Tree diversity effects on soil microbial biomass and respiration are context-dependent across forest diversity experiments

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Data availability statement

The data and R codes supporting the results are available from GitHub after acceptance: https://github.com/SimoneCesarz/Drivers-of-soil-microbial-properties

Conflict of interest disclosure

All authors declare no conflict of interest

Ethics approval statement

Not applicable. No humans or animals were included in this study

Patient consent statement

Not applicable. No patients

Permission to reproduce material from other sources

All databases were cited

Clinical trial registration

Not applicable. No clinical study

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12   **Tree diversity effects on soil microbial biomass and respiration are context-**

13   **dependent across forest diversity experiments**

14

15   **Running title:** Drivers of soil microbial properties

16

17   **Abstract**

18   **Aim**

19   Soil microorganisms are essential for the functioning of terrestrial ecosystems. Although soil

20   microbial communities and functions are linked to tree species composition and diversity,

21   there has been no comprehensive study of how general or context-dependent these

22   relationships are. Here, we examine tree diversity–soil microbial biomass and respiration

23   relationships across environmental gradients using a global network of tree diversity

24   experiments.

25

26   **Location**

27   Boreal, temperate, subtropical, tropical forests

28

29 **Time Period**

30 2013

31

32 **Major Taxa Studied**

33 Soil microorganisms

34

35 **Methods**

36 Soil samples collected from eleven tree diversity experiments were used to measure microbial

37 respiration, biomass, and respiratory quotient using the substrate-induced respiration method.

38 All samples were measured using the same analytical device, method, and procedure to

39 reduce measurement bias. We used linear mixed-effects models and principal component

40 analysis (PCA) to examine the effects of tree diversity (taxonomic and phylogenetic),

41 environmental conditions, and interactions on soil microbial properties.

42

43 **Results**

44 Abiotic drivers, mainly soil water content, but also soil carbon and soil pH, significantly

45 increased soil microbial biomass and respiration. High soil water content reduced the

46 importance of other abiotic drivers. Tree diversity had no effect on the soil microbial

47 properties, but interactions with phylogenetic diversity indicated that diversity effects are

48 context-dependent and stronger in drier soils. Similar results were found for soil carbon and

49 soil pH.

50

51 **Main conclusions**

52 Our results point to the importance of abiotic variables, and especially soil water content, for

53 maintaining high levels of soil microbial functions and modulating the effects of other

54 environmental drivers. Planting tree species with diverse water-use strategies and structurally

55 complex canopies and high leaf area may be crucial for maintaining high soil microbial

56 biomass and respiration. Since higher phylogenetic distance alleviated unfavorable soil water

57 conditions, reforestation efforts accounting for traits improving soil water content or choosing

58 more phylogenetically distant species may assist in increasing soil microbial functions.

59

60 **Keywords:**

61 Aboveground-belowground interactions, Biodiversity-ecosystem functioning, Biodiversity

62 loss, Context-dependency, Global change, Soil biota, Soil microbial functions, Soil

63 microorganisms, Tree diversity, TreeDivNet

64

65

66 # Introduction

67 Soil microorganisms are the functional backbones of terrestrial ecosystems (van der Heijden

68 et al., 2008), as they underpin crucial ecosystem functions and services that humankind relies

69 upon (Wall et al., 2015). Given the critical role of soil microorganisms in carbon dynamics

70 and soil feedback effects on climate, improving current understanding of the drivers of

71 microbial biomass and activity is an essential step towards predicting global change impacts

72 (Serna-Chavez et al., 2013; Xu et al., 2013, 2017; Chen et al., 2019). Soil microbial biomass

73 can serve as a proxy for nutrient cycling and soil enzyme dynamics, such as soil organic

74 matter (SOM) turnover as well as for secondary productivity. In addition, in-situ

75 measurements of microbial activity have been shown to correlate with rates of soil C

76 sequestration (Lange et al., 2015). Together, microbial biomass and activity provide critical

77 information on a range of important soil ecosystem functions.

78 Globally, abiotic factors are thought to be the main driver of soil microbial biomass and

79 microbial activity (Serna-Chavez et al., 2013; Xu et al., 2017; Smith et al., 2021; Wan et al.,

80 2021). Optimal soil water content (i.e., soil water holding capacity of around 60%), neutral

81 soil pH, and high soil organic carbon content (here summarized as high soil quality) are

82 among the most important factors that directly increase soil microbial biomass and activity

83 (Schimel, 2018). In contrast, climatic conditions such as temperature may influence soil

84   microbial biomass indirectly via evapotranspiration and changes in soil organic matter

85   content (Serna-Chavez et al., 2013). These patterns become less clear when taking

86   interactions among different drivers into account. For instance, the positive effects of high

87   soil nutrient content may be constrained by stressful environments (Serna-Chavez et al.,

88   2013) or become even stronger (Guerrero-Ramírez et al., 2017), highlighting the importance

89   of context-dependent effects or microclimatic conditions regulated by the vegetation

90   (Gottschall et al., 2019). Moreover, the effects of abiotic drivers may further be modulated by

91   local biotic conditions. For example, studies in grasslands and forests have demonstrated that

92   plant diversity affects soil microbial community composition, activity, and biomass (Lange et

93   al., 2015; Chen et al., 2019) with significant effects on ecosystem functions, such as soil

94   carbon storage (Lange et al., 2015),. However, global analyses of plant diversity effects on

95   soil microbial communities have had limited scope, focusing either on soil communities but

96   not on soil functions, or on grasslands only (Prober et al., 2015; Thakur et al., 2015). In

97   addition, the magnitude and direction of plant diversity effects on soil microbial communities

98   were inconsistent, probably due to strengthening of these effects with time (Thakur et al.,

99   2015) and different environmental contexts, such as different soil conditions (Guerrero-

100   Ramírez et al., 2017). So far, plant diversity effects on soil microbial functions have been

101   studied mostly in grasslands while little is known about tree diversity effects on soil

102   microbial functions in forests (Chen et al., 2019; Xu et al., 2020). This is a major knowledge

103   gap because there might be substantial differences between ecosystems in terms of soil

104   microbial function and potential climate feedback effects on soil communities (Chen et al.,

105   2018).

106   Previous studies on tree diversity effects on soil microorganisms mainly compared

107   monoculture stands with mixtures of two tree species in different environments, making it

108   difficult to disentangle site conditions from tree diversity and tree identity effects (Liang et

109   al., 2016). One of the first studies using data from a tree diversity experiment with

110   homogeneous abiotic conditions found soil microbial activity and biomass to increase with

111   tree species richness in a saturating relationship, while soil microbial community composition

112   did not vary significantly (Khlifa et al., 2017). One of the potential mechanisms underlying a

113   positive plant diversity effect on microorganisms is the increased input of diverse resources

114   (Eisenhauer et al., 2017). In line with the view that the quality of plant inputs is essential for

115   soil microbial processes, the chemical composition of leaf litter determines nutrient

116   mineralization, microbial respiration, and microbial biomass (Pei et al., 2017), whereas

117   species diversity per se was shown to have little effect (Meier & Bowman, 2008). This

118   finding suggests that an increase in species richness may not increase soil microbial biomass

119   and activity if not accompanied by a simultaneous increase in the functional dissimilarity of

120   co-occurring species (Heemsbergen et al., 2004). While much debated, research in grasslands

121   suggests that functional diversity is of higher importance than species richness for soil

122   microbial biomass and activity (e.g., Ebeling et al., 2014), while there is even less conclusive

123   information for forest ecosystems (Scherer-Lorenzen et al., 2007) using belowground traits

124   (but see Guerrero-Ramírez et al., 2021). Unfortunately, access to and measuring the above-

125   and belowground traits on the same plants is often not possible for logistical reasons. To

126   overcome this lack of data, phylogenetic diversity can be used as a proxy for functional

127   diversity (Tucker et al., 2018), which has been used successfully for aboveground ecosystem

128   functions (Cadotte et al., 2009).

129   Here, we present the first coordinated sampling and analysis of soil microbial properties

130   across eleven tree diversity experiments distributed across four biomes. To explore potential

131   tree diversity effects on three key soil microbial properties - soil microbial basal respiration,

132   biomass, and carbon-use efficiency - we tested effects of tree species richness (the

133   biodiversity measure most frequently manipulated in tree diversity experiments; Verheyen et

134   al., 2016) and tree phylogenetic diversity. We expected that phylogenetically diverse

135   experimental forests will provide more dissimilar resources and niches to soil

136   microorganisms, thereby increasing soil ecosystem functioning. We investigated three

137   hypotheses: (1) Both tree species richness and phylogenetic diversity are predicted to increase

138   soil microbial processes, but phylogenetic diversity is expected to have stronger effects. (2)

139   Abiotic drivers strongly influence soil microbial functions, because high soil carbon (soil C)

140   concentration, high soil water content (SWC), and more neutral soil pH are hypothesized to

141   increase the biomass, activity, and carbon use efficiency of soil microorganisms. (3)

142   Interactions among abiotic and biotic drivers may influence soil microbial properties, given

143   the context-dependency of biodiversity-ecosystem function relationships.

144

145   **Materials & Methods**

146   Soil samples were taken in 2013 from eleven tree diversity experiments that are part of the

147   global network TreeDivNet (Verheyen et al., 2016; http://www.treedivnet.ugent.be/).

148   Experiments are independent of each other with different experimental designs and plot

149   configurations (Table 1). Experiments are distributed across four continents (Asia, Europe,

150   North, and South America) and four different biomes (boreal, temperate, tropical, subtropical,

151   Olson et al., 2001), and differ in age, with the youngest experiments running for three years

152   and the oldest for fourteen years as of 2013 (i.e., the year of the sampling campaign; Fig. 1;

153   Table 1). In total, 106 tree species were included in this study (see Appendix S1 in

154   Supporting Information as Table S1.1). Experiments had a mean ± SD number of diversity

155   levels of 3.7 ± 1.0, with diversity levels ranging from monocultures to 18 tree species in

156   sub/tropical regions. All experiments had an experimental gradient in tree species richness,

157   with the exception of one (BIOTREE-FD, see Table 1) which manipulated functional

158   diversity at a constant level of tree species richness.

Soil sampling

160   Soil samples were taken from a depth of 0 – 10 cm, excluding the litter layer using a soil

161   corer. Temperate and boreal experiments were sampled in the summer season in the Northern

162   hemisphere, while subtropical and tropical experiments were sampled in the wet summer

163   season. All experiments were sampled between June and September 2013. Depending on the

164   size of the experimental plot, different numbers of subsamples were taken per plot to create

165   one composite sample. For plots <100 m², three subsamples were taken , while ten

166   subsamples were taken for plots >100 m2. These subsamples were taken to capture the spatial

167   heterogeneity of the plot and to represent as many different combinations of tree species as

168   possible. Soil samples were always taken in the center of surrounding tree stems.

169   Immediately after sampling, soil samples were stored at 5°C until sieving at 2 mm and then

170   were stored at -20°C until and during shipping to minimize changes in microbial activity,

171   biomass, and composition. Alternatively, if shipping at -20°C was not possible, samples

172   defrosted during shipping and were measured shortly after arrival. Altogether, 1010 plots

173   were sampled across the eleven tree diversity experiments (Table 1).

Measurement of soil microbial properties

175   Before the start of microbial measurements, samples were kept at +20°C for five days to

176   unfreeze and to adapt the soil microbial community to a constant and standardized

177   temperature. Three different soil microbial community properties were assessed using an

178   automated O_2 micro-compensation system (Scheu, 1992). First, basal respiration (μl O_2 h^{-1} g^{-1}

179   dry soil) was measured as the mean oxygen consumption per hour without the addition of any

180   substrate. The mean oxygen consumption was measured for hours 15 to 20. Basal respiration

181   reflects the active part of the soil microbial community at the time of sampling. Second,

182   microbial biomass carbon was measured by substrate-induced respiration, i.e., the respiratory

183   response of microorganisms to glucose and water addition. To saturate catabolic microbial

184   enzymes, 8 mg glucose g^{-1} soil dry weight was added as an aqueous solution to the soil

185   samples. The lowest substrate-induced respiration of three contiguous hours within the first

186   10 h was taken as the maximum initial respiratory response (MIRR) – a period when

187   microbial growth has not started. Microbial biomass (μg C g^{-1} dry soil) was calculated as 38

188   $\times$ MIRR (μl O_2 h^{-1} g^{-1} dry soil) following Beck et al., (1997). By providing water and

189   glucose, the maximum potential of the living microbial biomass is activated that is able to use

190   glucose, whereas for basal respiration only a fraction of the entire community is active. Third,

191   the microbial-specific respiratory quotient (μl O_2 mg^{-1} Cmic h^{-1}) was calculated as the ratio of

192   basal respiration and soil microbial biomass. The specific respiratory quotient is a measure of

193   soil microbial carbon-use efficiency. Carbon-use efficiency is high when microbial biomass

194   can be built up without high investment in basal respiration, which is indicated by a lower

195   specific respiratory quotient. All measurements were conducted at +20°C in an air-

196   conditioned laboratory using the same analytical devices (RMS Schuller, Darmstadt,

197   Germany).

198

199   **Diversity metrics**

200   In addition to tree species richness, we aimed at testing a tree diversity metric that captures

201   the functional diversity of each experimental forest plot. However, no comparable trait

202   measurements were available from all experiments nor from the TRY database. Instead, we

203   used phylogenetic diversity as a proxy for multi-trait functional diversity (Tucker et al.,

204   2018). Phylogenetic diversity indices have been shown to be powerful predictors of

205   biodiversity-ecosystem functioning relationships (e.g., Craven et al., 2018) and are suggested

206   to work when key functional traits are not available (Paquette et al., 2015). We used the

207   molecular phylogeny from previous studies (Pietsch et al., 2014; Zanne et al., 2014) as a

208   backbone to build a phylogeny of all species within the tree diversity experiments,

209   conservatively binding species into the backbone using dating information from congeners in

210   the tree. We used the comparative.comm function in the R package pez to calculate a set of

211   phylogenetic diversity indices, specifically MPD (mean phylogenetic diversity), MNTD

212   (mean nearest taxonomic distance), and the standardized version of both to account for

213   correlation with species richness (Pearse et al., 2015). MNTD was found to correlate less

214   (using Pearson correlation) with log species richness and, therefore, was used in all following

215   analyses (see Appendix S2 in Supporting Information as Table S2.1). Taxonomic names of

216   tree species were standardized using the website

217   http://tnrs.iplantcollaborative.org/index.html.

218

219   **Soil characteristics**

220   We included a set of explanatory variables to describe the experimental sites, which were

221   shown to have an effect on soil microbial properties and reflect the designs and local

222   conditions of the different experiments (Fig. S2.1). Gravimetric soil water content was

223   measured as % H_2O from fresh soil weight by drying the whole sample at 75°C for three

224   days. Soil pH and soil C (%) were measured at the block level to obtain information about

225   soil quality characteristics of each experiment. Therefore, equal proportions of dry soil were

226   weighed from each sample to form a composite sample. The whole sample was ground, and a

227   fraction of 10 g was used for pH measurements by adding 0.01 m $CaCl_2$. Soil C

228   concentrations were analyzed by using the ground soil with an elemental analyzer (Vario EL

229   Cube, Elementar). We further extracted clay (%), sand (%), and silt (%) content from the

230   SoilGRIDS database (Hengl et al., 2014).

231

232   **Environmental conditions**

233   For each experimental site, we extracted mean annual temperature (MAT), the seasonal

234   variability of temperature (Season Temp.), annual precipitation (MAP), and the seasonal

235   variability of precipitation (Season Prec.) from the WorldClim database

236   (http://www.worldclim.org/current) with 2.5 arc-minutes resolution. Potential

237   evapotranspiration (PET) and the aridity index (MAP/PET) were extracted from CGIAR-CSI

238   (https://srtm.csi.cgiar.org/). In addition, we obtained the age of the experiment (years) and

239   tree density (trees m^{-2}) from publications associated with each experiment (Table 1) and the

240 TreeDivNet website (http://www.treedivnet.ugent.be/). Biomes were assigned based on Olson

241 et al., (2001).

242

243 **Data analysis**

244 Prior to analysis, all data were centered and standardized (i.e.; re-scaled to variance = 1)

245 using the scale function from the base package in R, and the distributions of response

246 variables were checked visually. We only included abiotic variables to our linear-mixed-

247 effects models where block or plot-level data were available (i.e., soil water content, soil pH,

248 and soil C), allowing us to test all possible two-way interactions. In addition, we included

249 logarithmized tree species richness (log SR) and MNTD as fixed effects in our models. We

250 tested if our models were overfitted by calculating the variation inflation factor (VIF) for

251 each model, which were simplified removing interactions with VIF>3 (Montgomery et al.,

252 2012). Since only one interaction of soil pH and soil C for basal respiration and the

253 respiratory quotient showed a VIF between 3.0 and 3.6, we used model comparison with

254 anova() to check AICs. Both models, the reduced and the full model, did not differ by more

255 than two units. Therefore, the full models were kept and are presented here.

256 The random effect structure accounted for the hierarchical data structure, with block nested

257 within site and site nested within experiment. The Kenward-Rogers approximation was used

258 to test for the significance of fixed effects and degrees of freedom. Marginal and conditional

259 R^2 were calculated using the function r.squaredGLMM from the MuMIn package. Marginal

260 R^2 represents the variance explained by the fixed effects, whereas conditional R^2 represents

261 the variance explained by both fixed and random effects. Collinearity among explanatory

262 variables in mixed-effects models was below r =|0.7| as suggested by Dormann et al., (2013)

263 (Figure S2.1). We checked model assumptions of the most parsimonious models by fitting

264 model residuals versus the results of fitted models. Basal respiration and the respiratory

265 quotient were log-transformed to achieve the requirements of parametric statistical tests.

266 Model fits of the mixed-effects models were used to plot estimates using the function

267 plot_model from the package sjPlot. Significant interactions were plotted using ggpredict

268 from the package ggeffects.

269 Principal component analysis (PCA) was used to visualize how every single experiment is

270 characterized by the explanatory variables and their relation to the dependent variables. Basal

271 respiration, microbial biomass, and the respiratory quotient were treated as active variables as

272 well as the explanatory variables available. PCA was computed using prcomp from the stats

273 package. Visualization was done using the function fviz_pca_biplot from the factoextra

274 package. In addition to linear mixed-effects models and the PCA we included a piecewise

275 structural equation model (pSEM , Lefcheck, (2016)) to investigate causal relationships

276 among variables as there is uncertainty about underlying mechanisms (see Appendix S3 as

277 Fig. S3.2, Table S3.2). pSEM further allows to account for the nested structure of the

278 underlying data. We accounted for correlated errors of all microbial properties, as well as for

279 tree species richness and MNTD. Because of the mismatch in data resolution, we cannot test

280 some potential effect pathways. For instance, we are unable to test if increasing sand content

281 reduces soil C content, which – in turn – is negatively related to soil water content, - in turn –

282 is positively related to soil microbial properties but with an overall negative effect on soil

283 microbial biomass. Independent claims were all non-significant. All variables were scaled as

284 mentioned above. All statistical analyses were performed in R (version 4.0.3) (R Core Team,

285 2016).

286

Results

288 Mean soil basal respiration ($\pm$ SD) was 2.06 ± 1.94 μl O_2 h^{-1} g soil dw^{-1}, with the lowest

289 values in the FORBIO experiment in Belgium (min: 0.08 μl O_2 h^{-1} g soil dw^{-1}) and the

290 highest values in the SATAKUNTA experiment in Finland (max: 15.26 μl O_2 h^{-1} g soil dw^{-1};

291 see Appendix S4 as Fig S4.3). Similarly, we found the lowest soil microbial biomass values

292 in the FORBIO experiment (min: 11.85 μg Cmic g soil dw^{-1}) and the highest values in the

293 SATAKUNTA experiment (max: 2501.54 μg Cmic g soil dw^{-1}). Mean soil microbial biomass

294 was 435.51 ± 325.03 μg Cmic g soil dw^{-1}. The respiratory quotient was lowest (i.e., highest

295 carbon-use efficiency) in the BIOTREE-FD in Germany (min: 0.008 μl O_2 μg^{-1} Cmic h^{-1}) and

296 the highest respiratory quotient was measured in the ORPHEE experiment in France (max:

297 0.0395 μl O_2 μg^{-1} Cmic h^{-1}). The grand mean across experiments for the respiratory quotient

298 was 0.0052 ± 0.0031 μl O_2 μg^{-1} Cmic h^{-1}. Mean soil water content was $17.2 \pm 11.5\%$, and the

299 driest soil was found in the IDENT Cloquet experiment in Minnesota, USA (min: <0.1%),

300 whereas the highest values were measured in the experiment SATAKUNTA (max: 58.5%).

301 Principal component analysis highlighted the strong relationship of basal respiration and

302 microbial biomass to soil water content, experimental age, and soil C that correlated strongest

303   with the first PCA axis and explained 59.3% of the variance (Fig. 2, Table S2.3). These three

304   variables reached maximum values in the boreal experiment Satakunta. The high microbial

305   biomass values found in the Biotree-FD experiment could be linked with higher clay content

306   and lower potential evapotranspiration. The second axis explained 36.2% of the variance and

307   was mainly affected by the seasonality of temperature, soil pH, and tree density, variables

308   that strongly increased the carbon use efficiency.

309

310   Hypothesis 1: Tree diversity increases soil microbial properties

311   Overall, tree species diversity and phylogenetic tree diversity did not significantly influence

312   basal respiration, microbial biomass, or carbon use efficiency (Fig. 3; Table 2). Similarly, a

313   detailed examination of each experimental forest revealed only one positive significant effect

314   that was found in the ORPHEE experiment, where carbon-use efficiency increased (i.e., the

315   respiratory quotient decreased) with increasing MNTD (Fig. S4.3f, Table S4.4), whereas the

316   other two significant effects showed negative relationships (Sardinilla: Fig.S4.3a and BEF-

317   China: Fig.S4.3e, Table S4.4). Consequently, the R^2 of the models analyzing individual

318   experimental forests were consistently low (Table S4.4), indicating that soil microbial

319   properties are not well explained by tree diversity. Across all experimental forests, marginal

320   R^2 of the linear mixed-effects models were 17% for basal respiration and for microbial

321   biomass, and 38% for the respiratory quotient (Table 3). Conditional R^2 was roughly twice as

322   high as marginal R^2.

323   Hypothesis 2: High soil water content, soil C content, and soil pH increase soil microbial

324   properties

325   Soil water content increased all microbial properties significantly when all the experiments

326   were considered together (Fig. 2, Table 2); this positive effect was seen in all but one

327   (ORPHEE) experimental site (Fig. S4.4a). In contrast, soil C alone did not affect any of the

328   microbial properties investigated in the linear mixed models nor in the piecewise SEM (Fig.

329   2, Table 2, Fig. S3.2, Table S3.2). Interestingly, the relationship between soil C, soil

330   respiration, microbial biomass, and carbon use efficiency was not positive as expected, but

331   negative for many of the experiments as mentioned above (Fig. S4.5). However, the very

332   high C values in the boreal Satakunta experiment led to a generally positive effect that was

333   removed in the linear mixed-effects models. Higher soil pH significantly increased microbial

334   biomass and carbon use efficiency (i.e., a negative effect on the respiratory quotient) but not

335   basal respiration (Figs. 3h,l).

336   Hypothesis 3: Context dependency

337   Although all three microbial properties were not affected by tree diversity itself, there was a

338   significant effect of the interaction of MNTD and soil water content on all three properties,

339   and basal respiration was also affected by the interaction effect of MNTD and soil C. The

340   interactive effects of tree species richness with abiotic factors were, however, not statistically

341   significant (Fig. 3, Table 2). Generally, all soil microbial properties increased with increasing

342   soil water content. At low soil water content, we detected significant positive effects of

343   MNTD on soil microbial properties. In contrast, the effects of MNTD were not statistically

344   significant at high levels of soil water content (Fig. 3b,f,j). High MNTD increased basal

345   respiration and microbial biomass at low levels of soil water content but decreased carbon-

346   use efficiency.

347   The significant interaction effect of MNTD and soil C for basal respiration showed a different

348   pattern. Generally, the fitted model predicted basal respiration to be highest when soil C was

349   low (Fig. 3c). Increasing MNTD increased basal respiration only at high soil C levels,

350   whereas increasing MNTD decreased basal respiration at low soil C levels.

351   In addition to interactions with MNTD, we found additional interactions between the abiotic

352   variables. A significant interaction for all three microbial properties was found for soil C and

353   soil water content. At high soil water content, differences in soil C had less impact on all soil

354   microbial properties (Figs. 3d,g,k). The increase in basal respiration and microbial biomass

355   along the soil water gradient was steepest when soil C was high, and the reverse was true for

356   the respiratory quotient.

357   Microbial biomass and carbon-use efficiency were significantly affected by the interaction of

358   soil pH and soil water content (Figs. 3h,l). Effects of soil pH on soil microbial properties

359   were negligible when soil water content was high.

360

361   **Discussion**

362 Leveraging a global network of experimental forests, we found that variation in soil microbial

363 properties was mediated by abiotic factors to a greater extent than by biotic factors. Effects of

364 tree diversity on soil microbial respiration were largely context-dependent, only emerging

365 when soil water content was low.

366 Our first hypothesis posited that – independent of environmental context - taxonomic and

367 phylogenetic diversity would increase soil microbial properties. Yet, we found that tree

368 diversity did not have statistically significant effects on any soil microbial property. Recent

369 studies in experimental plots found weak tree diversity effects on soil microorganisms,

370 suggesting that tree species identity may be a more important driver of soil microorganisms

371 and soil functions (e.g., Gottschall et al., 2019; Khlifa et al., 2017). Individual tree species

372 can affect the structure of the litter layer by influencing microclimatic conditions that drive

373 soil microbial functions (Gottschall et al., 2019). Therefore, improved data on litter and root

374 traits, as well as their influence on soil quality and microclimate, are need to improve our

375 mechanistic understanding of tree identity effects on soil functions (Laliberté, 2017; Beugnon

376 et al., 2021). Using belowground traits, rather than aboveground traits, is essential to predict

377 soil functions, as different mechanisms likely operate belowground. Unfortunately, no

378 representative above- or belowground trait data were available for the eleven tree diversity

379 experiments to investigate tree identity effects in greater detail. We were not able to use data

380 from trait databases, since relevant traits were not available for many subtropical and tropical

381 tree species. Instead, we used phylogenetic diversity (MNTD) as a proxy for differences

382 among tree species (Craven et al., 2018). A notable drawback of using MNTD (or any other

383 measure of phylogenetic diversity) is that it lacks a clear mechanistic link to soil microbial

384 properties, nor can it be used to explore mechanistic identity effects. A targeted trait approach

385 paired with phylogenetic information may help to better understand underlying mechanisms.

386 The growing network of global tree diversity experiments (Verheyen et al., 2016), and trait

387 syntheses (Guerrero-Ramírez et al., 2021) will allow for coordinated approaches and should

388 aim to directly measure belowground traits to identify abiotic and biotic drivers of soil

389 microbial functions.

390 We did not find any interactive effects of tree species richness and abiotic factors on soil

391 microbial properties, confirming that tree species richness per se does not necessarily

392 influence belowground ecosystem functions (Guerrero-Ramírez et al., 2016) and/or that tree

393 species richness did not interact with abiotic factors. Using phylogenetic diversity instead of

394   tree species richness provided the advantage of having a more even data distribution. This is
395   because the temperate and boreal experiments did not contribute to medium and high levels
396   of tree species richness, while the limited number of tropical and subtropical experiments had
397   higher levels of tree species diversity. The species pool of the sub- and tropical experimental
398   forests had a more constrained phylogeny than that of the temperate experimental forests,
399   possibly reflecting the absence of coniferous species and including multiple species of the
400   same genera (Table S1.1). This may explain the weaker (or more variable) effects of MNTD
401   in tropical experimental forests than in temperate or boreal experimental forests.

402   Our study confirmed that soil microorganisms are mainly influenced by abiotic drivers
403   (hypothesis 2), which were also important in modulating tree diversity effects (hypothesis 3).
404   Soil water content was the dominant abiotic driver, affecting all soil microbial functions and
405   interacting significantly with all mentioned abiotic drivers. The strong impact of soil water
406   content on soil microbial properties has been shown in many studies (see the review by
407   Schimel, 2018) and could be more important than nutrient availability (Singh et al., 2009).
408   We found that at high soil water content, changes in soil pH and soil C had minimal effects
409   on the overall high values of soil microbial properties. For instance, positive effects of high
410   temperature on soil biological activity can only be achieved when soil water is not limiting
411   (Thakur et al., 2018), and nutrient availability can be increased by higher soil moisture via
412   increasing diffusion of soluble organic substrates (Schimel, 2018). This suggests that optimal
413   soil water availability (i.e., between 50 – 70% of the field capacity, Manzoni et al., (2012))
414   can mitigate the unfavorable effects of other abiotic factors on soil ecosystem functioning.
415   For a better mechanistic understanding, microclimatic parameters (e.g., soil humidity and
416   temperature) should also be included, which can provide new insights (Gottschall et al.,
417   2019). Therefore, to maintain soil ecosystem functioning, especially when faced with more
418   frequent dry periods due to global change, tree species or communities may be selected that
419   directly use water more efficiently, have a higher diversity in hydraulic traits (Anderegg et
420   al., 2018), and/or have traits that indirectly maintain higher soil water levels, e.g., via higher
421   leaf area, denser canopies, or leaf litter traits that build a thick litter layer (Gottschall et al.,
422   2019). In addition, further management practices, e.g., leaving leaf litter on the ground,
423   applying mulch, planting a cover crop, may be needed to enhance soil water content, and
424   thus, to increase soil functioning.

425   The present study shows that tree diversity effects, as captured by phylogenetic diversity in

426   the present study, on soil microbial properties were statistically significant at low soil

427   moisture levels, confirming earlier findings of an observational study across European forests

428   (Ratcliffe et al., 2017). In contrast, a recent study investigating the interaction with water

429   availability and tree diversity on similar microbial properties did not find positive diversity

430   effects at low soil water availability (Strukelj et al., 2021). This study, however, only

431   investigated context-dependency at two experimental sites. Using eleven tree diversity

432   studies, our findings suggest that biodiversity may function as a buffer against harsh

433   environmental conditions and maintain ecosystem functioning under drought. As plant

434   diversity increases soil microbial diversity, diverse soil microbial communities can increase

435   the resilience of plants after drought (Prudent et al., 2020). For instance, biodiversity may

436   enhance drought resistance due to strengthened biotic interactions, for instance, via

437   mycorrhiza (van der Heijden et al., 2008), especially when mycorrhization rates increase with

438   increased tree diversity (Ferlian et al., 2021). Including microclimatic information would

439   further help to understand underlying mechanisms as shown by Gottschall et al. (2019) who

440   found higher night temperature associated with a specific tree species to increase wood

441   decomposition.

442   One mechanism by which tree diversity increases soil microbial properties is via enhanced

443   inputs of soil carbon since microorganisms are generally carbon limited (Soong et al., 2020).

444   A recent global study investigating the effect of plant diversity (including 92 forest sites) on

445   carbon stocks and microbial biomass C found significant higher soil organic carbon in

446   mixtures compared to monocultures but did not detect a generally positive effect of

447   increasing species diversity on soil carbon stocks and microbial biomass C (Chen et al.,

448   2020). In the aforementioned study, carbon stocks increased with time, suggesting that more

449   time is needed for tree diversity effects to increase carbon stocks and likely cascading effects

450   on microbial functions. Therefore, the lack of a consistent diversity effect on soil carbon in

451   the present study could be due to the young age of most experimental forests (average 7.2 ±

452   3.9 years). This also suggests that belowground responses to tree diversity may take much

453   longer than aboveground responses (Guerrero-Ramírez et al., 2017). Our dataset did not

454   allow testing for three-way interactions, but the PCA together with the interactions of soil

455   water suggest that high amounts of soil carbon and soil water increase soil microbial

456   respiration and biomass the most. These two variables are strongly affected by soil texture at

457   one experimental site (Guenet et al., 2011) but can also be affected by tree diversity, likely

458 over the long term. We were not able to include soil texture data in our linear-mixed effects
459 models, although other studies found strong effects (Xu et al., 2017). A statistical analysis
460 (not presented) showed no effect of soil texture, likely reflecting the limited number of
461 experimental sites and the data source (SoilGRID database, Hengl et al., (2014)).
462 Consequently, the benefits of afforestation efforts in terms of soil ecosystem functioning will
463 not be immediate and will likely take more than a decade to manifest. However, planting
464 trees with specific traits combined with targeted management practices may promote this
465 effect.

466

467 **Conclusion**

468 Global analyses of biodiversity-ecosystem functioning relationships aim to identify general
469 patterns, context-dependencies, and underlying mechanisms to predict and mitigate the
470 consequences of biodiversity loss for human well-being. Our results indicate that tree
471 diversity effects on soil microbial biomass and respiration in young plantations are generally
472 weak but are strongest under dry soil conditions. Notably, the results of tree diversity
473 experiments may have important practical implications, as many degraded ecosystems are in
474 the process of being reforested, and recommendations regarding how to enhance the
475 multifunctionality of these restored ecosystems are urgently needed to mitigate climate
476 change. Because the potential impacts of high-diversity reforestation efforts will likely
477 manifest over the long term, especially with regards to soil ecosystem functioning, we
478 recommend management practices that maintain soil water content. Doing so will require
479 addressing key gaps in biodiversity data, particulary belowground functional traits..
480 Exploring interactions between abiotic and biotic factors in driving soil microbial properties
481 and carbon storage in future studies is pivotal in order to get a more mechanistic
482 understanding of the driving forces of and management options for soil carbon storage.

483

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706

Data availability statement

708 The data that support the findings of this study are openly available in DRYAD at

709 https://datadryad.org/stash/share/1jo89ZCsSXdGLlvO79rDRxPvB5DPe1n1zP6JBI83tP8

710

Biosketch

712 The research team is part of TreeDivNet a large network of tree diversity experiments

713 investigating how different facets of tree diversity affects ecosystem functioning in major

714 forest types around the world. http://www.treedivnet.ugent.be/

715 **Tables**

716

Table 1. List of tree diversity experiments that contributed to the study (alphabetical order) to investigate abiotic and biotic drivers of soil microbial functions. All experiments differ in their plot architecture, as indicated by different numbers of diversity levels and the gradient of diversity. Further, experiments differ in experimental age (in years), number of sites and blocks, as well as in plot size, tree distance, tree density, and species pool. For the BEF-China and Sabah experiment, only a fraction of the whole diversity gradient was sampled (the respective missing richness levels are indicated by square brackets). The BIOTREE-FD experiment has only one species richness level (with four species per plot), but mixtures differ in their functional diversity (FD; indicated by a * in the Table). The number of plots only considers plots that entered the analysis, i.e., controls without trees and plots with missing measurements were excluded. The total number of existing plots is given in square brackets. A list of the full references is found in Appendix 1.

Experi-ment	Country	Biome	Age (y)	Altitude (m)	Former land use	n sites	n blocks	n diversity levels	Species richness levels	Plot size (m²)	n plots	Minimal tree distance (m)	Tree density (trees m⁻²)	Reference
Bangor	UK	temperate	9	1	forest	1	2	3	1, 2, 3	from 45 to 196	80 [92]	1	1.0	http://www.treedivnet.ugent.be/ExpBangor.html
BEF-China	China	subtropical	4	190	forest	2	NA	5	1, 2, 4, 8, 16, [24]	666.6	60 [566]	1.29	0.6	Bruelheide et al., 2014
BIOTREE-FD	Germany	temperate	10	400-415	pasture	1	4	4*	4 (+FD)	1700	24 [25]	1	0.7	Scherer-Lorenzen et al., 2007b
FORBIO	Belgium	temperate	3	398, 56, 13	forest, arable land	3	6	4	1, 2, 3, 4	1764	126 [127]	1.5	0.4	Verheyen et al., 2013
IDENT Auclair	Canada	temperate	3	333	pasture	1	4	3	1, 2, 6	14.4	187 [192]	0.4	8.2	Tobner et al., 2013
IDENT Cloquet	USA	temperate	3	383	forest	1	4	3	1, 2, 6	14.4	190 [192]	0.4	8.2	Tobner et al., 2013
Kreinitz	Germany	temperate	8	115	agricultural	1	2	5	1, 2, 3, 5, 6	25	96 [98]	0.8	1.2	Hantsch et al., 2014

Drivers of soil microbial functions

ORPHEE	France	temperate	5	60	forest	1	2 [8]	5	1, 2, 3, 4, 5	400	61 [256]	2	0.3	Castagneyrol et al., 2013
Sabah	Borneo	tropical	11	102	forest	1	NA [2]	2	1, [4,] 16	40000	27 [124]	3	0.003	Hector et al., 2011
Sardinilla	Panama	tropical	10	70	forest	2	6	3	1, 2, 5, 9, 18	2025	46 [46]	3	0.1	Scherer-Lorenzen et al., 2007a
Satakunta	Finland	boreal	14	35	forest	3	NA	4	1, 2, 3, 5	400	113 [163]	1.5	0.4	Vehviläinen & Koricheva, 2006
Mean± SD			**7.2 ± 3.9**	**183.6 ± 163.8**				**3.7 ± 1.0**		**3937.5 ± 11381.2**	**total : 1010**	**1.6 ± 0.9**	**1.1 ± 1.2**	

717

Drivers of soil microbial functions

Table 2: ANOVA table of linear mixed effects models testing the effect of biotic and abiotic factors on three microbial properties in 11 tree diversity experiments. All variables were scaled. NumDF: numerator degrees of freedom, DenDF: denumerator degrees of freedom. SR = tree species richness, MNTD = mean nearest taxonomic distance, SWC = soil water content. Significant effects (P< 0.05) are highlighted in bold.

Fixed factor	NumDF	Basal respiration			Microbial biomass			Metabolic quotient		
		DenDF	F value	Pr(>F)	DenDF	F value	Pr(>F)	DenDF	F value	Pr(>F)
scaled log SR	1	950.92	0.49	0.485	946.76	0.28	0.594	855.34	0.01	0.937
scaled MNTD	1	948.32	0.96	0.328	944.48	0.41	0.521	964.10	2.02	0.156
scaled log SWC	1	897.22	120.46	**<.001**	969.62	108.42	**<.001**	328.67	6.26	**0.013**
scaled soil C	1	20.55	0.90	0.354	24.12	2.54	0.124	6.49	1.76	0.230
scaled soil pH	1	18.05	0.56	0.464	43.15	5.76	**0.021**	14.79	38.00	**<.001**
scaled log SR : scaled log SWC	1	954.09	3.37	0.067	948.70	0.96	0.327	714.17	1.24	0.265
scaled log SR : scaled soil C	1	945.70	0.36	0.550	945.07	0.40	0.526	956.39	1.66	0.198
scaled log SR : scaled soil pH	1	968.33	0.01	0.936	965.28	0.61	0.435	653.79	0.63	0.426
scaled MNTD : scaled log SWC	1	957.07	31.44	**<.001**	951.79	6.16	**0.013**	963.34	12.93	**<.001**
scaled MNTD : scaled soil C	1	949.95	7.04	**0.008**	945.72	1.49	0.223	961.91	2.17	0.141
scaled MNTD : scaled soil pH	1	953.69	0.19	0.663	951.73	0.02	0.891	960.43	0.11	0.740
scaled log SWC : scaled soil C	1	780.09	21.30	**<.001**	819.94	37.87	**<.001**	129.03	9.27	**0.003**
scaled log SWC : scaled soil pH	1	916.36	0.50	0.481	889.17	20.53	**<.001**	90.30	6.85	**0.010**
scaled soil C : scaled soil pH	1	14.98	2.34	0.147	16.70	0.12	0.729	2.97	1.57	0.300

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719

720 **Table 3:** AICc and R^2 for mixed-effects models for three microbial properties as affected by tree diversity and abiotic factors in 11 tree diversity
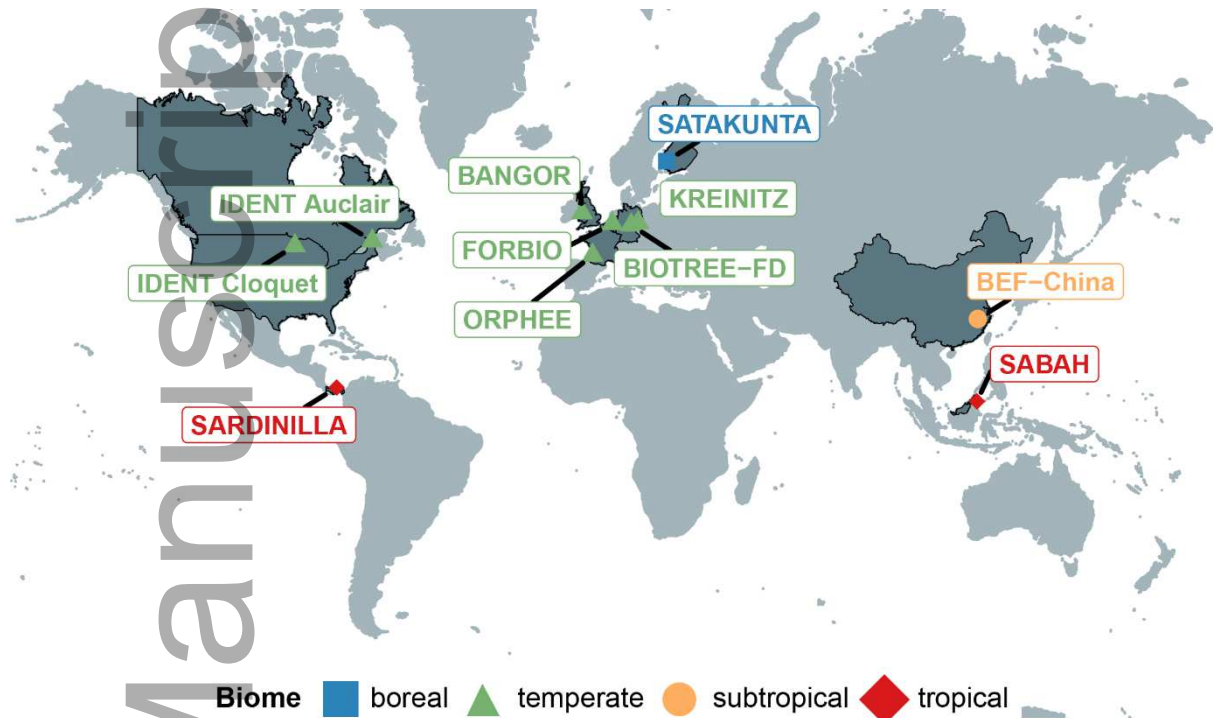
721 experiments. df: degrees of freedom.

Response variable	df	AICc	R^2 fixed (marginal)	R^2 random (conditional)
Basal respiration	19	1522.34	0.33	0.78
Microbial biomass	19	979.26	0.17	0.90
Respiratory quotient	19	2065.99	0.38	0.69

722

723 **Figures**

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726

727 **Figure 1:** Locations of the eleven tree diversity experiments and assignments to biomes
728 (Olsen et al., 2001). Details on the locations and experimental designs are shown in Table 1.
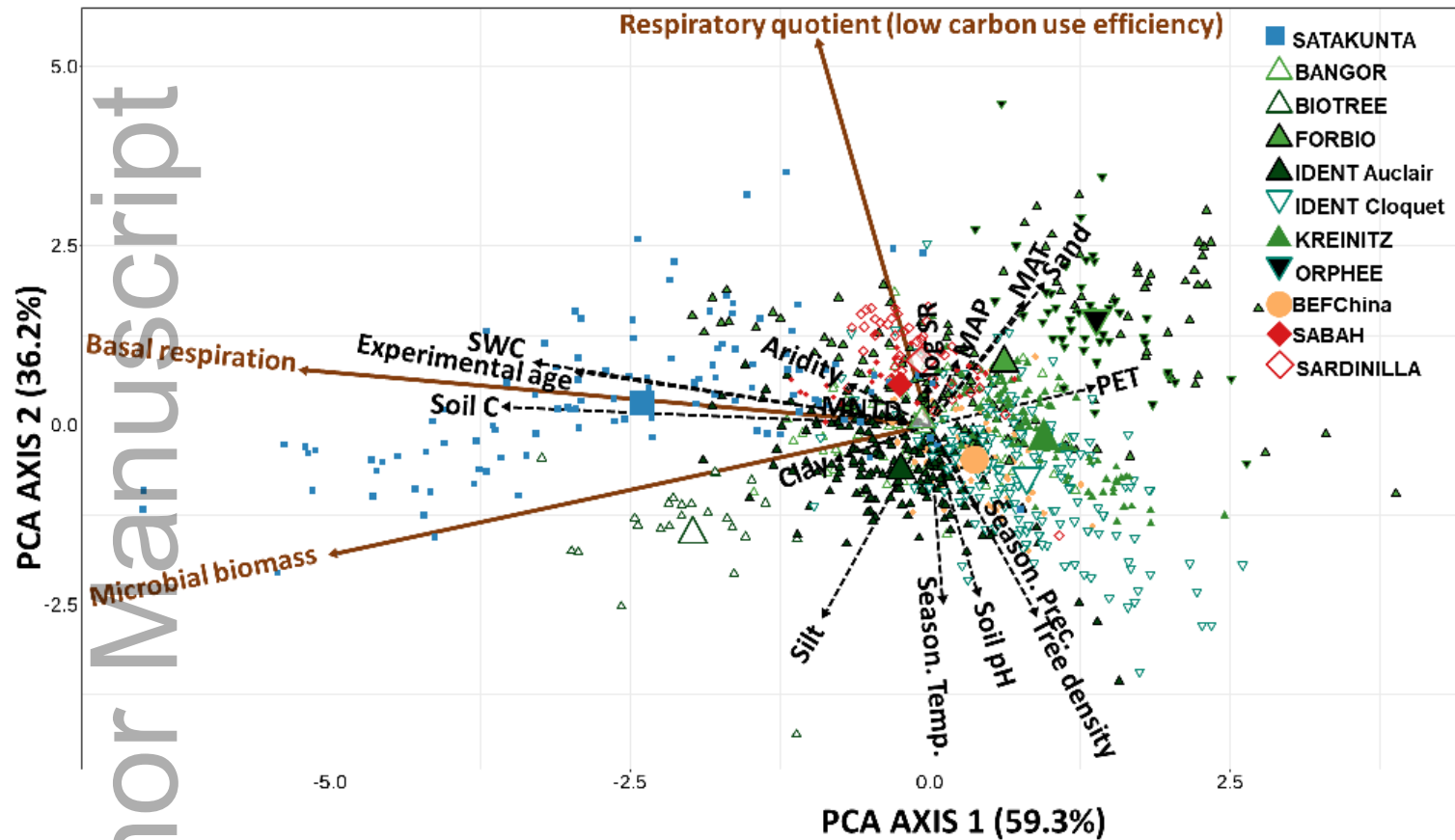
729

730   **Figure 2:** Principal component analysis (PCA) with the three microbial properties in focus (given in brown: basal respiration, microbial

731   biomass, and the respiratory quotient), as well as the abiotic variables soil water content (SWC), soil C, sand, silt, clay, seasonality of

732   temperature (Season. Temp) and precipitation (Season. Prec), soil pH, potential evapotranspiration (PET), mean annual temperature (MAT),

733   mean annual precipitation (MAP), aridity, and biotic variables experimental age, tree density, tree species richness (log SR) and mean nearest

734   taxonomic distance (MNTD) in eleven tree diversity experiments in boreal (blue, n = 1), temperature (green, n = 7), subtropical (yellow, n = 1),

735   and tropical (red, n = 2) biomes. All variables were scaled. Percent values in brackets give the variance explained by the different PCA axis.

736   Large symbols represent the centroids of the samples for each experiment. Correlations between moderators and the principal components can be

737   found in Table S2.
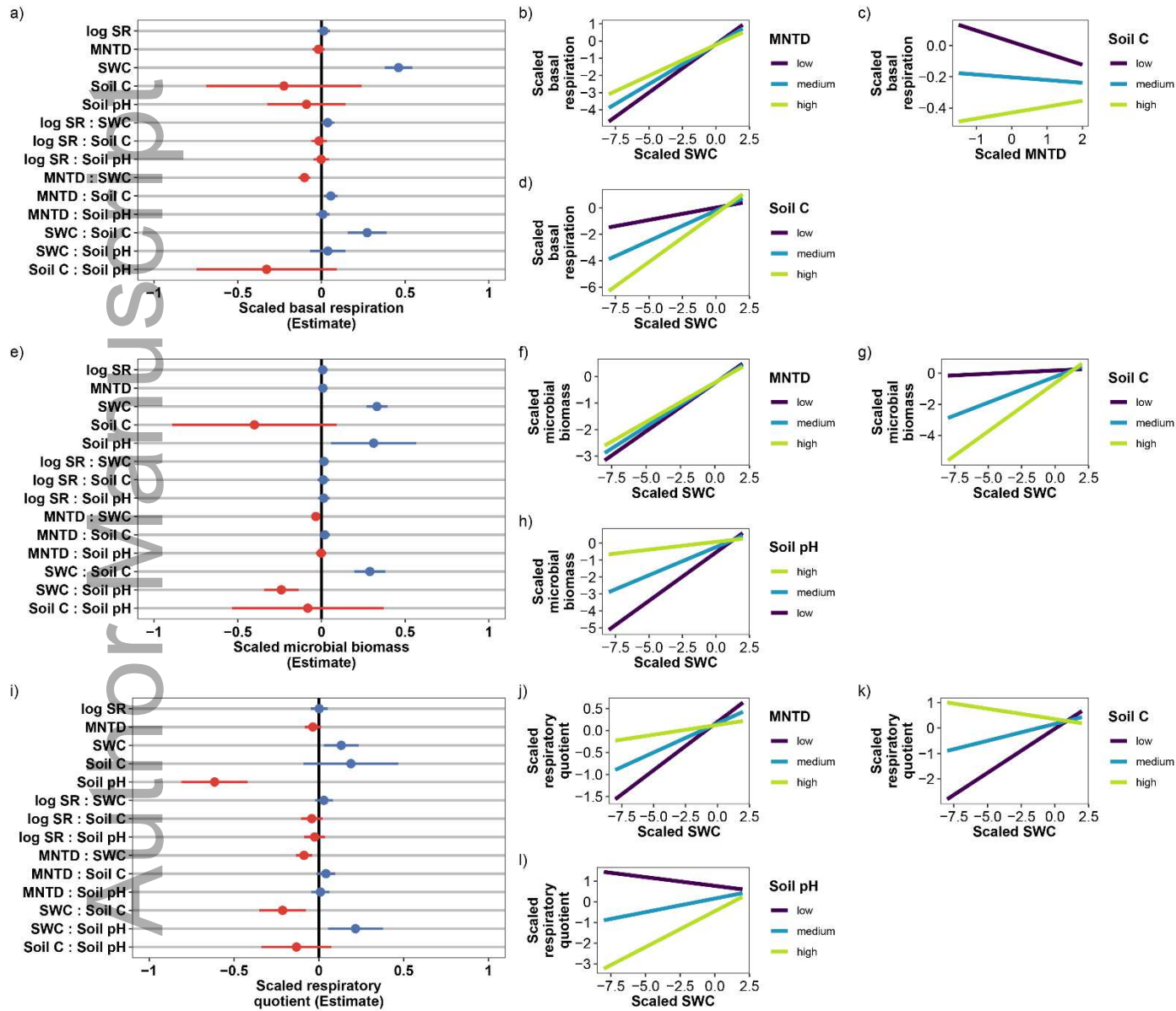
739   **Figure 3:** Coefficient estimates of linear mixed-effects models for three soil microbial properties (basal respiration, microbial biomass, and the

740   respiratory quotient) as affected by two tree diversity metrics, namely tree species richness (SR) and mean nearest taxonomic distance (MNTD),

741   and abiotic variables affecting soil microbial properties the most, i.e., soil water content (SWC), soil C, and soil pH. The blue color indicates a

742   positive effect of the fixed factor on the response variable, whereas red indicates a negative effect. For significant interactions (not crossing the

743   zero line), interaction plots were given as smaller panels, where one of the variables was categorized into low, medium, and high levels.

Supplementary Information

Appendix S1 - List of tree species occurring in the tree diversity experiments studied

Appendix S2 - Correlations

Appendix S3 - piecewise SEM

Appendix S4 - Single experiments

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