxy for decomposition rates. Studies on the vegetation shift from

321 Scots pine to beech forests in Germany (Rumberger et al., 2004; Bens et al., 2006) have shown

322 Scots pine forests to be associated with more and thicker humus layers and less active mor-like

323 moder and mor forms compared to more active mull and moder under beech vegetation. Previous

324 studies in the study area have shown that the humus profiles of the Scots pine sites vary with

325 increasing elevation from Dysmull to Hemimoder classifications as a consequence of a reduction

326 in soil biological activity (Badía-Villas & Girona-García 2018); however, under beech and Scots

327 pine forests located at mid-elevations, the same humus form (Hemimoder) was observed,

328 exhibiting few differences (Girona-García et al., 2018). In the present study, we found that the

329 beech sites exhibit thicker OL layers, which often include the vOL layers not present in the Scots

330 pine sites. However, OH layers are only found at the higher elevation Scots pine locations. This

331 suggests that recently shed litter under Scots pine vegetation has higher decomposition rates that

332 then considerably slow during the late stages of decomposition at high elevations.

333 **4.2. Biochemical drivers of decomposition**

334   Carceller et al. (1989) investigated the litterfall of beech and Scots pine forests in the study area

335   and found the litter to be mainly (70-80%) composed of leaf litter with only very minor

336   contributions from ligneous debris. Our results on the elemental composition of the nOL layer in

337   beech forests are similar to those observed in leaf litter in the study area by Roca & Rovira (1989),

338   and for both beech and Scots pine sites, they tend to resemble the typical values for these species'

339   leaf litter according to Berg & McClaugherty (2014), with an important exception of N content

340   under Scots pine being noticeably higher in our study. In turn, we found lower CC levels and

341   higher HEM and AUR levels in the Scots pine sites than those reported for the litter of this species

342   by Berg et al. (1982) and by Hobbie et al. (2006). McTiernan et al. (2003) found that levels of N,

343   HEM, and AUR increased and the CC level decreased during the decomposition of Scots pine

344   litter; thus, our results suggest a further degree of decomposition of the nOL layer in Scots pine

345   sites compared to beech sites.

346   The C:N, C:P, and N:P values of the nOL layer indicated a stronger limitation by P than by N,

347   especially in Scots pine sites, which are conducive to the preferential immobilization of P over N

348   during decomposition (Prescott, 2005). The average N:P ratios (23 in beech sites, 28 in Scots pine

349   sites) were close to the highest values within the range of 9-27 reported for the litter of beech

350   growing on various substrates, as reported by Leuschner et al. (2006), who found such high values

351   in strongly acidic soils. The elevated values of the AUR:N and AUR:P ratios highlight a tendency

352   of both N and P to be preserved within recalcitrant substances (i.e., AUR) during decomposition

353   (Osono & Takeda, 2001), which leads to the division of the decomposition process into two

354   stages: an early stage characterized by the degradation of the most easily decomposable

355   components, during which only small amounts of nutrients would be released, and a later stage

356   in which the recalcitrant substances would be degraded and a larger nutrient release would take

357   place. This sequence is consistent with the two-phase model of litter decomposition described by

358   Berg & McClaugherty (2014), according to which litter mass loss is initially controlled by the

359   degradation of relatively labile polymers (i.e., CEL and HEM) and nonstructural carbohydrates

360   but is controlled by lignin degradation in later stages. Kalbitz et al. (2006) found that the decay

361   of beech and Scot pine litter fits this two-phase model well, although Scots pine litter showed

362 larger mass losses and a shorter first phase of decay (5 vs. 9-12 months), which is consistent with

363 our above suggestion of a faster initial decay in Scots pine sites than in beech sites.

364 The buildup of OM within the individual layers (which we can use as an inverse proxy for

365 decomposition rates) had the highest and most consistent correlations with the Ca levels and the

366 Ca ratios to other elements and to AUR. Calcium is an important component of plant cell walls

367 and can be selectively retained during decomposition in the slowly degrading cell wall structures

368 (Berger et al., 2015); this process, in the present study, was evidenced by the correlation between

369 the AUR:Ca ratio and the OM buildup for the humus layers. Calcium is a very scarce element in

370 acidic soils, but beech trees can capture it from the subsoil and transfer it to the soil surface via

371 litterfall (Ammer et al., 2006; Błońska et al., 2018). We found the Ca content to be higher in beech

372 sites than in Scots pine sites in the nOL layer but not in the POM, which highlights a higher Ca

373 retention during decomposition of pine litter compared to that of beech litter, as was reported by

374 Berger et al. (2015). The diminishing effect of Ca on OM buildup can be explained by its

375 improvement of habitat conditions for decomposer organisms. Acidic environments are

376 unfavorable to deep-dwelling invertebrates such as earthworms (Judas et al., 1997; Curry, 2004),

377 resulting in poor mixing of decaying OM with the underlying soil, which is largely responsible

378 for humus accumulation in acidic forest soils (Meier & Leuschner, 2010; Fahey et al., 2011).

379 Indeed, Reich et al. (2005) and Ammer et al. (2006) found that the litter of Ca-rich species, such

380 as beech, was associated with a higher abundance and diversity of earthworms and considered

381 this to be responsible for higher decomposition rates.

382 Only for the humus layers were significant relationships also observed between the OM buildup

383 and the levels of K and S. The release of K and S from decaying litter has been described to follow

384 a two-stage sequence with an initial phase of rapid change and a later phase marked by nutrient

385 immobility (Laskowski et al., 1995; Stevenson & Cole, 1999). Studies on the decomposition of

386 beech litter have reported high and rapid K losses (Roca and Rovira, 1989; Berger et al., 2015),

387 which coincide with the low K enrichment ratios found in the present study in the POM relative

388 to the nOL layer of the beech sites. Potassium is generally not a structural component in plant

389 tissues, which favors its loss in large amounts during the first phase of decay (Berg and

390   McClaugherty, 2014). For Scots pine, however, we observed a marked immobilization of K in

391   the POM, which was described by Laskowski et al. (1995) to occur in Scots pine litter as a

392   consequence of initial low K levels, resulting in considerable shortening of the initial phase and

393   early K immobilization. In turn, S was found to be immobilized (i.e., showed higher enrichment

394   ratios) in the POM of both the beech and Scots pine sites, albeit more markedly in the latter. Sulfur

395   occurs in plants primarily as sulfate and in proteins, and part of the latter is attached to AUR

396   (Reeves 1993), which contributes to their retention during decomposition, as was observed for

397   beech by Berger et al. (2015). Once released, the dissolved forms of both K and S are highly

398   mobile and readily leached, which is consistent with their levels in the humus layers being

399   proportional to their release rates from decaying OM.

400   The buildup of OM in the humus layers was positively correlated with the LCI and CC levels and

401   negatively correlated with the CEL levels. Nonstructural components (i.e., CC) are generally

402   considered to be labile (McKee et al., 2016) and thus can be expected to be rapidly removed from

403   litter (Hobbie et al., 2006), as we observed in the beech sites. However, the CC had a much greater

404   preservation tendency in the OM during decomposition in the Scots pine sites than in the beech

405   sites. This finding is consistent with the observation by Strukelj et al. (2013) of an enrichment in

406   nonstructural components during the decomposition of coniferous woody debris as a result of an

407   abundance of resin acids, which are highly resistant to degradation and are particularly abundant

408   in Scots pine litter (Lindberg et al. 2004). Decomposition rates were therefore better reflected by

409   the CEL level and the LCI (which measures the ratio of CEL and HEM to AUR) than by the CC.

410   This coincides with the observation by Kalbitz et al. (2006) that the CEL component was better

411   correlated to the decomposable fraction of fresh litter in several species, including beech and

412   Scots pine. The degradation of CEL was seemingly responsible for most of the C losses from the

413   decaying litter, as can be inferred from the correlations of the humus buildup to the CEL:C ratio

414   (negative) and the AUR:C ratio (positive), and contributed to N enrichment in the remaining

415   residue, as highlighted by the positive correlation between the CEL:N ratio and the buildup of

416   OM in the humus and the POM. Overall positive correlations were also found between the OM

417   buildup and the level of free lipids, which is in agreement with earlier reports in the literature that

418   lipids are highly decomposable and exhibit rapid losses during decay (Ziegler & Zech, 1989;

419   Marseille et al., 1999). Most of these relationships between the OM buildup and various organic

420   fractions were no longer significant for the POM, likely because the organic composition is more

421   stable and changes much slower in the organomineral layer.

422   **4.3. Effects of the tree species**

423   In the present study, Scots pine and European beech stands that differed considerably in their

424   altitudinal ranges were examined. These differences might imply some limitation to the

425   ANCOVA since they can decrease its ability to detect significant relationships (Hsu & Sebatane,

426   1979). Nevertheless, elevation and tree species were both found to significantly affect the stocks

427   of OM in the humus and POM. Conversely, differences in the values of the covariation have little

428   effect on the ANCOVA when the covariate is highly correlated with the dependent variable (Hsu

429   & Sebatane, 1979), as we found to be the case for the stocks of most of the humus components.

430   According to our results, the total stocks of OM in the humus and POM were higher in the Scots

431   pine forests than in the beech forests. The buildup of OM depends on the balance between litter

432   production and decay rates and thus can be affected by tree species as a result of differences in

433   the quantity and composition of litter. A review of several studies by Vesterdal et al. (2013)

434   indicated that differences in litterfall rates between different tree species growing under similar

435   conditions are small, so they cannot explain the variations in OM buildup. In a nearby area of the

436   Iberian ranges, Santa Regina & Tarazona (2001) found that similar amounts of leaf litter were

437   produced in beech and Scots pine forests, but other less abundant debris (twigs, bark, fruits) was

438   produced in larger amounts in Scots pine forests, which could have contributed to the higher OM

439   buildup in the Scots pine sites in our study.

440   Conversely, larger humus stocks found under Scots pine than under beech vegetation have been

441   repeatedly reported and generally attributed to differences in litter decomposition (e.g., Schulp et

442   al., 2008; Leuschner et al., 2013; Simková et al., 2014). Litter decomposition rates are often

443   assumed to be higher for broadleaved tree species than for conifers mainly because of the greater

444   content of nutrients and easily decomposable organic compounds in broadleaf litter compared

445   with coniferous litter, which is conversely richer in relatively stable organic compounds, such as

446   lignin and cuticular waxes (i.e., AUR) (Berg & McClaugherty, 2014). However, several studies

447   have shown that beech litter decays more slowly than that of other broadleaved species (Hobbie

448   et al., 2006; Jacob et al., 2010) or even that of Scots pine (Santa Regina & Tarazona, 2001; Hobbie

449   et al.; 2006; Kalbitz et al., 2006). Our analysis of the composition of the nOL layer also indicated

450   a faster decay of Scots pine litter than that of beech litter during the earliest stages of

451   decomposition. It is also in agreement with the proposal by Girona-García et al. (2019) that the

452   degree of decomposition, as assessed from the N isotope composition ($\delta^{15}N$) of the OM of the

453   Scots pine sites of the study area, is maintained from the OL to the OF layers and enhanced

454   towards the OH and Ah layers, especially compared with beech sites. The low decomposability

455   of beech litter has been explained by the high abundance of lignin, cutin, and suberin (Hobbie et

456   al., 2006; Berg & McClaugherty, 2014). We found the AUR levels to be higher in the POM but

457   not in the nOL layer of the beech sites compared to Scots pine sites, which indicates that AUR

458   was not the cause of the slower decomposition of beech litter, but rather that it became

459   concentrated in the POM of the beech sites during decomposition. However, the concentration of

460   AUR might arise as an important factor limiting the decomposition of POM, whose buildup was

461   found to be strongly influenced by the tree species.

462   Whereas the humus stocks of OM and C were larger for the Scots pine sites, the stocks of nutrients

463   (N, P, S, K, Ca) were similar between the humus of the beech and Scots pine sites, and the stock

464   of ASA was larger for the beech sites. The cases of S, K, Ca, and ASA can be explained by their

465   higher concentrations in the beech litter, in agreement with the finding by Leuschner et al. (2013)

466   of larger pools of base cations in the humus layers of beech forests compared to Scot pine

467   afforestation despite the pine humus layers being much thicker.

468   **4.4. Effects of elevation**

469   Elevation is a surrogate of several covarying climate parameters (precipitation, temperature,

470   radiation). In the present study, the influence of elevation differed from that of the tree species by

471   significantly affecting the humus stocks of several nutrients (N, P, S, K, and Ca) and certain

472   organic components (HEM and CEL), which increased with increasing altitude.

473   On a global scale, litter production decreases with elevation due to decreasing temperatures

474   (Lonsdale, 1988), but at regional and local scales, the distinct climate parameters related to

475   elevation can exert opposite effects on litterfall, which can result in no clear altitudinal

476   dependence (Kobler et al., 2019). Therefore, Kobler et al. (2019) found little change in the litter

477   production of beech forests along an altitudinal gradient (550 to 900 m a.s.l, 9.8 to 7.3°C) in the

478   Austrian Alps, and Pausas et al. (1994) found no relationship between elevation (gradient from

479   1,380 to 1,610 m.a.s.l., 8.1 to 7.1°C, 757 to 1,280 mm year^{-1}) and litterfall in the Scots pine forests

480   of the Moncayo and Pyrenees ranges. In contrast, Blanco et al. (2006) found indications that the

481   litter production of Scots pine forests decreased with increasing elevation (650 to 1336 m.a.s.l.,

482   12.0 to 8.2°C, 612 to 1,268 mm year^{-1}) in the Pyrenees. None of these effects can explain the

483   increased accumulation of OM and OM components in the humus layers with increasing altitude

484   in the present study (gradient from 1,185 to 1,605 m.a.s.l., 10.4-7.2°C, 549 to 1,413 mm year^{-1}).

485   Decomposition is expected to vary with increasing altitude, either increasing with increasing mass

486   loss due to leaching or decreasing due to lower rates of biological activity at low temperatures

487   (Swift et al., 1979). Our finding that labile organic components, such as CEL or HEM, increase

488   their stocks with higher altitudes is indicative of the diminishing effect of elevation on

489   decomposition rates. Berger et al. (2015) examined the decay of litter of beech and black pine

490   (*Pinus nigra,* J. F. Arnold) along an altitudinal gradient similar to that of the Moncayo area and

491   found a delay in decomposition at higher altitudes, which was attributed to lower temperatures.

492   Additionally, Meier & Leuchner (2010) examined a precipitation gradient with a uniform relief

493   in the center of the beech distribution range and found the C stocks of the humus layers were

494   unaffected by temperature but increased with increasing rainfall due to lower decomposition rates

495   with increasing moisture. In the present study, we found climate effects to be more important

496   during the initial stages of decomposition and to show little effect on the stocks of POM.

497   The concentrations of several nutrients (N, K, Ca) in plant tissues have frequently been reported

498   to decrease with increasing altitude as their bioavailability is reduced by slow nutrient turnover

499   and/or strong leaching (He et al., 2008; Kang et al., 2011). Phosphorus may increase its levels and

500   availability with altitude because of its heightened release from freeze-thaw weathering (He et

al., 2008) and its lower use by plant cells under conditions less favorable to growth (González de Andrés et al., 2019) or may decrease under low pH ranging 4.0-4.7 in the topsoils at the highest locations of the study area (Badía et al., 2016). None of these altitudinal trends were evident in the present study for the nutrient concentrations in the humus; therefore, their stocks per area simply paralleled those of total OM and C in increasing with increasing elevation.

For the POM, the concentration of Ca (and to a much lower degree, that of K) was found to visibly decrease with increasing elevation, and as a consequence, the Ca stock per area decreased with elevation. Berger et al. (2015) found that the K and Ca concentrations decreased in decaying beech and pine litter with increasing altitude seemingly as a result of the higher leachability of these highly soluble elements, as was also observed for the exchangeable K in the soils of the study area by Badía et al. (2016).

Altitudinal gradients are suitable models to evaluate the effects of climate on ecological processes (Körner 2007). The altitudinal gradient examined in the present study represents an intermediate value within the 1.7-4.8°C range of global warming and approximately twice the largest reduction of up to 25% in annual rainfall that is forecasted to affect the forests of the study region during the XXIth century (Sánchez-Salguero et al., 2017). Therefore, the altitudinal variations found here can be indicative of some of the changes that may affect OM turnover and storage in these forests in the near future, particularly for the Scots pine stands at the highest elevations, where the largest OM storage was found to occur under particularly cold and humid conditions.

5. CONCLUSIONS

Our findings only partially supported our hypothesis that at the southernmost boundaries of the distribution of beech and Scots pine forests, the effects of climate on humus and POM buildup would dominate over those resulting from the tree species. Elevation considerably affected the early and intermediate stages of decomposition represented by humus, whose stocks per area of OM, C and nutrients exhibited clear increasing trends with increasing elevation. When controlling for the influence of elevation, the tree species showed no effects on the humus stocks of nutrients (although it did affect those of OM and C, which were found to be larger in the Scots pine sites). In contrast, for POM decomposition, the forest type became the most important driver, and the

529 effects of elevation were mostly negligible. We found the buildup of OM in both the humus and

530 the POM to be tightly inversely related to the contents of Ca, whose levels were found to decrease

531 with increasing altitude and to increase under beech vegetation. Our findings suggest that humus

532 and POM buildup depends less on the recalcitrance of organic substrates than on the effect of Ca

533 in improving the conditions of these acidic soils for biological activity.

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815

816   **Table 1** Dominant plant species of the stands of beech forests and Scots pine

817   forestations studied at different elevations.

m.a.s.l.	Canopy	Undergrowth
1,120	*Pinus sylvestris* L.	*Crataegus monogyna* Jacq., *F. sylvatica*, *Juniperus communis* L., *Quercus pirenaica* Willd.
1,185	*Fagus sylvatica* L.	Mosses
1,230	*Fagus sylvatica* L.	*Abies alba* Mill., *Erica arborea* L., *Ilex aquifolium* L., *P. sylvestris*, *Vaccinium myrtillus* L.
1,310	*Fagus sylvatica* L.	*Erica arborea*, *V. myrtillus*, mosses
1,430	*Pinus sylvestris* L.	*Deschampsia flexuosa* (L.) Trin., *E. arborea*, *I. aquifolium*
1,605	*Pinus sylvestris* L.	*E. arborea, E. vagans* L., *I. aquifolium, V. myrtillus*

818

819

820 **Table 2** Thickness (cm) and number of samples (n) studied of humus and topsoil layers

821 under beech forests and pine forestations at different elevations.

	Beech forest						Scots pine forest					
m.a.s.l.	1,185		1,230		1,310		1,120		1,430		1,605	
Layer	n	cm	n	cm	n	cm	n	cm	n	cm	n	cm
nOL + vOL	3+1	1.5–3.6	3+2	1.5–3.3	3+1	2.5–4.0	3+0	0.5–0.9	3+0	0.7–0.9	3+0	1.0–2.3
rzoOF	3	1.5–2.3	3	1.0–2.5	3	1.5–5.3	3	0.7–2.0	3	0.6–1.1	3	1.0–3.1
mzoOF	3	0.2–0.2	3	0.0–0.3	3	0.0–1.0	2	0.0–2.9	2	0.0–4.9	3	0.0–1.1
OH											3	1.0–22.0
meAh	3	(>) 5	3	(>) 5	3	(>) 5	3	(>) 5	3	(>) 5	3	(>) 5

822

823 **Table 3** Ranges (minimum and maximum) for the stocks of OM ($Mg\ ha^{-1}$) in the humus

824 layers and the POM in the meAh layer (0–10 cm depth) under beech forests and pine

825 forestations at different elevations.

	Beech forest						Scots pine forest					
m.a.s.l.	1,185		1,230		1,310		1,120		1,430		1,605	
Layer	n	$Mg\ ha^{-1}$	n	$Mg\ ha^{-1}$	n	$Mg\ ha^{-1}$	n	$Mg\ ha^{-1}$	n	$Mg\ ha^{-1}$	n	$Mg\ ha^{-1}$
nOL	3	2.8–8.5	3	3.7–5.8	3	3.2–5.6	3	4.4–10.9	3	5.1–6.3	3	6.8–23.9
vOL	1	2.0	2	0.7–0.8	1	0.9						
rzoOF	3	5.2–8.9	3	5.2–9.2	3	6.4–9.9	3	5.5–14.6	3	8.4–13.9	3	6.0–26.2
mzoOF	3	2.2–2.5	3	1.7–6.3	3	2.8–4.9	2	0.8–8.8	2	3.7–13.1	3	11.2–17.1
OH											3	4.0–111
POM	3	6.3–17.8	3	13.2–137	3	10.5–27.6	3	36.0–43.2	3	15.1–59.0	3	23.1–50.6
Non POM	3	27.3–51.5	3	60.5–80.4	3	32.3–101	3	53.8–79.0	3	19.3–64.9	3	27.6–58.1

826

Table 4 Comparative values (descriptive and t–statistics) of the concentrations (g kg^{-1})

and some relevant stoichiometric ratios of the components of the nOL layer between

beech and Scots pine forest sites.

	Beech forest (n=9)						Scots pine forest (n=9)						Critical	Transf.	$t_{(16)}$
	Mean	S.D.	Min.	Max.	Skw.	Typ.	Mean	S.D.	Min.	Max.	Skw.	Typ.			
CC	193	16	165	221	0.02		257	24	207	290	−0.81			n.t.	−6.65***
HEM	185	26	151	220	−0.39		142	21	114	181	0.26			n.t.	3.84**
CEL	255	29	208	293	−0.50		249	10	227	262	−1.09			1/x^2	−0.13
AUR	368	26	329	400	−0.40		352	11	336	371	0.28			1/x^2	−1.58
Free lipids	29.5	10.5	12.7	46.8	0.14		33.1	7.2	25.0	46.5	0.79			n.t.	−0.83
ASA	64.2	10.4	45.0	85.0	0.29		17.3	13.0	0.5	37.0	0.16			n.t.	8.72***
C	467	29	430	508	0.17		507	18	489	540	0.89			n.t.	−3.42**
N	9.11	0.76	8.20	10.69	1.09	9.1†	9.89	1.24	8.51	11.82	0.74	3.6†		n.t.	−1.61
P	0.42	0.11	0.26	0.60	0.25	0.6†	0.36	0.07	0.28	0.50	0.87	0.2†		n.t.	1.41
S	0.72	0.23	0.40	1.02	−0.23	1.2†	0.35	0.11	0.23	0.60	1.41	0.4†		ln (x)	4.58***
K	2.62	0.52	1.73	3.40	−0.42	2.7†	0.59	0.14	0.43	0.84	0.72	0.5†		ln (x)	14.4***
Ca	16.2	1.8	13.5	19.3	0.41	10.0†	9.9	2.1	7.3	12.8	−0.06	5.6†		n.t.	6.83***
C:N	51.6	5.6	44.6	61.1	0.34		51.8	6.0	41.8	59.2	−0.39		23–80‡	n.t.	0.07
C:P	1173	303	783	1656	0.18		1440	245	984	1735	−0.40		350–480‡	n.t.	2.05#
C:S	725	256	423	1129	0.62		1565	407	828	2131	−0.49		200–400§	n.t.	−5.24***
N:P	23.1	7.1	14.6	36.6	0.71		27.8	4.0	22.5	34.9	0.35		15–16‡	n.t.	−1.75#
AUR:N	40.7	4.8	34.3	48.8	0.41		36.0	4.7	29.8	41.9	−0.11		23–25††	n.t.	2.07#
AUR:P	915	197	665	1258	0.33		1000	176	701	1240	0.01		500–620††	n.t.	0.97#

Typical nutrient concentrations (Typ.) for beech and Scots pine as reported by Berg & McClaugherty (2014)† and critical values for stoichiometric ratios in foliar litter as compiled by Prescott (2005)‡ and reported by van Wesemael (1993)§ and Osono & Takeda (2004)†† are given as references. CC = cell content, HEM = hemicellulose, CEL = cellulose, AUR = acid-unhydrolyzable residue, ASA = acid-soluble ash, S.D. = standard deviation, Min. = minimum, Max. = maximum, Skw. = skewness. Transf. = transformations applied to variables for t-testing, including natural logarithm [ln (x)] and inverse square (1/x^2) transformations, or no-transformation (n.t.). # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

831 **Table 5** Comparative values (descriptive and t–statistics) of the concentrations (g kg^{-1}
832 organic matter) and some relevant stoichiometric ratios of the components of POM of
833 the meAh (topsoil) layer at 0-10 cm depth between beech and Scots pine forest sites.

	Beech forest (n=9)					Scots pine forest (n=9)					Critical	Transf.	t$_{(16)}$
	Mean	S.D.	Min.	Max.	Skw.	Mean	S.D.	Min.	Max.	Skw.			
CC	24	25	15	26	0.19	135	88	0	59	0.13		$\sqrt{x}$	−3.71**
HEM	135	23	106	162	−0.04	124	34	76	164	−0.38		n.t.	0.81
CEL	326	73	219	432	0.16	286	47	227	359	0.18		n.t.	1.37
AUR	547	90	427	697	0.21	454	64	352	579	0.41		n.t.	2.53*
Free lipids	47.3	0.3	2.1	98.3	−0.02	23.1	0.2	3.1	61.7	0.98		n.t.	1.91#
ASA	247	93	167	457	1.62	170	116	7	355	0.22		n.t.	1.56
C	528	46	465	605	0.25	528	64	402	587	−1.42		x^3	0.15
N	17.5	2.9	14.9	23.5	1.54	16.2	2.6	12.0	19.6	−0.12		ln (x)	1.07
P	1.36	0.62	0.58	2.36	0.41	0.96	0.32	0.61	1.64	1.49		ln (x)	1.56
S	2.30	0.38	1.70	2.96	0.03	1.73	0.61	0.84	2.44	−0.39		n.t.	2.39*
K	3.35	1.36	2.29	6.67	2.14	2.92	1.25	1.39	5.22	1.01		n.t.	0.70
Ca	13.8	4.3	7.0	20.2	0.20	9.62	5.06	5.4	20.2	1.54		n.t.	1.90#
C:N	30.8	5.0	21.6	39.0	−0.23	33.0	4.1	28.5	39.5	0.80	23–80†	n.t.	−1.05
C:P	473	218	214	805	0.39	600	190	332	964	0.35	350–480†	n.t.	2.30
C:S	235	48	180	315	0.64	336	103	221	480	0.44	200–400‡	1/x^2	−2.82*
N:P	15.6	7.5	7.3	28.0	0.74	18.5	6.8	8.8	32.2	0.68	15–16†	n.t.	−0.88
AUR:N	32.1	8.0	22.0	44.7	0.61	29.0	5.3	24.0	40.5	1.53	23–25§	n.t.	0.97
AUR:P	482	205	219	732	0.01	522	159	294	772	−0.05	500–620††	n.t.	−0.47

Critical values for stoichiometric ratios in foliar litter as compiled by Prescott (2005)† and reported

by van Wesemael (1993)‡ and Osono & Takeda (2004)§ are given as references. CC = cell content,

HEM = hemicellulose, CEL = cellulose, AUR = acid-unhydrolyzable residue, ASA = acid-soluble

ash, S.D. = standard deviation, Min. = minimum, Max. = maximum, Skw. = skewness. Transf. =

transformations applied to variables for t-testing, including cubic (x^3), root ($\sqrt{x}$), natural logarithm

[ln (x)] and inverse square ($1/x^2$) transformations, or no-transformation (n.t.). [#] $P < 0.10$, [*] $P < 0.05$,

[**] $P < 0.01$, [***] $P < 0.001$.

834

835 **Table 6** Results of the correlations of the OM and C storage within the individual layers

836 with the composition of OM, separately considering the humus layers and the POM in

837 the meAh layer (0–10 cm depth) and altogether.

	Humus (n=59)		POM (n=18)		Humus and POM (n=77)	
	OM	C	OM	C	OM	C
CC	0.297*	0.292*	0.216	0.173	–0.141	–0.151
HEM	–0.256#	–0.251#	0.015	–0.036	–0.255*	–0.266*
CEL	–0.307*	–0.305*	–0.131	–0.112	0.111	0.116
AUR	0.058	0.061	–0.214	–0.164	0.254*	0.276*
Free lipids	–0.255#	–0.246#	–0.273	–0.285	–0.236*	–0.237*
ASA	–0.222#	–0.209	–0.258	–0.193	–0.131	–0.113
C	0.147	0.176	–0.071	0.033	0.160	0.213
N	0.016	0.025	0.593**	0.631**	0.174	0.184
P	–0.087	–0.079	0.382	0.358	0.290*	0.280*
S	–0.307*	–0.305*	–0.131	–0.112	0.136	0.149
K	–0.295*	–0.286*	0.075	0.113	0.161	0.174
Ca	–0.425**	–0.412**	–0.474*	–0.497*	–0.487***	–0.487***
C:N	–0.047	–0.047	–0.554*	–0.525*	–0.167	–0.163
C:P	0.028	0.026	–0.188	–0.136	–0.153	–0.145
C:S	–0.004	–0.008	0.044	0.022	–0.154	–0.158
C:K	0.296*	0.289*	–0.097	–0.089	–0.025	–0.025
C:Ca	0.685***	0.677***	0.437#	0.474*	0.645***	0.658***
N:P	0.143	0.139	–0.070	–0.025	–0.112	–0.096
N:S	0.046	0.040	0.274	0.243	–0.131	–0.139
N:K	0.394**	0.385**	0.128	0.127	0.018	0.017
N:Ca	0.673***	0.666***	0.694**	0.718**	0.740***	0.747***
P:S	0.002	–0.004	0.315	0.244	–0.018	–0.041
P:K	0.324*	0.316*	0.179	0.147	0.103	0.094
P:Ca	0.497***	0.490***	0.838***	0.823***	0.760***	0.747***

S:K	0.292*	0.294*	−0.099	−0.080	0.092	0.102
S:Ca	0.540***	0.543***	0.480*	0.515*	0.639***	0.652***
K:Ca	0.116	0.115	0.412#	0.445#	0.510***	0.519***
CEL:C	−0.315*	−.325*	−0.098	−0.136	0.044	0.026
CEL:N	0.698***	0.686***	0.407#	0.438#	0.664***	0.674***
CEL:P	−0.167	−0.172	−0.314	−0.311	0.246*	0.249*
CEL:S	−0.086	−0.092	−0.200	−0.171	−0.148	−0.146
CEL:K	−0.062	−0.068	0.009	−0.027	−0.148	−0.156
CEL:Ca	0.149	0.139	−0.159	−0.177	−0.066	−0.071
AUR:C	0.344**	0.341**	−0.127	−0.095	0.211#	0.228*
AUR:N	−0.070	−0.088	−0.267	−0.273	0.189	0.190
AUR:P	−0.072	−0.079	−0.294	−0.288	0.202	0.208
AUR:S	0.020	0.014	−0.315	−0.266	−0.137	−0.129
AUR:K	−0.011	−0.018	−0.070	−0.100	−0.148	−0.155
AUR:Ca	0.313*	0.301*	−0.190	−0.186	−0.005	−0.006
LCI	0.318*	0.316*	−0.069	−0.031	0.229*	0.246*

CC = cell content, HEM = hemicellulose, CEL = cellulose, AUR = acid-unhydrolyzable residue, ASA = acid-soluble ash, LCI = lignocellulosic index. # $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

838

839

840 **Table 7** Results of the analysis (ANCOVA) of the effects of elevation and forest type

841 (beech, Scots pine) on the stocks of OM and of the various OM components in the

842 humus and the POM. [#] $P < 0.10$, [*] $P < 0.05$, [**] $P < 0.01$.

	Humus					POM/meAh layer				
		Elevation		Forest type			Elevation		Forest type	
	Transf.	F (1)	P	F (1)	P	Transf.	F (1)	P	F (1)	P
OM	$1/x^2$	4.78	0.045[*]	6.43	0.023[*]	x	0.02	0.890	14.76	0.002[**]
CC	$1/x^2$	0.03	0.867	6.50	0.022[*]	$1/x$	0.10	0.763	6.60	0.026[*]
HEM	$1/x^2$	5.63	0.032[*]	0.13	0.729	ln (x)	1.41	0.254	13.94	0.002[**]
CEL	$1/x$	10.44	0.006[**]	2.03	0.174	x	0.59	0.454	9.27	0.008[**]
AUR	$1/x$	9.23	0.008[**]	4.65	0.049[*]	x	0.01	0.932	8.48	0.011[*]
ASA	x	7.77	0.014[*]	6.69	0.021[*]	$1/x^2$	0.78	0.391	2.24	0.156
Free lipids	ln x	8.96	0.009[**]	0.01	0.937	ln (x)	3.49	0.081[#]	2.13	0.165
C	$1/x$	8.97	0.009[**]	7.45	0.016[*]	ln (x)	0.00	0.997	13.08	0.003[**]
N	$1/x^2$	7.30	0.017[*]	1.96	0.181	ln (x)	0.10	0.759	9.60	0.007[**]
P	$1/x^2$	9.73	0.007[**]	0.00	0.981	ln (x)	0.00	0.986	3.88	0.068[#]
S	$1/\sqrt{x}$	11.75	0.004[**]	0.13	0.728	ln (x)	0.32	0.580	2.76	0.118
K	x^2	8.67	0.010[*]	0.01	0.944	ln (x)	2.27	0.152	8.58	0.010[*]
Ca	$1/x$	6.48	0.022[*]	0.89	0.360	x^2	4.97	0.042[*]	7.70	0.014[*]

CC = cell content, HEM = hemicellulose, CEL = cellulose, AUR = acid-unhydrolyzable residue, ASA = acid-soluble ash. Transf. = transformations applied to variables for the ANCOVA, including natural logarithm (ln x), inverse (1/x), inverse square ($1/x^2$), and square (x^2) transformations, or no-transformation (x). [#] $P < 0.10$, [*] $P < 0.05$, [**] $P < 0.01$.

843

844 **FIGURE CAPTIONS**

845 **Figure 1** Location of the study area and the sampling sites.

846 **Figure 2** Changes in mean temperature and annual rainfall with altitude and altitudinal

847 distribution ranges of beech and Scots pine in the study area, elaborated from data by

848 Martínez del Castillo et al. (2019), and altitudinal range of the study stands.

849 **Figure 3** Biplot of the first two axes of a principal component analysis of the levels of

850 the major fractions in decomposing OM across the humus layers and the topsoil.

851 Elevation is plotted as a passive variable.

852 CC = cell content, HEM = hemicellulose, CEL = cellulose, AUR = acid-unhydrolyzable

853 residue, ASA = acid-soluble ash

854 **Figure 4** Biplot of the first two axes of a principal component analysis of the levels of C

855 and major nutrients in decomposing OM across the humus layers and the topsoil.

856 Elevation is plotted as a passive variable.

857 **Figure 5** Ratios of the concentrations of C and major nutrients in the POM to the

858 concentrations in the nOL layer in European beech and Scots pine sites.

859 **Figure 6** Buildup of OM (in log scale) against Ca levels in the individual humus layers

860 and the POM (0-10 cm depth).

861 **Figure 7** Stocks per area of major OM fractions in the whole humus and the POM (0-10

862 cm depth) and of free lipids in the whole humus and the topsoil (0-10 cm depth) in relation

863 to elevation and the dominant tree species.

864 CC = cell content, HEM = hemicellulose, CEL = cellulose, AUR = acid-unhydrolyzable

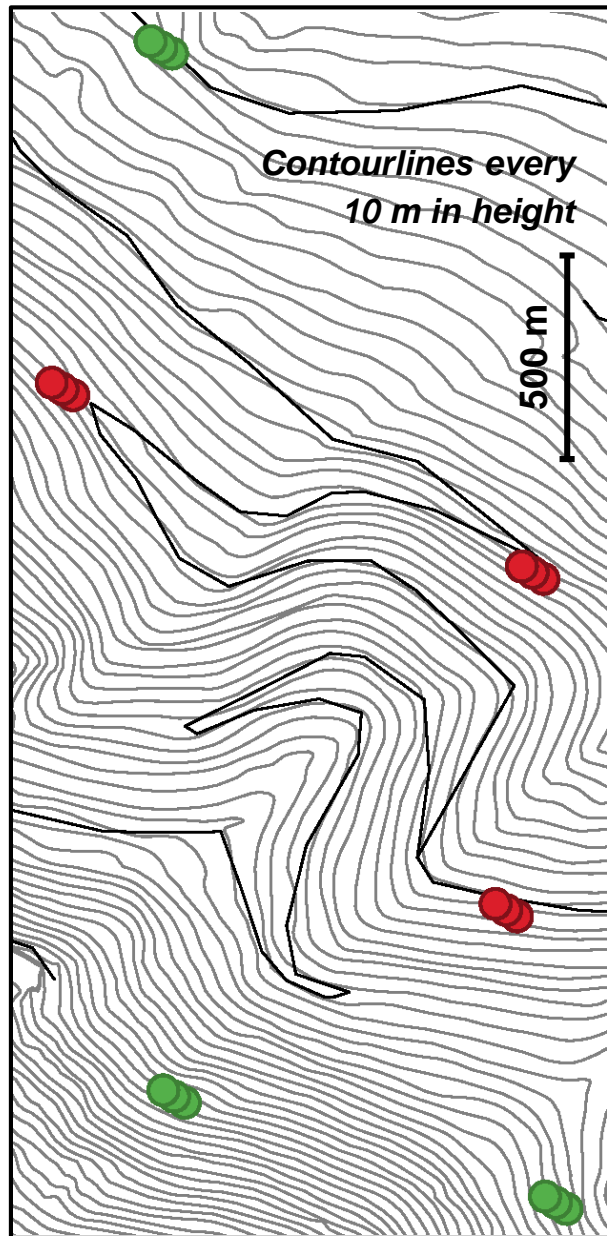865 residue, ASA = acid-soluble ash

866 **Figure 8** Stocks per area of C and major nutrients across the humus layers and the POM

867 (0-10 cm depth) in relation to elevation and the dominant tree species.

868

869   **Figure A.1** Concentrations of major OM fractions in the humus layers and the POM and

870   of free lipids in the humus layers and the topsoil in relation to elevation and the dominant

871   tree species. CC = cell content, HEM = hemicellulose, CEL = cellulose, AUR = acid-

872   unhydrolyzable residue, ASA = acid-soluble ash

873   **Figure A.2** Concentrations of C and major nutrients in the humus layers and the POM in

874   relation to elevation and the dominant tree species.
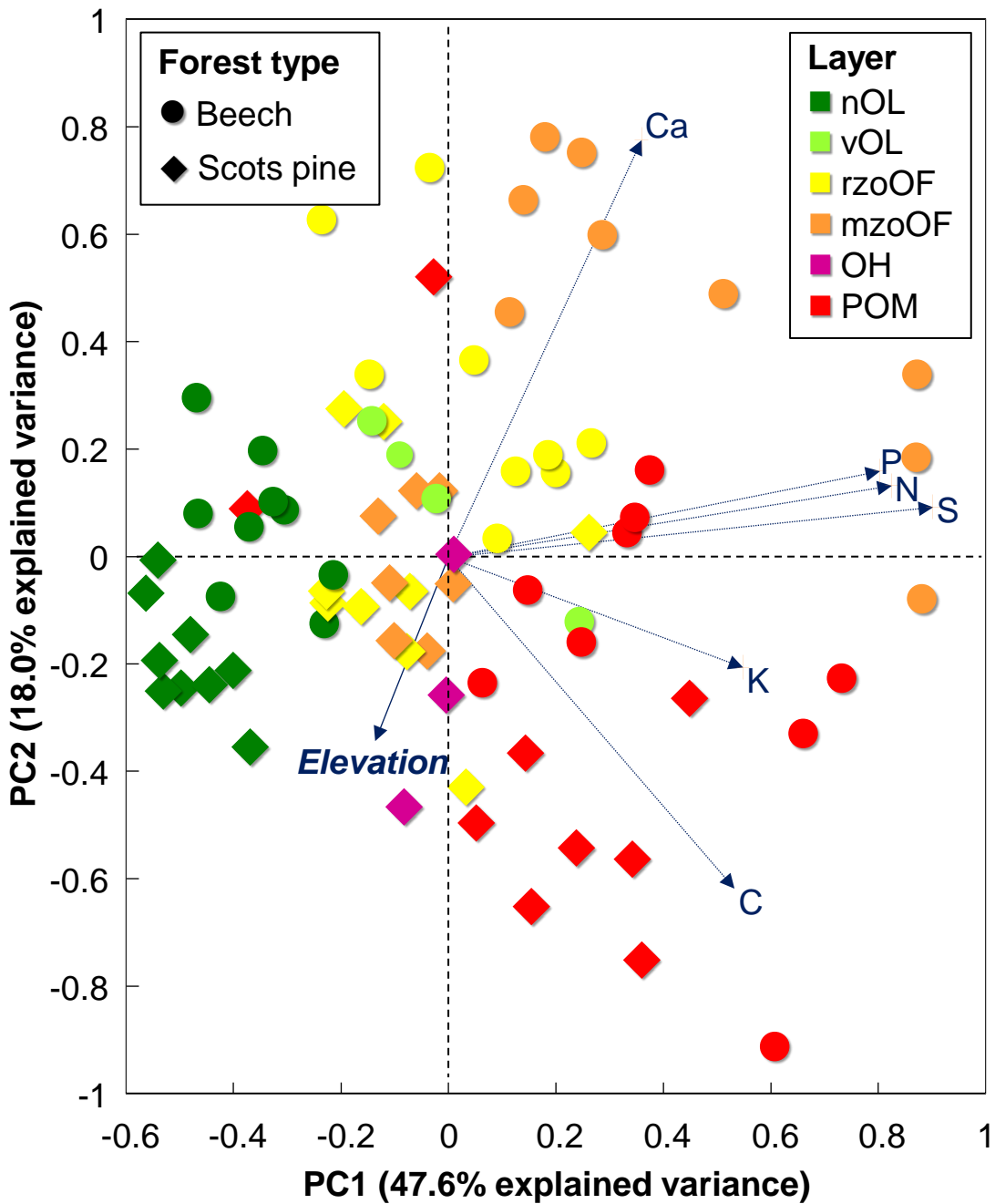
875

Beech forest
Scots pine forest

Contourlines every 10 m in height

500 m

SPAIN
Iberian Range

Litago
Vera de M
Añón de Moncayo
Talamantes
Moncayo Natural Park
Beratón
Purujosa
Calcena

N

Figure 2

Figure 3

Click here to access/download;Figure;

Figure 4

Figure 7

Figure 8