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1 **Overstory and understory vegetation interact to alter soil community composition and activity.**

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9 **Abstract**

10 Aim: To test if there is an interactive effect between tree and understory species on the soil microbial
11 community (SMC), community level physiological profiles (CLPP) and soil micro-fauna.

12 Method: A replicate pot experiment with five sapling tree species (*Betula pendula*, *Betula pubescens*,
13 *Sorbus aucuparia*, *Quercus petraea* and *Pinus sylvestris*) and a no-tree treatment with and without
14 *Calluna vulgaris* was established. After 21 months samples were taken for phospholipid fatty acid
15 (PLFA) analysis, CLPP and soil microfauna assessment.

16 Results: There was an interactive effect of tree species and *Calluna* on the SMC, CLPP and nematode
17 densities. *Calluna* addition changed the SMC composition (increase in fungal PLFAs) and the CLPP
18 (lower utilisation of most carbon sources but greater utilisation of phenolic acids). A multivariate test
19 for homogeneity of dispersion showed that while *Calluna* addition resulted in the presence of an
20 altered microbial composition, it did not result in there being less variability among the samples with
21 *Calluna* than among the samples without *Calluna*. Sapling trees with *Calluna* present grew less well
22 than trees without *Calluna.* Structural equation modelling showed that it is possible that *Calluna* had
23 an indirect effect on the SMC via below-ground tree biomass as well as a direct effect.

24 Conclusion: Interactions between trees and understory vegetation can impact on the composition of
25 soil biota and their activity.

26 Keywords: carbon utilisation, community level physiological profile, enchytraeids, foundation
27 species, nematode, plant-soil interaction, PLFA, structural equation modelling.

28 **Introduction**

29 Soil organisms play a key role in ecosystem processes by influencing decomposition and nutrient
30 cycling and have been observed to affect productivity, competition, nutrient uptake, diversity and
31 successional processes in the plant community (van der Heijden et al. 2008; Lavelle et al. 1997).
32 Several examples suggest that soil microbes and microfauna must be considered important drivers of
33 plant diversity and productivity in terrestrial systems (e.g. Reynolds et al. 2003; van der Heijden et al.
34 2008). Furthermore, the interaction between plants and soil organisms is two-way (Wardle et al.
35 2004; van der Heijden et al. 2008) and a number of studies provide evidence that above-ground plant
36 composition can influence soil biota (e.g. Williamson et al. 2005; Hogberg et al. 2006; van der Wal et
37 al. 2006; Chen et al. 2007; (Mitchell et al. 2007 & 2010a; Djukic et al. 2010) at a range of temporal
38 and spatial scales (Bardgett and Wardle 2010).

39 The impacts of plant composition on belowground communities are generally attributed to species
40 differences in litter quality, root exudates, herbivory and nutrient uptake (Hobbie 1992). Plant species
41 are known to vary in the quality and quantity of carbon types released in their litter and root exudates
42 (Langley and Hungate 2003; Sauheitl et al. 2010). Thus plants may directly impact root-associated
43 soil organisms through resources produced by the roots (root exudates) and the decomposer food web
44 by determining the quantity and quality of litter that enters the soil (Keith et al. 2009). While trees can
45 directly impact soil organisms by decreasing soil moisture and changing the amount and quality of the
46 litter available, they may also have indirect effects by driving changes in understory composition (eg
47 Miles and Young 1980). Species with the greatest biomass are assumed to be most important in
48 driving ecosystem processes and often termed foundation species ("a single species that defines much
49 of the structure of a community by creating locally stable conditions for other species, and by
50 modulating and stabilizing fundamental ecosystem processes (Dayton 1972)" as cited by Ellison et al.
51 2005). Thus in forest and woodland systems it is often the tree species that are studied and the
52 understory is ignored (but see Nilsson and Wardle 2005). However, the understory species may also
53 have important effects on soil organisms, either directly through root exudates and litter addition or
54 indirectly by reducing the growth and productivity of the tree species through allelopathic effects,
55 which leads to a reduction in the amount of litter and root exudates input from the trees. In addition
56 the understory species may associate with mycorrhizal fungi, which maybe the same or different from
57 those associated with the trees; this may affect plant growth (Diaz et al. 2006), the composition of the
58 soil microbial community (SMC) and result in complex nutrient dynamics.

59 Here, in a controlled experiment, we study how the presence of an ericaceous understory shrub layer
60 (*Calluna vulgaris*) with five different sapling tree species (*Betula pendula*, *Betula pubescens, Sorbus*
61 *aucuparia, Quercus petraea* and *Pinus sylvestris*) affects the soil biota. *B. pendula, B. pubescens, S.*

62 *aucuparia*, *Q. petraea* and *P. sylvestris* are native species to the Scottish Highlands in the UK. At low
63 densities these species typically occur with an understory of *C. vulgaris* (Rodwell 1991); thus this mix
64 of species is a natural combination of tree and understory species found in the UK. *Calluna* is an
65 ericoid-mycorrhizae species and the trees are ecto-mycorrhizae species.

66 As the SMC is known to be different under different tree species (Grayston and Prescott 2005; Ushio
67 et al. 2008) we would expect the soil community to be different under each of the 5 tree species in this
68 experiment; but the presence of *Calluna* may also drive changes in the soil community. The soil
69 community in *Calluna*-dominated moorlands is known to be fungal-dominated with low densities of
70 nematodes and a high density of enchytraeids (Keith et al. 2006; Mitchell et al. 2010a; Mitchell et al.
71 2010b). When *Betula* sp. colonises moorland the SMC changes to a bacterial-dominated community,
72 the density of soil nematodes increases and density of enchytraeids decreases (Keith et al. 2006;
73 Mitchell et al. 2010a; Mitchell et al. 2010b). However when *Betula* woodland establishes on
74 moorland the ground flora also changes from *Calluna* dominated to a grass and herb-dominated flora
75 and the relative roles of the trees versus the understory in driving this change is unknown. If the
76 *Calluna* is a foundation species we would expect the *Calluna* to drive the soil community towards that
77 typical of a moorland community (increased fungal PLFAs and enchytraeids and decreased
78 nematodes), irrespective of which tree species are present; as a result of this the variability among
79 samples with trees and *Calluna* will be less than among samples with just trees (Hypothesis 1).
80 Alternatively the *Calluna* may alter the soil community, but in conjunction with the particular tree
81 species present, leading to an interactive effect of tree species and *Calluna* presence. Thus our second
82 hypothesis is that there is an interactive effect between tree species and *Calluna*, whereby soil
83 community composition and physiological profiles will differ between tree species but that these
84 differences change when *Calluna* is present. We test if there is an indirect effect of *Calluna* on the
85 SMC by using structural equation modelling to test our third hypothesis that the presence of *Calluna*
86 reduces the growth of above and below ground biomass of the trees which in turn impacts on the
87 SMC.

88 **Methods**

89 *Experimental set up*

90 The experiment studied the interactions between six tree treatments and the presence/absence of a
91 single *Calluna vulgaris* plant. The tree treatments consisted of saplings of five species of tree (*Pinus*
92 *sylvestris*, *Sorbus aucuparia*, *Quercus petraea*, *Betula pendula* and *Betula pubescens*) and a "no-tree"
93 treatment. Each of the tree treatments were established with and without *C. vulgaris* giving 12
94 treatments in total. The no-tree treatment provided microbial studies with only *C. vulgaris* (no-tree,

95 *C. vulgaris* present) and a fallow treatment with no plant inputs (no-tree, *C. vulgaris* absent). There
96 were four replicates of each treatment combination, resulting in a total of 48 pots.

97 The experiment was set up in January 2003 with soil obtained from Glen Tanar Estate in North-east
98 Scotland, (Latitude 57°03′07.5″N, Longitude 002°48′54.45″W). The altitude of the soil collection site
99 is 240m, the soil type is on the borderline of the Raemoir Series (brown forest soil) and the
100 Countesswells Series (iron podzol) and the parent material is glacial till derived from granite. The soil
101 was sieved through a 6mm mesh sieve prior to planting to remove stones. The overlying vegetation on
102 the soil at the time of collection was *Pteridium aquilinum* with *Pinus sylvestris* and *Calluna vulgaris*
103 within 10m of the collection area. The soil included the organic layer and the mineral horizon down
104 to 25 cm but was bulked and thoroughly mixed in a cement mixer prior to the start of the experiment.
105 The organic horizon was only a few centimetres deep and therefore taking a mixture of organic and
106 mineral horizons represented the depth to which both species were rooted in the natural system. Six
107 soil samples were taken randomly from the bulk mixed soil to establish baseline soil chemistry. The
108 soil had the following mean (± standard error) characteristics: pH (in calcium chloride) 3.09 ± 0.02;
109 moisture 32.11% ± 0.17, carbon 8.2% ± 0.45, nitrogen 0.29% ± 0.02, phosphorus 0.02% ± 0.0004,
110 potassium 0.03% ±0.0005, calcium 0.02% ± 0.0005, magnesium 0.03% ± 0.0004 and sodium 0.01% ±
111 0.0002. Approximately 2kg of coarse sand was placed in the bottom of each of 15-litre pots to
112 provide drainage. The pots were then filled with the mixed soil until the soil was 2cm from the rim.

113 Individuals of the five tree species and *C. vulgaris* were obtained from a nursery. The trees had been
114 container grown for the previous two years and were selected for uniformity in size and structure. The
115 broadleaved trees were around 30cm in height and the pine trees were around 20cm in height. The
116 longest shoots of the *Calluna vulgaris* plants were around 30cm. The roots were washed thoroughly
117 prior to planting to remove the nursery soil. Visual inspection of a selection of the plants showed that
118 no mycorrhizal colonisation had occurred in the nursery.

119 The pots were laid out in a completely randomised design out of doors in an open caged area to avoid
120 any possibility of herbivory. Using natural outdoor growth conditions was important, as frost can
121 affect nutrient availability and the aim was to reflect natural conditions as closely as possible. All
122 pots were weeded regularly

123 The experiment ran for 21 months; in October 2005 two soil cores (each 3.5 cm diameter × 5 cm
124 depth) were taken from each pot, sieved to 5mm and bulked. A subsample of sieved soil was
125 removed and freeze-dried for further microbial analysis. Remaining soil was used to extract soil
126 microfauna (nematodes and enchytraeids). In addition a 100g soil sample was taken weighed and
127 dried for 48 hours at 105 °C for soil moisture measurement.

128 *Tree and Calluna biomass measurements*

129 At the start and end of the experiment the height and root collar diameter of each plant was measured;
130 the increase in these measures during the experiment was calculated. At the end of the experiment
131 each plant was cut at ground level. The soil was removed from the pot and all tree and *Calluna* roots
132 separated out, washed gently and blotted dry. The above and below-ground plant samples were oven
133 dried at 70°C for 72 hours and weighed.

134 *Phospholipid Fatty Acid Extraction (PLFA)*

135 The structure of the SMC was assessed by analysing the composition of extractable ester-linked
136 phospholipid fatty acids (PLFA). Lipids were extracted from 0.5 g freeze-dried soil in a chloroform-
137 methanol-citrate buffer mixture (1:2:0.8), and the phospholipids were separated from other lipids on a
138 silicic acid column using the procedure described by Frostegård et al. (1993). The phospholipids were
139 subjected to a mild-alkali methanolysis and the resulting fatty acid methyl esters (FAMES) were
140 separated and identified by gas chromatography. The separated FAMES were identified and
141 quantified by chromatographic retention time, initially confirmed by mass spectral comparison, using
142 a standard qualitative bacterial acid methyl-ester mix (Supelco; Supelco UK, Poole, Dorset, UK) that
143 ranged from C11 to C20. Analysis was carried out on a Hewlett Packard 6890N gas chromatograph.
144 Saturated and unsaturated FAMES were identified by making silver adducts and the position of
145 unsaturated bonds was determined using disulphide bridging. For each sample the abundance of
146 individual fatty acid methyl-esters was expressed as nm PLFA g^{-1} dry soil.

147 The PLFAs were classified according to Frostegård et al. (1996) for fungi and actinobacteria and
148 according to Frostegård et al. (1996) and Zogg (1997) for bacteria (Supplementary Table A). Zogg et
149 al. (1997) was used to classify the bacterial PLFAs into Gram-positive and Gram-negative bacteria
150 (Supplementary Table A). Those PLFAs listed by Frostegård et al. (1996) as actinobacteria were
151 added to the Zogg et al. (1997) list of Gram-positive bacteria. The PLFA 18:2ω6,9 is found in both
152 plants and fungi but as most roots were removed from the soil prior to analysis this marker was taken
153 to indicate the presence of fungi (Frostegård et al. 1993). By summing PLFAs known to be of
154 bacterial or fungal origin, it is also possible to quantify bacterial or fungal biomass separately (e.g.
155 Pennanen et al. 1998). The ratio of fungal to bacterial PLFA was calculated by taking the fungal
156 marker 18:2ω6,9 and dividing it by the sum of the predominant bacterial PLFAs (Supplementary
157 Table A; Frostegård and Bååth 1996).

158 *Biolog analysis*

159 Using the method of Campbell et al. (1997) with additional carbon sources (Grayston and Prescott
160 2005), the soil was analysed using Biolog to determine carbon utilisation profiles. A 10g sample of
161 soil was shaken with 100 mls ¼ strength ringer's solution (Oxoid) for 10 mins and then serially

162 diluted tenfold. A 50 ml sample of the dilution was centrifuged at 2000 rpm for 10 minutes and then
163 150 µl of the supernatant was inoculated into each well of the plates. The plates were then incubated
164 at 15°C and colour development (carbon utilisation) was measured at 590nm every 24 hours for 5
165 days using a microplate reader (Vmax, Molecular Devices, Oxford, UK). The colour response of the
166 control blank well was subtracted from the colour response of each well prior to data analysis. Only
167 data from day 5 are presented here as using this time interval revealed the clearest patterns.

168 *Soil microfauna*

169 Microfauna were extracted using a modified 'tray' version of the Baermann funnel method
170 (Whitehead and Hemming 1965). Briefly, 60g fresh sieved soil was added to a tray containing 400ml
171 distilled water with a coarse mesh support and a single layer of 'Kleenex' tissue paper. The extract
172 was removed after 40 hours and settled for a further 24 hours before nematodes and enchytraeids were
173 counted live at 40× magnification.

174 *Data analysis*

175 In order to test if the variability between samples (in terms of a dissimilarity metric) was less when
176 *Calluna* was present a dissimilarity index for the PLFA and CLPP data was calculated using the
177 Gower dissimilarity index (DG) (Anderson et al 2006) and a multivariate test for homogeneity in
178 dispersions was carried out using the PERMDISP program (Anderson, 2004). The dissimilarity index
179 was calculated as:

180 $$DG = \frac{\sum_{k=1}^{p} w_k \left| x_{ik} - x_{jk} \right| / R_k}{\sum_{k=1}^{p} w_k}$$

181 where x_{ik} is the value of the kth PLFA/carbon source in the ith sample and R_k is the range of the kth
182 PLFA/carbon. Weights (w_k) are used to provide the desired exclusion of joint absences by setting w_k
183 = 0 , whenever $x_{ik} = x_{jk} = 0$ and $w_k = 1$ elsewhere.

184 A permutational multivariate analysis of variance (PERMANOVA, Anderson 2005) was performed
185 on the PLFA (% mol) and CLPP data using the DG index. Results of 9999 permutation of the raw
186 data are presented.

187 Univariate data were analysed by analysis of variance on pot level data using the GLM procedure in
188 SASv9.1 (SAS 2008). Tree treatment, the presence of *Calluna* and the interaction between tree
189 treatment and *Calluna* presence were included as fixed effects. Tukey's pair-wise comparisons were
190 used to determine differences between treatments and adjusted using the Tukey–Kramer correction
191 for multiple tests. PLFA data was analysed as nmol g^{-1}.

192 The direct and indirect impacts of *Calluna* and the tree species on the SMC was assessed using
193 structural equation modelling (SEM). SEM allows for the both the direct and indirect theoretical
194 causal relationships between intercorrelated variables to be tested, and for potential multivariate
195 relationships to be identified (Grace 2006). The SEM corresponds to a series of regression models
196 but, unlike a series of separate regressions, it provides tests of whether the observed covariance matrix
197 is similar to the one implied by the model. The PLFA fungal:bacterial ratio was taken as the most
198 appropriate measure to assess change in the SMC. The SEM was carried out using the Proc CALIS
199 procedure in SASv9.1 (SAS 2008). An initial SEM model, Model 1, (Fig 1) was developed to test the
200 direct and indirect effects of *Calluna* and tree species on the SMC. The indirect effects were thought
201 to occur through the influence of *Calluna* and tree species on the above- and below-ground tree
202 biomass which in turn were thought to affect the SMC. Thus Model 1 had three equations or sets of
203 pathways: (1) the effects of tree species and *Calluna* on below-ground tree biomass (2) the effects of
204 tree species and *Calluna* on above-ground tree biomass and (3) the effect of below- and above-ground
205 tree biomass, tree species and *Calluna* on the SMC. In addition the correlation between above- and
206 below-ground tree biomass was included in the model. *Calluna* presence was coded as a binary
207 variable and tree species were coded as dummy variables so only 4 of the 5 species needed to be
208 included and *S. aucuparia* was randomly chosen to be omitted.

209 Model 1 was then improved by removing non-significant pathways from the model. The d-separation
210 claims implied by each model (Shipley 2009) were tested to assess if the regression coefficients for
211 *Calluna*, below- and above-ground biomasss and the tree species were zero and thus if these terms
212 could be removed from the model. The significance of the path coefficients for *Calluna* presence,
213 below-ground biomass and above ground biomass was tested using t-tests. The significance of all 4
214 tree species variables was tested simultaneously using an F-test. For each model the conditional
215 independence of all pairs of variables not joined by an arrow was tested in this way. The probabilities
216 from the independence claims implied by each model were combined and the resulting C value
217 compared to a chi-squared distribution. In Model 1 the path coefficient for the effect of root biomass
218 on the PLFA fungal:bacterial ratio had the highest p-value; removing this term resulted in Model 2.
219 The path coefficient for the effect of above-ground biomass was not significant in Models 1 or 2;
220 removing this term from Model 2 resulted in Model 3. In Model 1 the effect of tree species on the
221 PLFA fungal:bacterial ratio was also not significant; removal of tree species from Model 1 gave
222 Model 4. A fifth model (Model 5) was tested that dropped the direct effect of *Calluna* on the
223 fungal:bacterial ratio from Model 4. The final model (Model 6) removed the direct effect of above-
224 ground tree biomass from Model 4. A χ^2 test was used to determine whether the covariance structure
225 implied by the modified models adequately fitted the actual covariance structures of the data (a non-
226 significant χ^2 ($P>0.05$) indicates adequate model fit). The null hypothesis is that the observed and
227 predicted variances and covariances are equal, where the covariance matrix is a 9x9 matrix

228 corresponding to the SMC, above ground biomass, below ground biomass, and 5 indicator variables
229 for tree species and *Calluna*.

230 **Results**

231 *The soil microbial community: PLFA results.*

232 The PERMANOVA analysis of the % mol PLFA data showed that while the addition of *Calluna* had
233 no significant impact on the PLFAs ($F_{1,30}$ = 1.21, *P*= 0.266) the different tree species did have an
234 impact ($F_{4,30}$ = 2.86, *P*= 0.005). Pair-wise a posterior comparisons showed that the PLFAs under *B.*
235 *pubescens* were significantly different from those under *Sorbus aucuparia, B. pendula and Q. petraea*
236 *and* the PLFAs under *P. sylvestris* were significantly different from those under *S. aucuparia, and Q.*
237 *petraea.* There was no significant interaction between *Calluna* presence and tree species. Analysis of
238 the dissimilarity indices showed that there was no significant difference in multivariate dispersion
239 between the groups with and without *Calluna* present (*P*>0.05). Thus, *Calluna* addition did not result
240 in there being less variability between the samples with *Calluna* than between the samples without
241 *Calluna*.

242 Univariate analysis of the PLFA data (nmolg^{-1} data) following classification into microbial groups
243 (Supplementary Table A; Frostegård and Bååth 1996) showed that the presence of *Calluna* had a
244 significant effect on the amount of fungal PLFA and the fungal:bacterial ratio, with more fungal
245 PLFA and a higher fungal:bacterial ratio in the pots with *Calluna* than in those without (Table 1, Fig.
246 2). Tree treatment had a significant effect on the total amount of PLFA, bacterial PLFA, Gram
247 positive PLFA and Gram negative PLFA (Table 1, Fig.2); in each case values were greater under *Q.*
248 *petraea* than under *P. sylvestris* (*P*<0.05). In addition, Gram positive PLFA was greater under *Q.*
249 *petraea* than in the no-tree treatment (*P*<0.05). There was also a significant effect of tree treatment on
250 the Gram-positive:negative ratio, with the ratio being significantly greater in the *Q. petraea* and the *B.*
251 *pubescens* than in fallow pots. There was no effect of tree treatment or *Calluna* presence on the
252 amount of actinomycete PLFA present (Table 1). There was a significant interaction between tree
253 treatment and *Calluna* for the fungal:bacterial and Gram-positive:negative ratios (Fig.3). The
254 fungal:bacterial ratio was significantly greater for *P. sylvestris* with *Calluna* than *B. pubescens* and *Q.*
255 *petraea* with *Calluna*, when *Calluna* was absent there was no difference between these species in
256 their fungal:bacterial ratio. The Gram positive:negative ratio showed that *Q. petraea* with *Calluna*
257 had a higher ratio than *P. sylvestris* with *Calluna,* when *Calluna* was absent there was no difference
258 between these tree species. *Q. petraea* with *Calluna* also had a higher Gram positive:negative ratio
259 than the fallow and *Calluna* only treatments.

260 *Nematode and enchytraeids*

261 The impact of tree treatment on nematode density was influenced by *Calluna* addition (tree treatment
262 by *Calluna* interaction: Table 1; Fig. 4a). When *Calluna* was absent *B. pubescens* and *P. sylvestris*
263 had significantly greater density of nematodes than fallow soil (no-tree, *Calluna* absent) but when
264 *Calluna* was present there was no difference in densities between these tree species and that in fallow
265 soil (no-tree, *Calluna* absent). In contrast, *Q. petraea* had greater densities of nematodes than fallow
266 soil (no-tree, *Calluna* absent) when *Calluna* was both present and absent. Pots without *Calluna* had a
267 much higher enchytraeid density than pots with *Calluna* but enchytraeid density did not vary
268 significantly between tree treatments (Table 1, Fig. 4b).

269 *Soil moisture*

270 Soil moisture at sampling ranged from 37% in the fallow pots (no-tree, *Calluna* absent) to 15.5% in
271 the *Calluna* only pots (no-tree, *Calluna* present) (Fig. 4c). *Calluna* presence had a significant effect
272 on soil moisture ($F_{1,36}$=108.26, P<0.0001) but this effect was modified by the tree treatment (tree
273 treatment by *Calluna* interaction: $F_{5,36}$=12.07, P<0.0001). For *P. sylvestris, B. pubescens, S.*
274 *aucuparia* and the no-tree treatments soil moisture was lower when *Calluna* was present (P<0.05), for
275 *Q. petraea* and *B. pendula* soil moisture did not vary significantly with the addition of *Calluna*. There
276 were also significant differences in soil moisture between tree treatments ($F_{5,36}$=9.45, P <0.0001) with
277 *Q. petraea* having significantly (P<0.05) lower soil moisture than all the other tree treatments.

278 *Community level physiological profiles (Biolog data)*

279 Multivariate analysis (PERMANOVA) showed that the presence of *Calluna* ($F_{1,30}$ = 2.957, P<0.0001)
280 and the different tree species ($F_{4,30}$ = 1.28 P=0.0127) had significant impacts on which carbon sources
281 were utilized. In addition there was a significant interaction between tree species and *Calluna*
282 presence on carbon utilisation ($F_{4,30}$ = 1.3, P=0.0058). Pair-wise a posteriori comparisons showed
283 significant differences in which carbon sources were utilized under *B. pendula* compared to *P.*
284 *sylvestris*, *Q. petraea and S. aucuparia.* The interaction term showed that carbon utilisation was
285 significantly different with and without *Calluna* for all of the deciduous tree species: *B. pendula, B.*
286 *pubescens, Q. petraea,* and *S. aucuparia* but not for *P. sylvestris*. Analysis of the dissimilarity indices
287 showed no significant difference in multivariate dispersion between the groups with and without
288 *Calluna* present.

289 Univariate analysis showed that total carbon utilisation was significantly lower when *Calluna* was
290 present (Table 1). However, there were differences between the different types of carbon sources (Fig
291 5). The utilisation of sugars, carboxylic acids, acidic amino acids, neutral amino acids, phenolic acid
292 signals, basic amino acids and secondary amino acids was lower when *Calluna* was present (Table 1).
293 The pattern was reversed for phenolic acids with the utilisation of phenolic acids being greater when
294 *Calluna* was present. There was no effect of *Calluna* on the utilisation of oligo-sugars and long chain

295 aliphatic acids. Total carbon utilisation was significantly different between tree treatments (Table 1)
296 with carbon utilisation being significantly greater under *Pinus sylvestris* than under *Betula pendula* or
297 in the no-tree treatment (P<0.05). There were significant interactions between tree treatment and
298 *Calluna* presence for aromatic amino acids and phenolic acids (Table 1, Fig. 6). When *Calluna* was
299 absent there was greater utilisation of aromatic amino acids in *Q. petraea* than *B. pendula* and greater
300 utilisation of phenolic acids in *B. pubescens* than *S. aucuparia* but no difference between the species
301 when *Calluna* was present.

302 *Biomass results*

303 *Calluna* presence had a significant effect on tree growth (Fig. 7). The increase in tree height in the 40
304 pots with trees present ($F_{1,30}$ = 11.10 P = 0.0023) and basal diameter ($F_{1,30}$ = 72.28, P <0.0001) was
305 greater in the absence of *Calluna*. Total above ground biomass ($F_{1,30}$ = 44.12, P <0.0001) and root
306 biomass ($F_{1,30}$ = 94.55, P <0.0001) was also greater in the absence of *Calluna*.

307 The total above-ground biomass of the *Calluna* plants at the end of the experiment was greater in
308 treeless pots than in pots with trees (above-ground: $F_{1,18}$ = 14.10, P =0.0014). There was a weakly
309 significant affect of tree presence on below-ground biomass ($F_{1,18}$ = 4.15, P =0.057) and there was no
310 significant difference in the increase in *Calluna* height and stem diameter between plants with and
311 without a tree present (height: $F_{1,18}$ = 0.03, P =0.86; stem diameter: $F_{1,18}$ = 0.001, P =0.97). The
312 interaction term between tree presence and tree species showed that when the *Calluna* was grown
313 with a tree the total above ($F_{4,18}$ = 1.62, P =0.21) and below-ground ($F_{4,18}$ = 0.06, P =0.99) biomass
314 and increase in *Calluna* height ($F_{4,18}$ = 1.47, P =0.25) and stem diameter ($F_{4,18}$ = 1.59, P =0.22) was
315 not significantly different between tree species.

316 *Structural equation modelling*

317 The initial model (Model 1) showed that tree species and *Calluna* significantly affected both above-
318 and below-ground biomass (P<0.05) with adjusted R^2 values of 0.74 and 0.88 respectively (Table 2).
319 The two equations relating tree species and *Calluna* to above- and below-ground biomass were not
320 changed in the sequence of models described below, thus the path coefficients for these variables and
321 the adjusted R^2 squares remained the same (Table 2). In Model 1 none of the variables were
322 significant in explaining the variation in the PLFA fungal:bacterial ratio. It was not possible to
323 calculate a χ^2 statistic for this initial model as it perfectly reproduces the sample covariance matrix and
324 the degrees of freedom are zero. Model 2, (Tables 2, 3 and 4) which removed root biomass from the
325 list of variables affecting the PLFA fungal:bacterial ratio, gave a χ^2 statistic of 0.02 and an adjusted R^2
326 of 0.151. The path coefficients in this model were -0.105 for above-ground biomass and 0.351 for
327 *Calluna* but again none of the variables were significant in explaining the variation in the
328 fungal:bacterial ratio. Model 3 which removed the direct effect of above-ground tree biomass on the

329 fungal:bacterial ratio had a χ^2 statistic of 0.14 (df=2) and an adjusted R^2 of 0.173. *Calluna* presence
330 had a significant, (*P*<0.0.5) direct impact on the fungal:bacterial ratio with a standardized path
331 coefficient of 0.408 but the direct effects of tree species were not significant (Table 2). Model 4,
332 which removed tree species from the direct effects on fungal:bacterial ratio that were in Model 1, had
333 a slightly lower goodness of fit index than Model 3, a χ^2 statistic of 2.16 (df=4) and an adjusted R^2 of
334 0.174. In this model below-ground biomass (standardized path coefficient = -0.263) had a weakly
335 significant (*P*<0.1) direct effect on the fungal:bacterial ratio and the presence of *Calluna*
336 (standardized path coefficient = 0.367) had a significant (*P*<0.05) direct impact on the fungal:bacterial
337 ratio. Model 5, (removal of the direct effect of *Calluna* on the fungal:bacterial ratio from Model 4)
338 led to a less acceptable model. The adjusted goodness-of-fit statistic for Model 5 was less than 0.8,
339 the adjusted R^2 was lower, one of the d-separation independence tests was weakly significant
340 (*P*=0.059, Table 4) and so was the C value for the two tests combined (*P*=0.108, Table 4). This
341 suggests that there was a direct effect of *Calluna* on the SMC. Model 6 had an adjusted goodness of
342 fit index of 0.871 and a χ^2 statistic of 2.79 (df=5) with *Calluna* presence (path coefficient 0.294) and
343 below-ground biomass (path coefficient -0.269) both having a weakly significant direct effect (*P*<0.1)
344 on the fungal:bacterial ratio (Tables 2, 3 and 4). Model 6 has the highest adjusted R^2 (0.184) of the
345 models tested but it is not possible to reject any of the other models with the possible exception of
346 Model 5.

347 **Discussion**

348 *Interactive effects of tree species and Calluna on the soil community*

349 This work has shown that there are interactive effects between understory species and tree species on
350 the SMC, nematode densities and community level physiological profile. The presence of *Calluna* did
351 result in some aspects of the soil community becoming more similar to that under a moorland as
352 predicted by Hypothesis 1. However, the variability between samples with trees and *Calluna* was not
353 lower than that between samples without *Calluna* indicating that although the *Calluna* was altering
354 the SMC the trees were also influencing the community; rejection of Hypothesis 1 and acceptance of
355 Hypothesis 2. As expected the SMC was different under different tree species (Aponte et al. 2010;
356 Grayston and Prescott 2005) but these differences changed when *Calluna* was present. When *Calluna*
357 was absent there was no difference between the tree species in the fungal:bacterial ratio or the Gram-
358 positive:negative ratios; however, when *Calluna* was present there were differences between tree
359 species. As the growth of the *Calluna* did not vary between tree treatments it is unlikely that
360 differences between *Calluna* plants caused these differences in treatment effects on the SMC, but
361 rather that there is an interactive effect occurring between tree species and *Calluna* presence. *Pinus*
362 *sylvestris* and *Calluna* both occur on 'mor' soils, which are acidic with low fertility (Gimingham

363 1960) and have fungal-dominated-food webs (Wardle 2005). *Betula* and *Quercus* species are known
364 to drive 'mor' soils towards a less acidic, relatively more fertile, 'mull' soil (Miles and Young 1980;
365 Nielsen et al. 1987; Nielsen et al. 1999; Mitchell et al. 2007) with a more bacterial-dominated food
366 web (Wardle 2005). These results suggest that even within a 21-month growth period a combination
367 of *P.sylvestris* and *Calluna* can drive the SMC towards a more fungal-dominated community
368 compared to pots with *Quercus* and *Betula* with *Calluna*.

369 Nematode density was greater under *B. pubescens, P. sylvestris* and *Q. petraea* than in fallow soil
370 when *Calluna* was absent. However, when *Calluna* was present nematode densities under *B.*
371 *pubescens* and *P. sylvestris* were at levels equal to those present in fallow soil; this was not the case
372 under *Q. petraea*. This implies that either the positive effects of *Q. petraea* on nematode densities
373 (root exudates and chemical composition of the litter) outweighed the negative effects of *Calluna* or
374 that any allelopathic effects of *Calluna* occur with *B. pubescens* and *P. sylvestris* but not *Q. petraea*.

375 *Calluna* presence also altered the CLPP with interactive effects between the presence of *Calluna* and
376 tree species. The CLPP was significantly different when *Calluna* was present under deciduous trees
377 but not under the one coniferous species used in the experiment. This suggests that *Calluna* and *P.*
378 *sylvestris* are providing the same types of carbon sources for the microbial community where as the
379 deciduous tree species are providing different types of carbon from the *Calluna*. When *Calluna* was
380 absent there was greater utilisation of aromatic amino acids in *Q. petraea* than *B. pendula* and greater
381 utilisation of phenolic acids in *B. pubescens* than *S. aucuparia* but there was no difference between
382 these species when *Calluna* was present. If the carbon utilisation pattern is a reflection of the carbon
383 types that the SMC are adapted to use, and hence the types of carbon being produced by the plants,
384 these results suggest that the presence of *Calluna* is altering the types or amount of carbon available to
385 the SMC, either directly or indirectly by effecting tree growth and the carbon sources produced by the
386 trees.

387 Trees with *Calluna* present grew less and had reduced above- and below-ground biomass. The
388 structural equation modelling showed that the *Calluna* may affect the SMC composition indirectly by
389 causing the trees to have lower below-ground biomass which in turn affected the SMC (Hypothesis
390 3). Therefore, *Calluna* may indirectly affect the SMC via allelopathic effects or direct competition for
391 light, water and nutrients reducing the growth of the trees (Mallik 1995). Ericaceous plants have been
392 shown to cause "growth check" in conifer plantations (Mallik 1995) and as early as 1961 it was
393 shown that *Calluna* differed from coexisting plants species because it could produce tannin-protein
394 complexes that inhibited mineralization of nitrogen, and therefore the nutrition of coexisting plant
395 species (Handley 1961). In the present experiment *Calluna* may also have reduced the growth of the
396 trees by reducing soil moisture. Whether the smaller trees were a result of competition or allelopathic

397 effects cannot be separated within this experiment. However, smaller trees will produce less litter
398 fall, root litter and root exudates, which are the main energy sources for the soil microorganisms,
399 resulting in a change in the composition of the SMC (Pietikainen et al. 2007). In addition,
400 competition may alter the resource allocation of carbon within the plant. In *P. sylvestris* stands with
401 strong competition the trees allocated more resources to stem wood and coarse roots and reduced the
402 amount allocated to fine roots and needles (Vanninen and Mäkelä 2005) which would provide the
403 more immediate source of carbon for soil microorganisms. In addition to driving the composition of
404 the SMC through smaller trees the *Calluna* may be inhibiting the trees from producing root exudates
405 that contain labile carbon sources such as sugars and amino acids and this may be one reason why the
406 utilisation of these carbon sources within the Biolog was lower when *Calluna* was present.

407 However, the results from the SEM were not clear cut; the correlation between below-ground biomass
408 and the PLFA fungal:bacterial ratio was only weakly significant and a model without this indirect
409 effect was also found to be consistent with the observed pattern of covariances. Whilst the causal
410 effect of the understory and tree species can be tested through a randomised experiment, above and
411 below ground biomass cannot be directly controlled and it was therefore necessary to use SEM.
412 However, the fact that SEM does not reject a hypothetical causal model does not mean that it is the
413 one true model; there may be additional models which fit as well or better (Shipley, 1999). Thus
414 further work is required to disentangle the direct and indirect effects of the understory and tree species
415 on the SMC. It may be that measures other than PLFAs would be more suitable to assess the SMC
416 and may better show the relative direct and indirect impact of the understory species.

417 *Direct effects of Calluna on the soil community*

418 The presence of *Calluna* had a significant impact on the SMC, the soil microfauna and community
419 level physiological profiles but did not result in the SMC under different tree species being more
420 similar to each other than when *Calluna* was absent. As predicted by our first hypothesis *Calluna*
421 presence increased the proportion of the PLFAs that were of fungal origin, this may, in part, be
422 because *C. vulgaris* will bring with it its own ericoid mycorrhiza (Diaz et al 2006) which will contain
423 the PLFA fungal marker 18:2ω6,9. The level of mycorrhizal infection was not assessed so it is not
424 possible to quantify the proportion of the increase in fungal PLFAs that was due to mycorrhiza.
425 Contrary to our first hypothesis, the density of enchytraeids declined when *Calluna* was added. These
426 changes may be driven via changes in soil moisture, soil chemistry or changes in the quality and
427 quantity of carbon sources available (Huhta et al. 1998; Gongalsky et al. 2008).

428 Twenty years after *Calluna* was planted in felled *Betula* woodland the fungal community in the
429 planted *Calluna* plots was different from the control plots (birch woodland) (Mitchell et al. 2010a) but
430 the soil chemical properties were not significantly different (Mitchell et al. 2007). This implies that

431 changes in soil chemistry driven by *Calluna* occur over decades, not over the few months of an
432 experiment such as this, suggesting that changes in soil chemistry are not the method through which
433 the *Calluna* is driving changes in the SMC within this study.

434 There were significant differences in carbon utilisation patterns when *Calluna* was present. Assuming
435 carbon utilisation patterns reflect the carbon resources that are available within the soil the results
436 suggest that *Calluna* is driving changes in the SMC by changing the quantity and quality of carbon
437 sources available through root exudates and litter (Bertin et al. 2003; Lambers et al. 2009). Root
438 exudates containing root-specific metabolites have critical ecological impacts on soil macro and
439 microbiota (Bertin et al. 2003). Through the exudation of a wide variety of compounds, roots impact
440 the SMC in their rhizosphere, support beneficial symbioses and alter the chemical and physical
441 properties of the soil (Bertin et al. 2003). *Calluna* produces phenolic compounds in both its litter and
442 root exudates (Hofland-Zijlstra and Berendse 2010). While sugars, amino acids and oligo sugars are
443 generally short lived in the soil, and used up by the soil community very quickly, phenolic acids are
444 more recalcitrant (Kanerva et al. 2008). Therefore an increase in soil organisms able to utilise
445 phenolic compounds when *Calluna* is present, as shown by the Biolog analysis here, is to be expected.
446 Waldrop and Firestone (2004) found that distinctly different plant communities (oak and grassland)
447 did not alter the microbial community profile responsible for decomposition of relatively labile carbon
448 substrates but did alter the profiles of microbial communities responsible for the decomposition of the
449 more recalcitrant substrates. Vauramo and Setälä (2010) found that the soil under plants producing
450 labile litter had a higher bacteria biomass than soil under plants producing more recalcitrant litter.
451 This concurs with our results which show that the presence of *Calluna*, which produces recalcitrant
452 substrates (phenols), altered the balance of fungi to bacteria within the microbial community. Root
453 exudates, particularly phenolics, influence the growth and development of soil micro-organisms
454 (Bertin et al. 2003) and it is possible that the *Calluna* root exudates may have inhibited the soil micro-
455 organisms that utilise the more labile carbon compounds (sugars and amino acids) explaining the
456 lower utilisation of these substrates in the Biolog analysis. However Biolog does provide a better
457 reflection of changes in the bacterial community than the fungal community. With increased
458 (mycorrhizal) fungi present, there might be less root exudates available for bacteria, and thus fewer
459 bacteria within the soil capable of utilising these more liable compounds.

460 *Understory vegetation as a foundation species?*

461 This work has shown that an understory species within a forest system can, have a direct effect on the
462 soil microbial and microfauna composition and substrate utilisation. *Empetrum hermaphroditum,* an
463 understory species in Swedish boreal forests, has been shown to drive changes in the SMC (Nilsson
464 and Wardle 2005) through slow decomposing litter containing lower levels of nitrogen than co-

465 existing ericaceous shrub species (Wardle et al. 2003a; Wardle et al. 2003b) and through the release
466 of phenols (Wardle et al. 1998; DeLuca et al. 2002). In the system studied by Nilsson and Wardle
467 (2005) the relationship between successional stage and decomposer activity is driven entirely by the
468 type of ericaceous dwarf shrubs present. The question of whether the understory species can also
469 have an indirect effect on the SMC via reduced tree growth requires further work but the results from
470 the SEM suggest that *Calluna* may also engineer the SMC through indirect effects on tree root
471 biomass as well as direct effects.

472 In the field, the direct effects of *Calluna* on the SMC may occur at all stages of tree development
473 during succession from *Calluna* dominated moorland to woodland, provided that the tree canopy
474 cover does not get so dense that the *Calluna* becomes shaded out (Hester et al. 1991). *Calluna* is
475 known to cause a growth check on establishing trees (Mallik 1995) and hence indirect effects of the
476 *Calluna* on the SMC, via allelopathic and competitive effects on the trees might be expected during
477 tree development; it is not know whether these effects continue once the trees are older and
478 competitive effects might be expected to be reduced. Future work in such upland systems could utilise
479 techniques to track compound specific carbon and assess the relative importance of direct and indirect
480 effects of *Calluna* in the field.

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Table 1 The impact of tree species and *Calluna* on soil microbial community (PLFA), soil micro-fauna and carbon utilisation. Summary statistics (F and *P* values) are derived from analysis of variance. Significant terms are in bold.

	Tree species		*Calluna* presence		Tree species x *Calluna* presence interaction	
	F	*P*	F	*P*	F	*P*
PLFAs						
Total	3.00	**0.020**	0.71	0.400	1.61	0.180
Bacteria	3.39	**0.010**	0.22	0.640	1.69	0.160
Fungal	0.82	0.540	12.66	**0.001**	2.36	0.059
Fungal:bacterial ratio	1.55	0.190	17.13	**<0.001**	3.32	**0.014**
Actinomycetes	1.48	0.220	0.04	0.848	1.40	0.247
Gram positive bacteria	4.25	**0.004**	0.31	0.580	1.71	0.156
Gram negative bacteria	2.98	**0.020**	0.13	0.719	1.81	0.135
Gram negative: positive ratio	4.06	**0.005**	0.03	0.861	3.32	**0.010**
Soil micro-fauna						
Enchytraeids	1.98	0.150	33.88	**<0.001**	1.98	0.106
Nematodes	5.45	**<0.001**	1.72	0.190	2.83	**0.030**
Carbon utilisation						
Total carbon	3.43	**0.014**	16.12	**<0.001**	1.55	0.198
Sugars	1.70	0.159	19.88	**<0.001**	1.53	0.206
Oligo-sugars	0.63	0.679	0.06	0.802	0.84	0.533
Carboxylic acids	3.99	**0.006**	8.19	**0.007**	2.42	0.055
Acidic Amino acids	2.34	0.060	11.18	**0.002**	1.32	0.279
Neutral amino acids	2.59	**0.040**	7.29	**0.010**	1.93	0.110
Basic amino acids	2.30	0.065	8.14	**0.007**	1.48	0.220
Aromatic amino acids	2.69	**0.036**	1.76	0.193	3.80	**0.007**
Long chain aliphatic acids	3.81	**0.007**	0.52	0.470	1.37	0.257
Secondary amino acids	0.58	0.717	5.55	**0.020**	0.40	0.844
Nucleic acid bases	0.11	0.988	3.19	0.080	0.45	0.810
Phenolic acids	2.45	0.052	7.52	**0.009**	2.61	**0.040**

Table 2. Standardized coefficients for the variables included within the three pathways or equations modelled by the structural equation modelling. Those in bold are significant at $P < 0.05$, those in bold italics are significant at $P < 0.1$. Where no coefficient is shown that pathway was not included in the model. Note the correlation between the error terms in equations 1 and 2 was 0.0475. CV= *Calluna vulgaris*, BPe = *Betula pendula*; BPu = *Betula pubescens*; PS = *Pinus sylvestris*; QP = *Quercus petraea.*

	Variables							R^2	Adjusted R^2
Equation 1:									
Root =	CV	PS	QP	BPu	BPe				
Models 1-6	**-0.425**	**-0.199**	**0.684**	**-0.198**	**0.16**			0.892	0.876
Equation 2:									
Above-ground biomass =	CV	PS	QP	BPu	BPe				
Models 1-6	**-0.545**	**0.573**	0.0322	**-0.214**	**-0.142**			0.776	0.743
Equation 3:									
Fungal:Bacterial ratio =	Roots	Above-ground biomass	CV	PS	QP	BPu	BPe		
Model 1	-0.066	-0.102	0.324	0.332	-0.039	0.02	0.17	0.282	0.125
Model 2		-0.105	0.351	0.343	-0.083	0.18	0.032	0.281	0.151
Model 3			**0.408**	0.286	-0.087	0.055	0.194	0.279	0.173
Model 4	***-0.263***	0.131	**0.367**					0.238	0.174
Model 5	***-0.385***	-0.045						0.157	0.111
Model 6	***-0.269***		***0.294***					0.225	0.184

Table 3. Goodness of fit statistics from structural equation modelling. AGFI = goodness of fit index adjusted for degrees of freedom (df). χ^2, df, and the *P* value from the χ^2 test are shown. Note a non-significant *P* values indicates that the actual covariance structures of the data are not significantly different from the covariance structure implied by the model and the model is an adequate fit. Data for Model 1 are not shown as it was not possible to calculate a χ^2 due to limited df.

	AGFI	χ^2	df	*P*
Model 2	0.99	0.02	1	0.89
Model 3	0.98	0.14	2	0.93
Model 4	0.87	2.16	4	0.71
Model 5	0.74	5.94	5	0.31
Model 6	0.871	2.79	5	0.73

Table 4. Tests of d-separation claims implied by structural equation models. Tests of whether the regression coefficients for *Calluna* (CV), below-ground biomass (roots) and above-ground biomass (A-G-BM) were zero were carried out using t-tests, while F-tests were used for testing the four species variables simultaneously. The probabilities from the independence claims implied by each model were combined and the resulting C value compared to a chi-squared distribution. The response variable is the Fungal:Bacterial Ratio (FB).

Model	Regression model	Variable tested	p-value	C-test p-value
2	FB ~ Species + CV + Roots + A-G-BM	Roots	0.887	0.887
3	FB ~ Species + CV + Roots	Roots	0.873	0.928
	FB ~ Species + CV + A-G-BM	A-G-BM	0.739	
4	FB ~ Species + CV + Roots + A-G-BM	Species	0.740	0.740
5	FB ~ Species + Roots + A-G-BM	Species	0.381	0.107
	FB ~ CV + Roots + A-G-BM	CV	0.059	
6	FB ~ Species + CV + Roots	Species	0.653	0.659
	FB ~ CV + Roots + A-G-BM	A-G-BM	0.456	

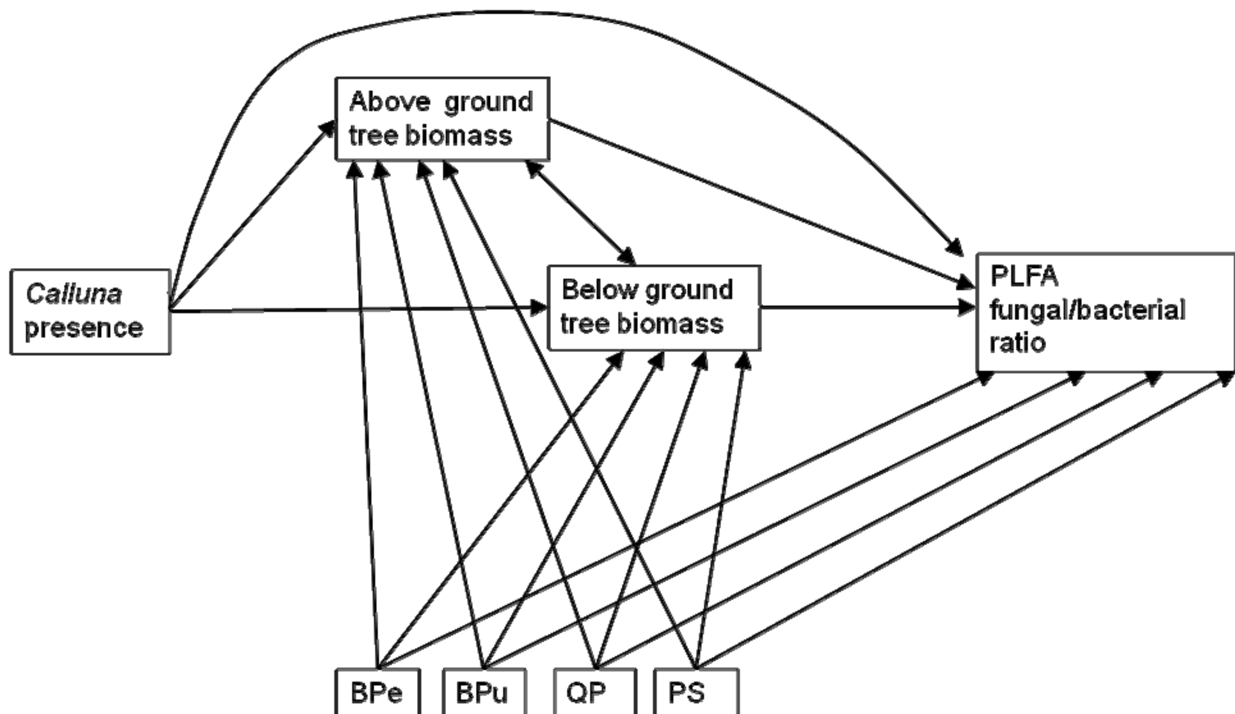

Figure 1 The initial structural equation model (Model 1). Each single headed arrow represents a hypothetical causal relationship such that the variable at the tail of the arrow is a direct cause of the variable at the head. A double headed arrow indicates an unresolved correlation between two variables. BPe = *Betula pendula*; BPu = *Betula pubescens*; PS = *Pinus sylvestris*; QP = *Quercus petraea.*

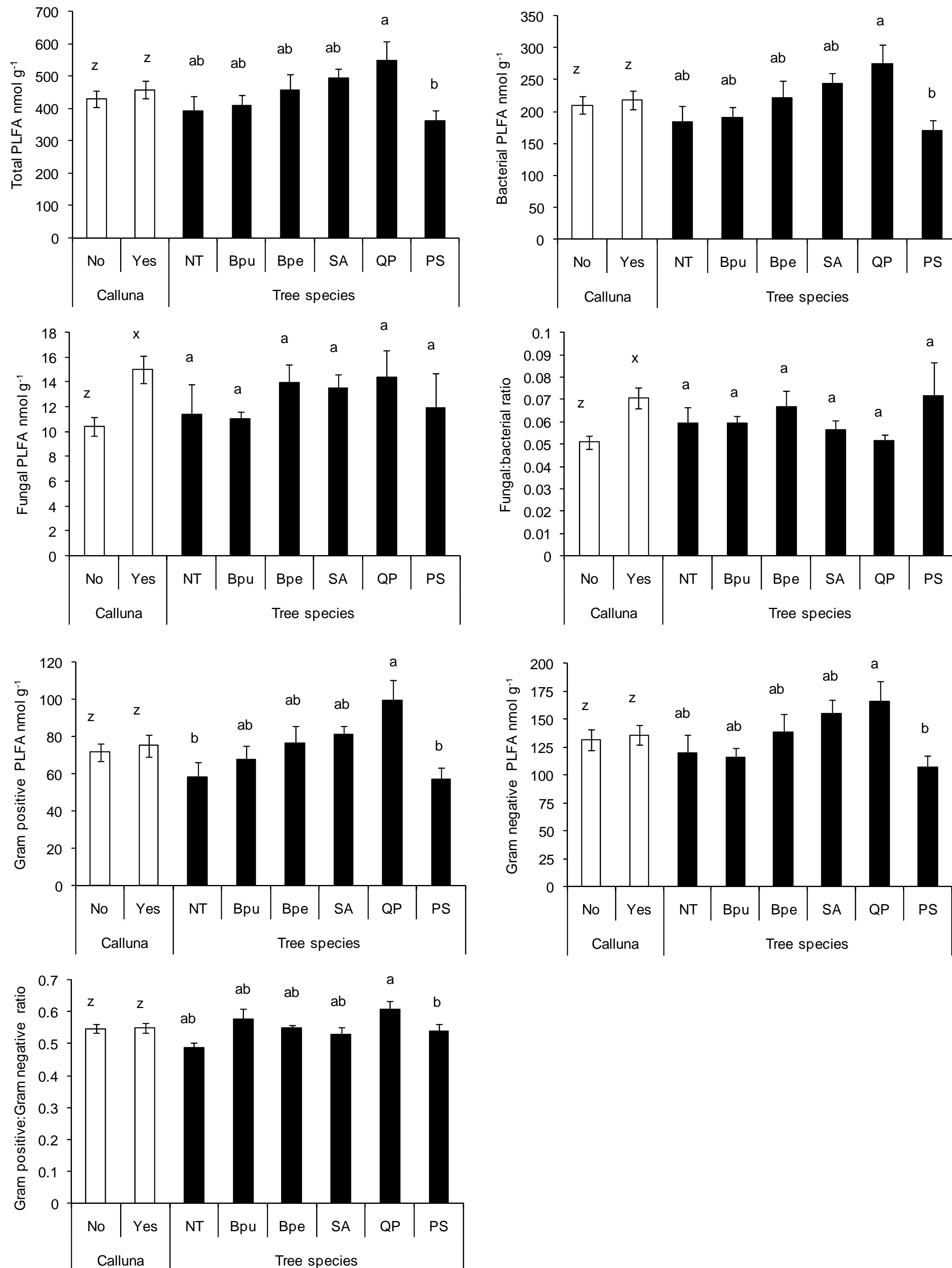

Figure 2 Influence of *Calluna* presence and tree species on phospholipids fatty acids (PLFA) content and diversity. Treatments within each main effect (*Calluna* presence or tree species) sharing letters are not significantly different at P<0.05. Means ± 1SE are shown (n=24 for *Calluna* and n=8 for tree species). NT = No-tree; BPu = *Betula pubescens*; BPe = *Betula pendula*; SA = *Sorbus aucuparia*; QP = *Quercus petraea*; PS = *Pinus sylvestris.*

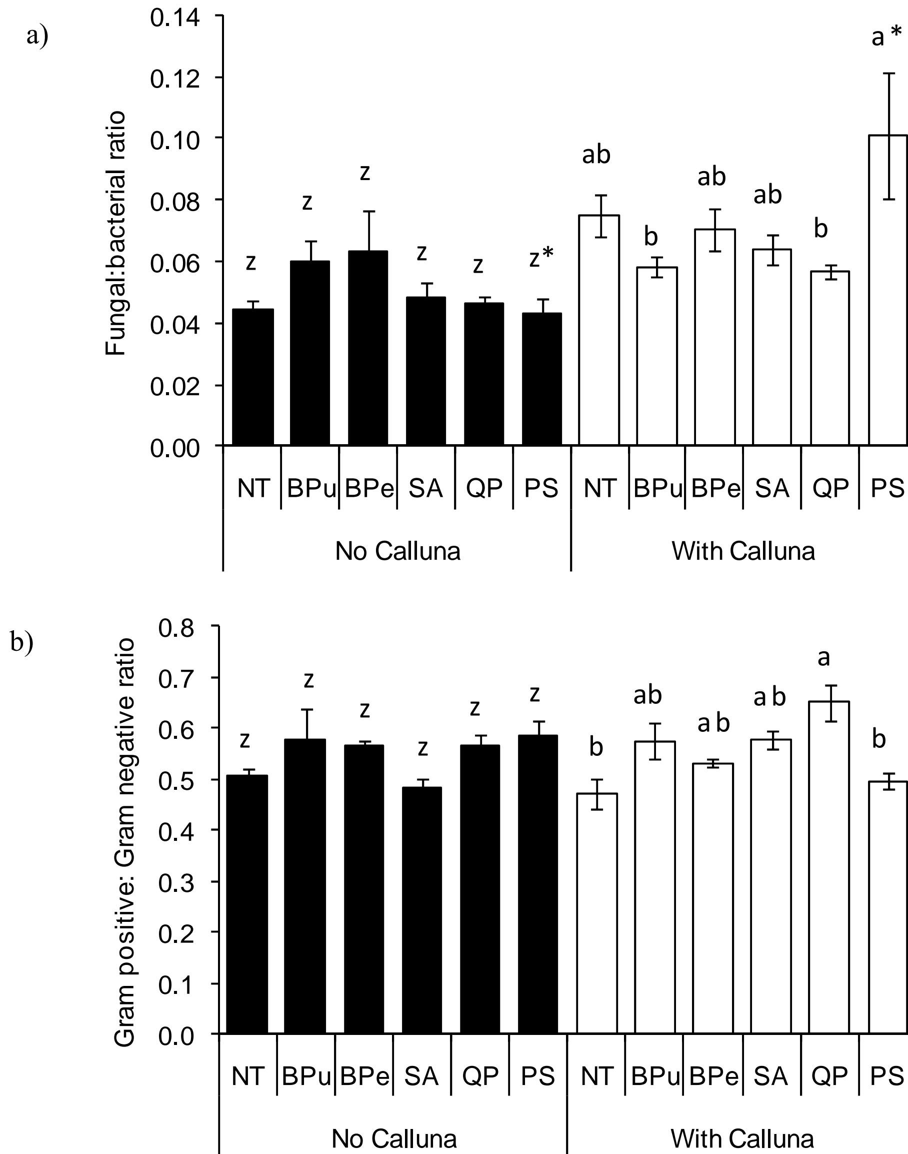

Figure 3 The impact of tree species and *Calluna* on Phospholipids fatty acids (PLFA). a) Fungal:bacterial ratio b) Gram positive:Gram negative ratio. Interaction are shown: within each Calluna treatment significant differences between tree species are indicated with letters, significant differences within a tree species with and without Calluna are indicated by an *. Means ± 1SE are shown (n=4). NT = No-tree; BPu = *Betula pubescens*; BPe = *Betula pendula*; SA = *Sorbus aucuparia*; QP = *Quercus petraea*; PS = *Pinus sylvestris.*

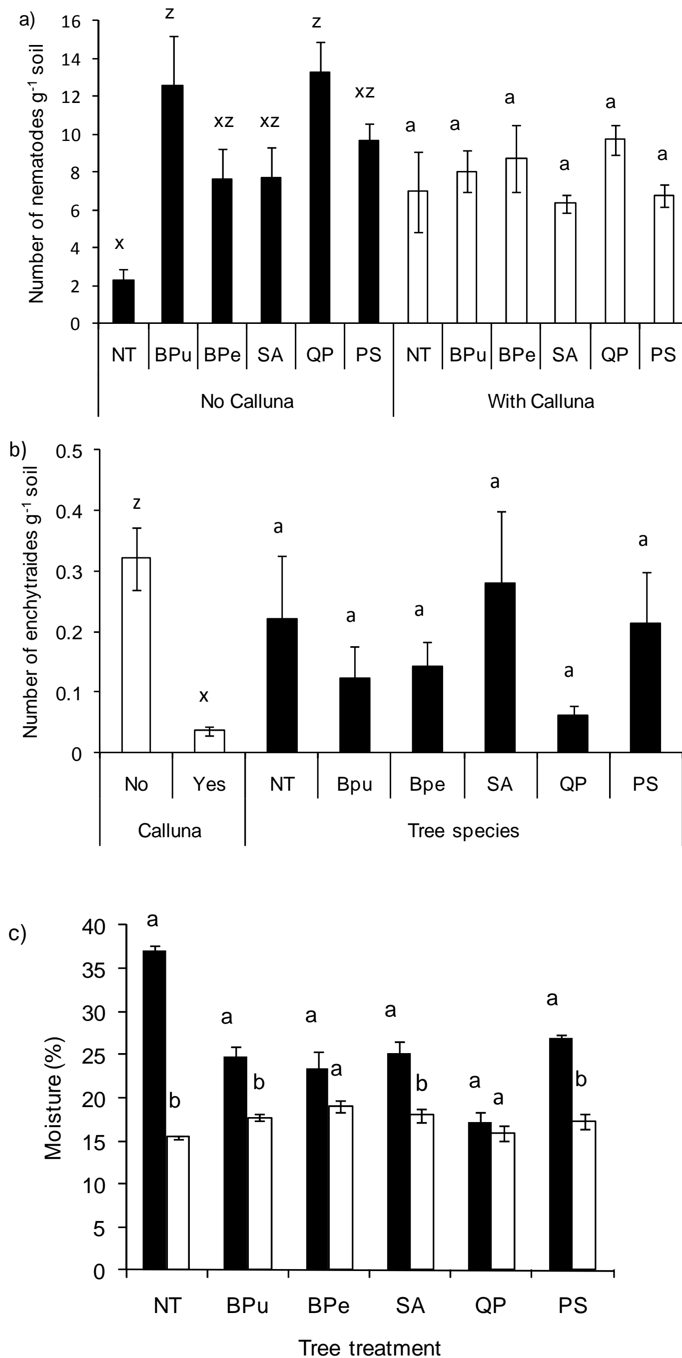

Figure 4 Impact of five different tree species with and without *Calluna* on: (a) nematodes density (b) enchytraeid density and (c) soil moisture. In (a) interactions are shown: within each *Calluna* treatment significant differences between tree species are indicated with letters. In (b) treatments within each main effect (*Calluna* presence or tree species) sharing letters are not significantly different at $P<0.05$. In (c) differences within a tree species with *Calluna* (white bars) and without *Callun*a (black bars) are indicated by different letters. Means ± 1SE are shown (n=4). NT = No-tree; BPu = *Betula pubescens*; BPe = *Betula pendula*; SA = *Sorbus aucuparia*; QP = *Quercus petraea*; PS = *Pinus sylvestris.*

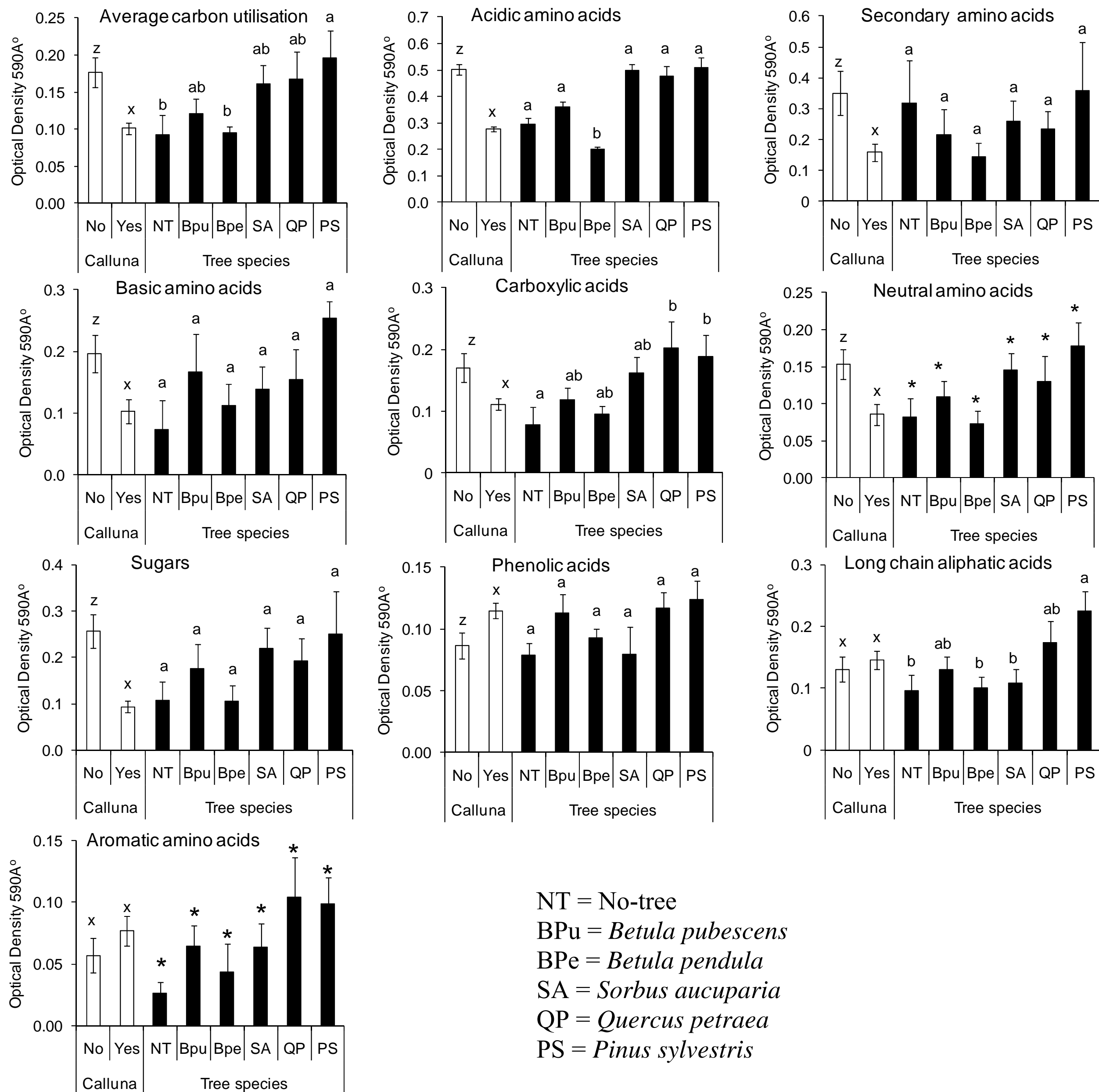

Figure 5 Carbon utilisation by the soil microbial community from soil under different tree species with and with out *Calluna vulgaris.* Treatments within each main effect (*Calluna* presence or tree species) sharing letters are not significantly different at $P<0.05$. Means ± 1SE are shown (n=24 for *Calluna* and n=8 for tree species). * = main effect significant but no pair wise comparisons significant once correction for multiple tests applied.

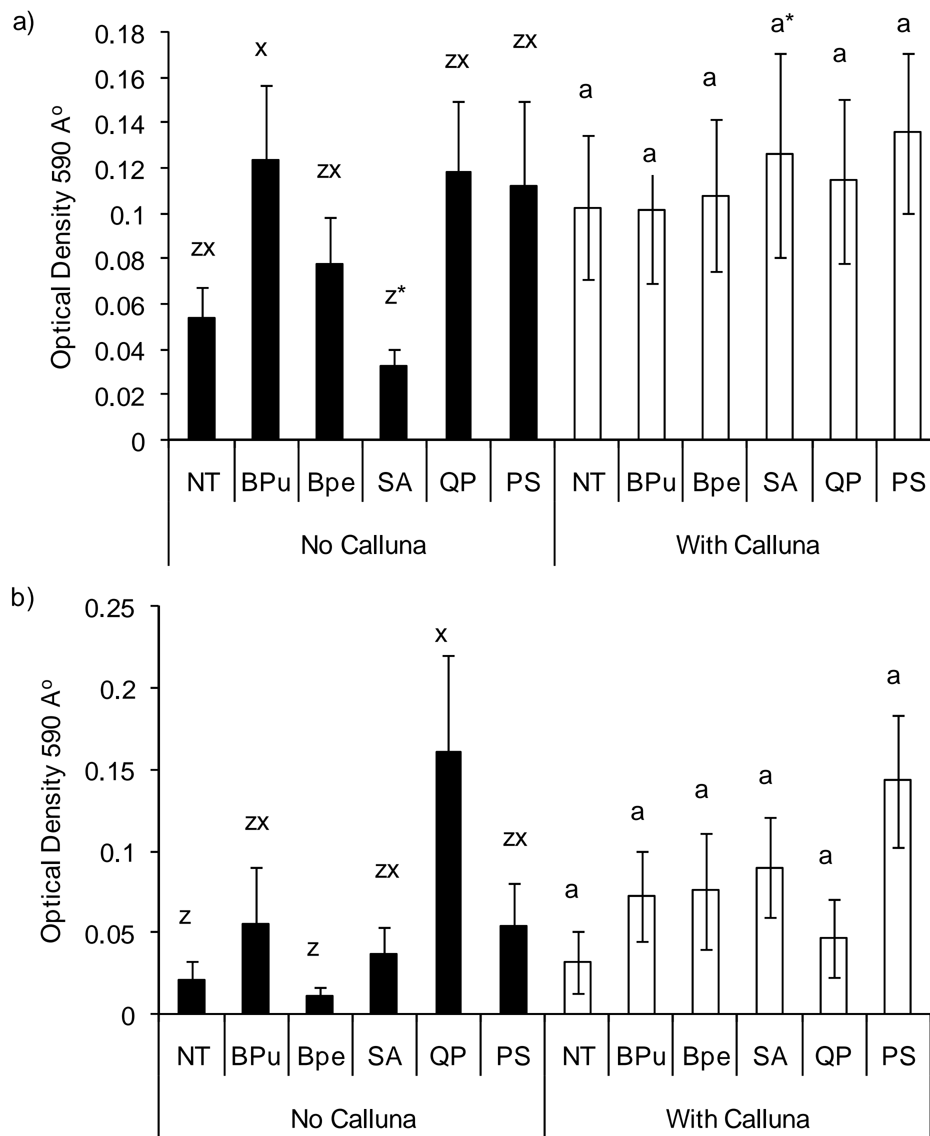

Figure 6 The impact of tree species and *Calluna* on carbon utilisation a) phenolic acids, b) aromatic amino acids. Interaction are shown: within each *Calluna* treatment significant differences between tree species are indicated with letters, significant differences within a tree species with and without *Calluna* are indicated by an *. Means ± 1SE are shown (n=4). NT = No-tree; BPu = *Betula pubescens*; BPe = *Betula pendula*; SA = *Sorbus aucuparia*; QP = *Quercus petraea*; PS = *Pinus sylvestris.*

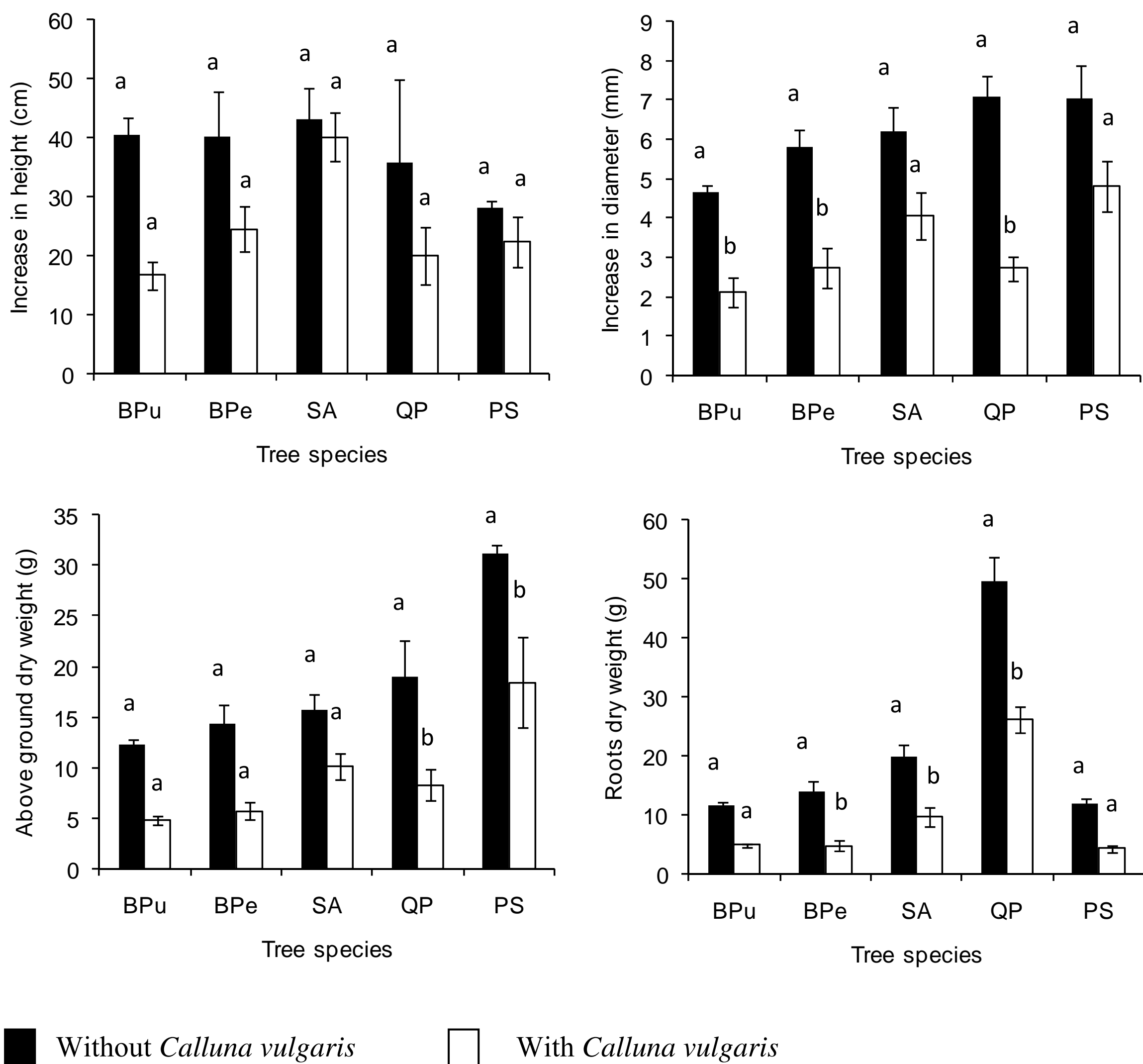

Figure 7. Growth and biomass of five tree species grown with and without *Calluna vulgaris*. Within each tree species *Calluna* treatments with different letters are significantly different from each other at P<0.05. Means ± 1SE are shown (n=4). BPe = *Betula pendula*; BPu = *Betula pubescens*; PS = *Pinus sylvestris*; QP = *Quercus petraea*; SA = *Sorbus aucuparia.*

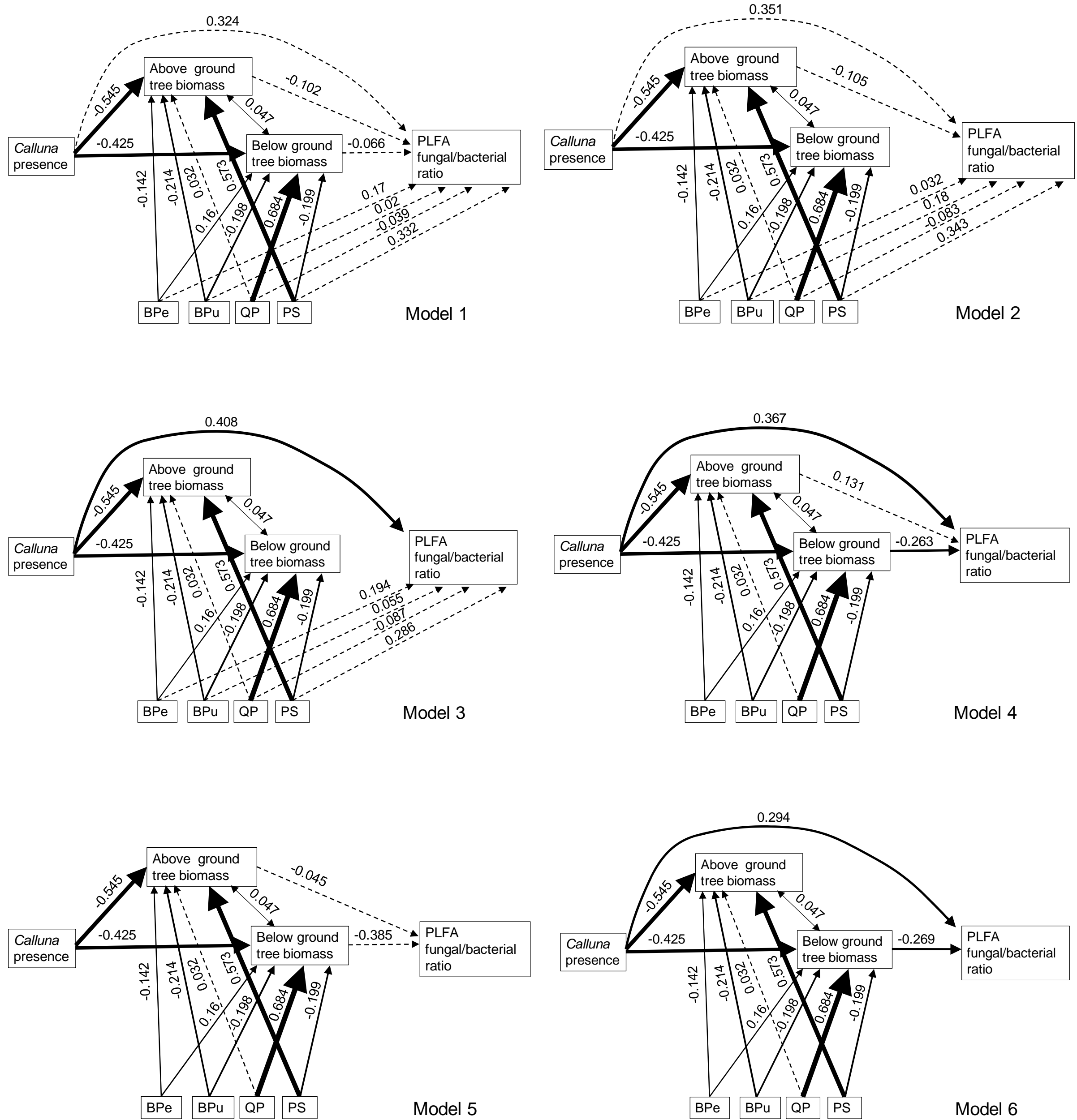

Figure 8 Results from structural equation modelling. Standardized path coefficients are shown on the figure. Paths that were not significant ($p \geq 0.10$) are indicated by dotted arrows. For significant pathways arrow widths are proportional to path coefficients.

Supplementary file Table A. Classification of the PLFAs as fungal, bacterial, or unclassified [*]. The list is limited to PLFAs extractable by the modified Bligh and Dyer method.

Fungal	Non specific eukaryotes[+]	Bacterial	Unclassified[*]
18:2ω6,9	20:4ω6,9,12,15[c]	15:0i[a]	13:0
	20:5ω3	15:0ai[a]	14:0i
	20:4ω2,6,10,14	15:0	14:0
	20:4ω3,6,9,12	16:0i[a]	14:1ω9c
	20:1ω9	16:1ω7c[b]	14:1ω9t
	20:1	16:1ω7t[b]	16:0
	20:0	16:1ω5c[b]	16:0br
		16:0(10Me)[a,d]	16:1i
		17:0(10Me)[a,d]	16:1ω11c
		17:0i[a]	16:1ω11t
		17:0ai[a]	16:0(12 Me)
		17:0cy[b]	17:0br
		17:0	17:1ω8t
		18:1ω7[b]	17:1ω8c
		18:0(10Me)[a,d]	17:1ω7
		19:0cy[b]	17:0(12Me)
			18:0
			18:3ω6,8,13
			18:2ω8,12
			18:1ω9
			18:1ω13
			18:1ω10 or 11
			19:1ω6
			19:1ω8

[+] PLFAs found in fungi and other eukaryotes. [*] Unclassified PLFAs occur in both prokaryotic and eukaryotic organisms and can not be classified as being either specifically fungal or bacterial. [a] Gram-positive bacteria, [b] Gram-negative bacteria, [c] Protozoa, [d] actinobacteria. The PLFAs were classified according to Frostegård *et al*. (1996) and Zogg *et al*. (1997).