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2
3   **Article Title:** Frequent fire slows microbial decomposition of newly deposited fine fuels in a
4   pyrophilic ecosystem
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8

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31   *We furthered fire ecology by creating a fire-microbe interaction model. The model shows the*

32   *importance of decomposition in pyrophilic systems, and provides a framework applicable to other*

33   *ecosystems.*

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36   **Author Contributions[1]**

Abstract

Frequent fires maintain nearly 50% of terrestrial ecosystems, and drive ecosystem changes that govern future fires. Since fires are dependent on available plant or fine fuels, ecosystem processes that alter fine fuel loads like microbial decomposition are particularly important and could modify future fires. We hypothesized that variation in short-term fire history would influence fuel dynamics in such ecosystems. We predicted that frequent fires within a short-time period would slow microbial decomposition of new fine fuels. We expected that fire effects would differ based on dominant substrates and that fire history would also alter soil nutrient availability, indirectly slowing decomposition. We measured decomposition of newly deposited fine fuels in a Longleaf pine savanna, comparing plots that burned 0, 1, 2, or 3 times between 2014 & 2016, and which were located in either close proximity to or away from overstory pines (Longleaf pine, *Pinus palustris*). Microbial decomposition was slower in plots near longleaf pines and, as the numbers of fires increased, decomposition slowed. =. We then used structural equation modeling to assess pathways for these effects (number of fires, 2016 fuel/fire characteristics, and soil chemistry). Increased fire frequency was directly associated with decreased microbial decomposition. While increased fires decreased nutrient availability, changes in nutrients were not associated with decomposition. Our findings indicate that increasing numbers of fires over short time intervals can slow microbial decomposition of newly deposited fine fuels. This could favor the fine fuel accumulation and drive positive feedbacks on future fires.

Keywords: Fire-microbe interactions, decomposition, microbial ecology, pine savanna, short-term fire history

Introduction

Fire is a consistent disturbance in terrestrial ecosystems that profoundly changes biological and biogeochemical processes. Although often thought of as rare, catastrophic events (Bowman et al. 2009), frequent fires are necessary to maintain nearly 50% of terrestrial ecosystems including grasslands, savannas, and many Mediterranean-type ecosystems (Archibald et al. 2018). Since wildfire frequency is expected to increase due to human influence (Balch et al. 2017) and climate change (Liu and Wimberly 2016, Schoennagel et al. 2017), understanding how ecosystems respond to frequent fire is important for their preservation and maintenance. While directly manipulating aspects of fire regime is impossible in many systems, prescribed fire in naturally fire-frequented ecosystems may represent a conservative model to predict the pathways through which increased fire frequencies can impact systems that otherwise rarely experience fire.

Frequent fires in grasslands and savannas alter organisms and their environment in ways that can impact subsequent fires. The fine fuels produced by fire-adapted plants (i.e. their litter) are key examples, as frequent fires favor plant species that rapidly recover following fire and produce biomass that fuels future fires (Whitlock et al. 2003, Beckage et al. 2009, Cornelissen et al. 2017). By favoring fire adapted plant species, characteristics of previous fires can create a feedback, through the rapid production of fine fuels that control the spread and intensity of new fires (i.e. short-term fire history; Neary et al. 1999, Ficken and Wright 2017). Fine fuel accumulation, however, is also governed by other processes like microbial decomposition, which may also produce feedbacks based on fire history (Butler et al. 2019). Quantifying the pathways by which fire history impacts decomposition is critical for the maintenance of fire-frequented ecosystems and predicting potential mechanisms by which frequent fires impact other systems.

82   Repeated fires directly govern microbial decomposition by altering fine fuel loads and

83   shaping the microbial communities that control fuel decay. The strength of fire's effect on

84   decomposition is constrained by fire regime components like fire history, which can determine the

85   quantity of available fine fuels and the intensity of future fires. For example, longer fire return

86   intervals result in increased fuel loads (Archibald et al. 2013, Harris et al. 2016) and longer

87   recovery times for microbes, while shorter fire return intervals, or frequent fires, can decrease plant

88   fuel loads and microbial recovery times due to repeated combustion (Platt et al. 2016). When

89   combined with natural variations in fuel load production (i.e. tree-grassland matrices of savannas;

90   Platt et al. 2016), this can produce fires of varying frequencies and intensities that drive differential

91   mortality of microbial decomposers and slow decomposition depending on location. Since fire

92   can cause the mortality of microbial decomposers and filter communities for particular functional

93   groups (Dooley and Treseder 2012, Ferrenberg et al. 2013, Brown et al. 2013), understanding how

94   fire-history and intensity alters microbial decomposition can clarify the dynamics of fire-microbe-

95   plant interactions in pyrophilic ecosystems. If microbial decomposition is strongly altered by fire,

96   this could directly shift new fine fuel accumulation rates and affect the intensity and likelihood of

97   future fires.

98   Fire regimes also influence the substrates and nutrients available for microbial

99   decomposition, which may create indirect pathways for fire feedbacks. Fine fuel traits (e.g. carbon

100   to nitrogen ratio and lignin content) directly govern decomposition (Manzoni et al. 2010), and also

101   determine the duration and intensity of fires (Demirbaş 2001). For example, the larger quantities

102   of needles near longleaf pines can increase local fire intensities (Platt et al. 2016), and are also

103   harder to decompose than grass and forb litter. As such, fire histories that change the composition

104   of new fine fuels may change both the substrates available to microbial decomposers, and fire's

105   direct effects on them. Fire history can also govern stoichiometry through fire effects on limiting

106   nutrients like nitrogen (N) and phosphorus (P) (Raison 1979, Butler et al. 2018). Rapid post-fire

107   decomposition may be favored by N and P mineralization if enzyme production and microbial

108   growth would be otherwise limited. Longer intervals between fires can allow fuels to build-up,

109   and increase fire intensity to the point where temperature-sensitive elements like N are volatilized

110   (Raison 1979). N availability then may vary with fire due to interactions between fire history and

111   intensity (i.e. maximum temperature and duration). Low N availability may a) slow decomposition

112   if N-limited microbes cannot make enzymes or b) accelerate decomposition if microbes can make

113   enzymes, and rapidly decompose new fuels to acquire N and other limiting nutrients lost with fire

114   (Parnas 1975). Apart from individual fire intensity, repeated fires decrease nutrient availability

115   (Bell and Binkley 1989), and drive leaching that could slow microbial decomposition.

116   These combined effects of short-term fire history on substrates and nutrients likely

117   combine with direct fire effects to determine microbial decomposition of fuels. While single fires

118   can slow decomposition and promote fuel accumulation (Semenova-Nelsen et al. 2019), increasing

119   the number of fires within a short period could produce synergistic effects that further slow

120   decomposition. These effects could result from both the direct and indirect effects of fire on

121   microbial decomposition mentioned above. We hypothesized that increasingly frequent fires

122   would slow decomposition, and that fire would impact decomposition through mechanisms related

123   to fire characteristics and nutrient availability. We also hypothesized that natural variations in the

124   type and quantity of fine fuels would slow decomposition independently of fire history due to

125   substrate differences (Taylor et al 1989).

126   We manipulated short-term fire history in an old-growth Longleaf pine savanna to evaluate

127   the effect of fire history on the microbial decomposition of fine fuels. Pine savannas offer ideal

128 systems for testing our hypotheses because: organisms there have long co-evolutionary histories

129 with fire (Noss et al. 2015), fire history can be experimentally manipulated, and the spatial

130 heterogeneity of the savanna produces variation in dominant vegetation and the fuels microbes

131 decompose. We used mesh litter bags to measure microbial decomposition of new, post-fire fine

132 fuels. Decomposition was assessed both near and away from pines, to reflect differences in fine

133 fuel substrates (Ellair and Platt 2013, Platt et al. 2016) and microbial communities (Semenova-

134 Nelsen et al. 2019). We first assessed the impact of fire history and pine proximity on microbial

135 decomposition rate constants (k) during the year following 2016 fires. We then used structural

136 equation modeling (SEM) to assess the relative importance of direct and indirect mechanisms on

137 decomposition following prescribed fires. In addition to fire history, edaphic properties, fire

138 characteristics, and fine fuel traits in 2016 were all analyzed as potential drivers of microbial

139 decomposition. The resulting SEM model allowed us to identify the primary pathways through

140 which fire history altered microbial decomposition of fine fuels.

141

142 **Methods**

143 *Field Site*: We conducted our study on the Wade Tract (30º 45' N; 84º 00' W; Thomas County,

144 Georgia, USA). Situated on moderately dissected terrain 25-50 m above sea level in the Red Hills

145 region of northern Florida-southern Georgia, the 80 ha preserve is characterized by a warm-

146 temperature climate, with a growing season of 10-11 months, a mean annual temperature of

147 19.6°C, and average precipitation of ca. 1,350 mm that tends to bimodally distributed during the

148 summer and winter months. Surficial soils are acidic, fine-textured sands with A horizons 50-100

149 cm deep over a clay hardpan (Typic and Arenic Kandiudults; Carr et al. 2009, Levi et al. 2010).

150 Natural fires in this site tended to occur every 1-3 years, generally during a fire season that spanned

151 dry springs to wet summers, when annual thunderstorms first occurred (Platt et al. 2015, Rother et

152 al. 2018). Historical "open-woods burning" and more recently prescribed fires, have maintained

153 the open savanna/woodland physiognomy (Platt et al. 1988, Gilliam and Platt 1999, Mugnani et

154 al. 2019). The ground layer vegetation and litter on the site has burned annually-biennially (return

155 intervals averaging 1.5 years) during prescribed fires between March and June using drip torches,

156 1-2 weeks after rain at relative humidity of 50-60% and winds 10-20 km/hr. Flame heights during

157 burns can reach 1-2 m, and generally result in 60-90% removal of accumulated fine fuels.

158 *2014 Field Plots*: We established experimental plots in mid-June 2014, following 2014 prescribed

159 fires. These fires produced large unburned patches in a matrix of burned vegetation. We randomly

160 selected 24 unburned patches, 12 in each of two fire management units. Within each fire

161 management unit, 6 patches were near (<5 m) and 6 patches were away (>10 m) from overstory

162 pines. We then randomly selected 24 similar sized burned patches (12 near pines, and 12 away

163 from pines), such that each burned plot was near an unburned patch. Thus, 24 unburned and 24

164 burned patches were evenly distributed across two fire blocks and relative to overstory pines

165 (Table 1). Each patch was at least 5 m in diameter to minimize fire-edge effects, and did not

166 contain large amounts of woody debris such as fallen trees or large branches. Within each patch,

167 we established randomly located, 1x1 m sampling plots for downstream measurements. Note that

168 these plots were same as used in Semenova-Nelsen et al. 2019. This allowed us to test both the

169 effects of increased fire frequency, as well as the presence/absence of fire on microbial

170 decomposition.

171 *Short-term fire regimes*: We generated differences in short-term fire histories (2014-2016) by

172 manipulating fire regimes. The different fire histories are depicted in Table 1. In 2014, unburned

173 and burned plots were selected based on patchiness of prescribed fires conducted that year. In

174   2015, we manipulated prescribed fires by burning only one fire block, so that half of the

175   experimental plots burned. Then, in 2016 all plots burned during prescribed fires. We thus

176   generated replicated plots with patterns of 1, 2, and 3 fires; six plots with each fire history were

177   located near and away from pines. Following the 2016 fires, we used fire maps to identify patches

178   that did not burn in 2014, 2015, or 2016; we randomly selected 12 of these patches, 6 near pines

179   and 6 away from pines, and established an additional plot in each. This generated a total of five

180   short-term fire histories that involved 0 (0-0-0), 1 (0-0-1), 2 (1-0-1, 0-1-1) and 3 (1-1-1) fires over

181   the three-year study period (Table 1).

182   We conducted prescribed fires similarly from 2014-2016. All were ignited and occurred

183   under similar conditions. In all three years, head and flanking fires were ignited in the two fire

184   management units between mid-March and early May under Keetch-Byram Drought Indices of

185   60-250 using drip torches. Fine fuel consumption in burned patches was estimated each year as

186   60-80%. Because fires were conducted under similar weather conditions and times of the year,

187   short-term fire histories in Table 1 were considered to differ mainly in the numbers of fires.

188   In 2016 we explored fuel-fire relationships and measured characteristics of fires in the

189   plots. First, we measured fine fuels, pre- and post-fire, in the 48 plots using procedures outlined

190   in Platt et al. 2016. We established pairs of 30 x 30 cm subplots adjacent to each of the $1m^2$ plots.

191   For each plot, we randomly selected one subplot and collected above ground fuels 1-2 days prior

192   to fires, then sorted those fuels into fine fuels using two categories: pine needles and non-pine fuels

193   (graminoid, forb, shrub, and other non-woody fuels). Additionally, we recorded the total amount

194   of fine fuels and proportion of fuel loads that were Longleaf pine needles. The fine fuels were air-

195   dried and weighed. One day after 2016 fires, we collected the fine fuels from the other subplot.

196   Remaining fine fuels were weighed to estimate fine fuel combustion. Average mass of woody

197 fuels in plots was similar before and after fires, so we did not examine woody fuel effects on fire

198 characteristics.

199   We assessed fire characteristics using temperatures recorded at the surface and in the soil

200 during the prescribed fires. We placed two thermocouples in the center of each plot. One was

201 placed 2-3 mm above the ground surface, not contacting litter or soil; the second was placed 1 cm

202 in the soil, close to the surface thermocouple. Thermocouples recorded temperatures every second

203 from the time of activation until 5-6 hours after prescribed fires. The temperature data were used

204 to estimate 1) *maximum surface & soil temperature increase* - the largest instantaneous rise in

205 temperature recorded and 2) *duration of heating* - the time (in seconds) that the temperature at the

206 soil surface remained >60°C (Platt et al. 2016).

207 *Quantifying Microbial Decomposition*: We measured microbial decomposition of recently

208 deposited litter experimentally in 2016. In October 2016, we collected recently deposited, intact

209 plant material (dead pine needles, grass culms, forbs, and oak leaves) from outside the 4 m^2 sample

210 plots. Litter collected from patches of the same type (i.e. near and away from pines) was pooled,

211 then shipped to the University of Kansas where it was stored at -20°C until processing. Near and

212 away litter was separated to account for inherent differences in litter chemistry (i.e. C:N ratios and

213 lignin content) and composition (i.e. more pine needles near pines) between litter types. Plant

214 litter was dried at 65˚C for 72 hours, ground using a Model 4 Wiley Mill (Thomas Scientific,

215 Swedesboro, USA) with a 6mm opening, and sterilized via gamma irradiation to ~32 kGy at the

216 Penn State Radiation Science & Engineering Center. Within a biological safety cabinet, we placed

217 the sterilized plant litter in 15 x 15 cm, 30 µM nylon mesh bags, following (Robertson and Paul

218 2000). This mesh excludes non-microbes and isolates microbial decomposition of plant litter

219   (Bradford et al. 2002). Each bag was filled with 5 g of plant litter collected either near or away

220   from pines. Initial bag masses were recorded, and bags were stored sterilely until deployment.

221   Bags were deployed in June 2016, 2-3 months after experimental fires. Four

222   decomposition bags with litter corresponding to pine proximity (i.e. near or away from pines),

223   were selected and randomly placed on the soil surface in each plot. The small mesh size used in

224   bag construction prevented photo degradation of bag contents. Bags were anchored along margins

225   with sod-staples so that one surface of the bag contacted litter and soil. One bag from each plot

226   was collected 2, 4, 6, and 8 months after deployment. Any soil or litter on the bag surface was

227   cleared, and then bags were placed in sterile plastic bags. Bags were shipped overnight to the

228   University of Kansas. Litter contents were then removed, dried at 65°C for 72 hours, and weighed

229   to determine mass loss. Decomposition rate constants (k) were determined by fitting

230   decomposition from 2 - 8 months in each experimental plot to a negative exponential curve using

231   the following equation:

232
$$\frac{M_t}{M_0} = e^{-k*t}$$

233   where the M_0 = starting mass, M_t = mass at time of collection, and t is the number of months the

234   bag was deployed in the field. A negative exponential curve was used to estimate k, as

235   decomposition is well known to follow an exponential decay function when measured over time

236   (Olson 1963, Karberg et al. 2008). This produced a decomposition rate constant (k) for each

237   experimental plot during the year following 2016 prescribed fires.

238   _Soil analysis_: Soil samples were collected from all plots in June 2016 to measure post-fire nutrient

239   flux. We collected soil at three randomly located points, avoiding ground layer plants. We

240   collected the upper 1.5 cm of soil within a 9 x 9 cm quadrat (i.e., depth potentially affected by

241 increasing fire temperatures; Mehlich 1984, Gagnon et al. 2015). Soil samples from each plot

242 were combined, and kept cool until frozen at -20°C within 6 hours of sampling. Samples were

243 overnighted to the University of Kansas, thawed, and homogenized by hand, before subsampling.

244 A 100 g subsample was sent to the Kansas State University Soil Testing Lab for analysis.

245 Soil phosphorus was measured using the Mehlich-3 method (Mehlich 1984) on a Lachat

246 Quickchem 8000 (Lachat Instruments, Loveland, USA). Total soil nitrogen and carbon were

247 measured on a LECO TruSpec CN Carbon/Nitrogen combustion analyzer (LECO Corporation, St.

248 Joseph, USA). Carbon to nitrogen ratio was also calculated. NH_4^+ and NO_3^- were extracted using

249 1 M KCl on 2 g of soil, then Cadmium reduction for nitrate and colorimetric procedures were used,

250 followed by flow analysis for ion quantification (Brown 1998).

251 *Data Analysis*: All analyses were conducted in R version 3.5.1 (R Core Team 2013). Analyses of

252 variance (ANOVAs) assessed the effect of short-term fire history and proximity to pines on

253 microbial decomposition rate constants (k). Differences in decomposition, fine fuels, nutrients,

254 and fire characteristics between short-term fire history and pine proximity treatments were first

255 assessed using Type III analysis of variance (ANOVA) using the "Emmeans" package (Lenth

256 2018). Note that pine proximity treatments were considered in these analyses to account for

257 inherent differences in litter chemistry, fuel traits, and flammability, between near pines fuels vs.

258 away from pines fuels. Following ANOVAs, apriori contrasts regarding differences in

259 decomposition based on the frequency of fires were assessed using the contrast function.

260 We then developed a structural equation model to assess the causal pathways by which

261 fires impacted plot-level microbial decomposition rate constants (k). Based on existing literature,

262 we hypothesized three specific pathways between fire history and microbial decomposition (Figure

263 1). These pathways included both direct fire history effects and indirect effects through 2016 fire

264   characteristics and initial changes to soil properties. Appendix Tables S1 and S1.5 describe

265   variables and justifications for model pathways included in the SEM analysis.   We also

266   hypothesized that fuel characteristics play an independent role in determining both fire

267   characteristics and decomposition. We hypothesized that frequent fires would 1) reduce microbial

268   decomposition rates (Figure 1; Path A; Ficken and Wright 2017), 2) reduce the severity of

269   individual fires thereby increasing decomposition rates (Figure 1; Path B; Ficken and Wright 2017,

270   Ellair and Platt 2013, and 3) modify the initial flux of nutrients mineralized by fire and slow

271   decomposition (Figure 1; Path C; Bell and Binkley 1989, Czimczik et al. 2005, Butler et al. 2018).

272   Distinct from short-term fire history effects, locational effects due to larger fine fuel loads and

273   larger amounts of pine needles near pines, should 4) increase fire intensity and slow decomposition

274   (Figure 1; Path D; Ellair and Platt 2013). Our SEM contained categorical, continuous, and ratio

275   variables. All continuous variables were transformed and scaled prior to analysis (appendix Table

276   S1).   After developing an initial model based on these hypotheses, the R Package: "lavaan"

277   (Rosseel 2012) was used to evaluate the preliminary SEM for convergence. Upon convergence,

278   fit measures and parsimony were used to assess the modification of model parameters. Further

279   models were then evaluated per Hooper et al. 2008.

280

281   **Results**

282   _Fine Fuels_: Pre-fire fine fuel loads varied based on proximity to overstory longleaf pines and

283   short-term fire history treatment. The largest differences were between pine needle fuels, with

284   near pines sites having larger amounts ($F_{1,59} = 33.4$, $p < 0.001$; Table S2, Fig.S1) and proportions

285   ($F_{1,59} = 22.3$, $p < 0.001$; Table S2, Fig.S1) of Longleaf pine needles. Total fine fuels ($F_{4,59} = 7.34$,

286   $p < 0.001$; Table S2, Fig.S1) and non-pine fuels ($F_{4,59} = 7.37$, $p < 0.001$; Table S2, Fig.S1) also

287 differed between short-term fire history treatments, with sites experiencing two fires in the final

288 two years having lower amounts of both. In summary, near pines sites had larger amounts of

289 Longleaf pine needles, and more frequently burned sites had smaller fine fuel loads and amounts

290 of non-pine fuels.

291 _Soil Nutrients_: Post-fire nutrients levels varied based on the short-term fire history. As the number

292 of fires increased, total soil nitrogen ($F_{4,59}$ = 5.17, p = 0.001; Table S3, Fig.S2), inorganic

293 phosphorus ($F_{4,60}$ = 4.49, p = 0.003; Table S3, Fig.S2), ammonium ($F_{4,60}$ = 10.6, p < 0.001; Table

294 S3, Fig.S2), and nitrate ($F_{4,60}$ = 3.39, p = 0.02; Table S3, Fig.S2) levels decreased. While nitrate

295 levels decreased when there were two fires in the final two years, it is worth noting that sites burned

296 in only the final year (0_0_1) or the first and final year (1_0_1) actually saw an increase in nitrate

297 levels. Total soil carbon however did not vary between experimental treatments ($F_{4,60}$ = 1.02, p =

298 0.4; Table S3, Fig.S2). While soil carbon did not vary between short-term fire history treatments

299 in this analysis, C:N ratios increased as fires became more frequent ($F_{4,60}$ = 10.4, p < 0.001; Table

300 S3, Fig.S2). Frequent fires were associated with lower amounts of soil nutrients, and changed

301 nutrient levels in ways that shifted stoichiometric ratios of carbon and nitrogen.

302 _Fire Characteristics_: 2016 prescribed burn characteristics differed between short-term fire history

303 treatments; however, these differences were primarily related to the presence or absence of fire.

304 While there was some natural variation between maximum surface ($F_{4,51}$ = 105.1, p < 0.001; Table

305 S4, Fig.S3) and soil temperature ($F_{4,51}$ = 3.75, p = 0.009; Table S4, Fig.S3) increases, surface fire

306 duration >60°C ($F_{4,51}$ = 25.3, p < 0.001; Table S4, Fig.S3), and percent fine fuel combustion ($F_{4,51}$

307 = 72.6, p < 0.001; Table S4, Fig.S3), between burned sites, prescribed fires generally did not very

308 in intensity between our experimental treatments.

309   *Microbial decomposition*: Short-term fire history and pine proximity independently affected

310   microbial decomposition rate. As fires increased in frequency, decomposition rates decreased

311   ($F_{4,48} = 3.971$, p = 0.007; Table S5, Fig.2a & S4) with contrasts revealing that burning at least once

312   during the 3-year period was associated with slower decomposition than not burning (P = 0.01;

313   Table S5). Additionally, decomposition rates were lower in sites that burned at least two times as

314   compared to sites that only burned once (P = 0.009; Table S5). There were no overall differences

315   in decomposition rates between sites that burned two times and sites that burned 3 times (P = 0.4;

316   Table S5).

317   Proximity to pines also altered microbial decomposition. During the year following 2016

318   prescribed fires, litter bags located near pines had slower decomposition rates than bags placed

319   away from pines ($F_{1,48} = 3.921$, P = 0.05; Table S5, Fig.2b). In summary, increasing the number

320   of fires during the study period and close proximity to pines slowed microbial decomposition.

321   *Structural equation modeling of causal pathways for fire history effects*: We initially began with a

322   highly saturated SEM based on our hypothesized pathways (Appendix; Table S1.5 and SEM model

323   fitting section). The first model converged, but was poorly supported ($X^2 = 102.392$, D.F. = 28, P

324   < 0.00). Through four iterations, poorly supported paths in the model were successively pruned

325   to improve model fit using an increasingly conservative threshold for relationships (e.g. P>0.5,

326   P>0.2). Model support was checked after each pruning step (support for each included in appendix

327   table S6) with fit statistics assessed according to Hooper et al. 2008. The final model was well

328   supported ($X^2 = 21.795$, D.F. = 23, P = 0.533; Table 2), and further removal of unsupported

329   pathways did not improve overall fit. Final SEM pathways and coefficients, along with literature

330   support for these pathways are presented in Table 2.

331 *SEM results*: The final SEM model supported our hypotheses that short-term fire history altered

332 the microbial decomposition of fine fuels. We used our initial hypotheses (Figure 1) to construct

333 pathways for relationships in our SEM model (Figure 3). In this way, we could distinguish the

334 underlying mechanisms through which fire history was postulated to modify microbial

335 decomposition in the final model. Numbers in parentheses are the standardized regression

336 coefficients (Table 2). These values indicate the direction (+/-) and strength of relationships

337 between variables, and allow for direct comparisons between model pathways.

338 *SEM-Direct Impact of short-term fire history*: In line with our causal model (Figure 1; path A),

339 short-term fire history was linked to microbial decomposition rate (-0.517; Figure 3). Specifically,

340 as the number of fires a plot experienced increased, the decomposition rate constants (k) decreased,

341 paralleling the ANOVA analyses above.

342 *SEM-Modification of Edaphic Factors*: While short-term fire history directly modified edaphic

343 pathways, changes to nutrient availability did not alter microbial decomposition rates (Fig.3).

344 Increased numbers of fires during the study period were associated with decreases in ammonium

345 (-0.77), nitrate (-0.37), phosphorus (-0.58), and total nitrogen (-0.55), and marginally significant

346 decreases in soil carbon (p = 0.08, -0.25). Overall, increasingly frequent fires were associated with

347 decreased nutrient availability, but these changes were not associated with decomposition rates

348 during the year following 2016 prescribed fires.

349 *SEM-Fire Characteristics*: As hypothesized in our causal model (Fig.1; Path B), short-term fire

350 history was associated with 2016 fire characteristics, but changes in fire characteristics were not

351 associated with microbial decomposition. More fires during the study period corresponded with

352 greater maximum surface temperature increases (0.55), although this was largely driven by the

353 presence vs. absence (0-0-0) of fire in the final year. Additionally, increased numbers of fires

354 during the study were associated with shorter fire durations (-0.22). Greater surface fire

355 temperature increases were also correlated with increased surface fire durations (1.03) and larger

356 soil temperature increases (0.65). Surface fire temperature increases and durations also altered

357 edaphic properties as fires became hotter and longer. Hotter surface temperatures were associated

358 with decreased nitrate (-0.69) and phosphorus (-0.49). Longer fire durations however, were

359 associated with increased phosphorus (0.86), ammonium (0.2), and nitrate (1.1), and decreased

360 carbon (-0.28). In summary, increasing the number of fires shifted 2016 fire characteristics, which

361 were associated with altered edaphic properties, but not microbial decomposition.

362 *SEM-Fuel traits:* Fuel traits were directly linked to microbial decomposition and 2016 fire

363 characteristics (Figure 1; path D). As shown in previous work (Ellair and Platt 2013), sites located

364 near pines had more pine needles (0.53), which were directly linked with greater increases in

365 maximum surface temperature (0.34) and indirectly linked to longer fire durations (0.34) and

366 higher soil temperatures (0.22) through changes to surface temperatures. Fuel traits also had

367 indirect effects on nutrient availability through their modification of fire characteristics (Figure 3).

368 Additionally, near pine sites had lower decomposition rate constants (k) than those located away

369 from pines (-0.27). Taken together, fuel traits modified the intensity of 2016 fire characteristics,

370 and slowed decomposition in sites located near pines.

371

372 **Discussion**

373 Microbial decomposition of new fine fuels was slower in frequently burned sites during the year

374 following 2016 prescribed fires. These fire driven changes are consistent with studies that show

375 repeated fires shift microbial community structure, cause the loss of key functional groups (Hart

376 et al. 2005, Ferrenberg et al. 2013, Brown et al. 2013), and are associated with slower

377   decomposition (Ficken and Wright 2017, Butler et al. 2019). While fire history in our system did

378   not suppress total fungal abundance (Hansen et al 2019), it likely impacted microbial community

379   structure (Semenova-Nelsen et al. 2019) in ways that slowed decomposition. This demonstrates

380   that short-term variations in fire history are as important as single fires (Ficken and Wright 2017,

381   Semenova-Nelsen et al. 2019) or long-term fire regime differences (Butler et al. 2019), in

382   determining ecological functions like decomposition. Moreover, decomposition differences arose

383   quickly (i.e. within 3 years) in this pyrophilic ecosystem, so rarely burned systems, which lack

384   fire-adapted organisms, may respond more strongly to repeated fires. It is important to note

385   however, that the pathway linking fire history to microbial decomposition includes other

386   unmeasured processes besides direct fire effects on microbial decomposers.

387   Although short term fire history impacted nutrients, these effects were not linked to shifts

388   in microbial decomposition. Our study confirms well known impacts of fire on the availability of

389   soil carbon and nutrients (Raison 1979, Neary et al. 1999, Certini 2005).   While nutrient

390   availability influences decomposition (Manzoni et al. 2010), significant fire-driven changes to

391   carbon, nitrogen (NO3 and NH4), and phosphorus did not slow decomposition rates during the

392   year following 2016 prescribed fires. Two key factors may explain the absence of this relationship.

393   First, short term nutrient effects directly after fire may have been obscured when evaluated on

394   decomposition rate constants (k) that integrate seasonal variation. Stoichiometric controls on

395   decomposition vary seasonally (Schmidt et al. 2007), and fire-induced differences in nutrient

396   availability may have had decomposition effects that were balanced out at other time points. For

397   example, frequent burning that reduced nutrients for decomposition directly after the 2016 spring

398   fire, also likely reduced plant production throughout the year perhaps leaving greater soil nutrients

399   for fall microbial decomposition. Second, the high frequency of fires at this site (return interval of

400   ~1 year) may cause long term nutrient limitations (Knicker 2007, Toberman et al. 2014, Butler et

401   al. 2019), which could mask the effect of short term changes in nutrient levels following single

402   fires. Despite immediate C, N, and P losses in our study, the associations between microbial

403   decomposition and fire history were stronger than associations between decomposition and altered

404   nutrient availability. The long term adaptation to frequent fires and low nutrient availability at this

405   site likely had a stronger effect on decomposition rates (Butler et al. 2019) than short term nutrient

406   effects following the most recent fire. Other unmeasured factors like soil moisture, pH, and

407   temperature, are also shifted by fire and can modify microbial decomposition, but past studies have

408   not shown these factors were linked to microbial communities or decomposition at this site

409   (Semenova-Nelsen et al. 2019). In summary, short term fire history altered nutrient availability,

410   yet these changes were not linked to variation in microbial decomposition.

411   Short-term fire history also modified the intensity of 2016 fires, but this was not strongly

412   linked to microbial decomposition rates. Larger temperature increases and longer burn durations

413   are expected to kill more microbes (Bárcenas-Moreno and Bååth 2009, Dooley and Treseder

414   2012), and alter microbial decomposition rates due to microbial mortality. However, even in

415   "long" unburned plots, prescribed 2016 fires at this site may not have reached sufficient intensity

416   to cause significant microbial mortality (Hansen et al. 2019). As with nutrient effects, it is also

417   possible that fire intensity related effects on microbial decomposition are strongest immediately

418   after fires, and dissipate with time (or are even offset) as microbial communities recover

419   (Bárcenas-Moreno et al. 2011). Consistent with this interpretation, while fire characteristics did

420   not impact microbial decomposition, they did have strong effects on nutrient availability. High

421   intensity fires can increase nutrient volatilization (Neary et al. 1999), while longer, low intensity

422   fires (i.e. $< 200°C$) may favor the release of nutrients from fine fuels (Certini 2005). These were

423 born out by our data, as hotter fires (i.e. higher surface temperatures) were associated with

424 decreased N and P, while longer fires (i.e. longer durations above 60°C) were associated with

425 increased N and P. Fire characteristics, including intensity and duration, may play a larger role in

426 decomposition after wildfires, since wildfire intensity commonly surpasses that of prescribed fires

427 (Certini 2005). Overall, short-term fire history modified fire characteristics and nutrient

428 availability, but these changes were not associated with shifts in microbial decomposition rates.

429   The types of fuels present determined both fire characteristics and postfire decomposition.

430 While location and fuel composition covary, we show that microbial decomposition rates were

431 slower in near pines sites. The direct link between pine proximity and decomposition suggests

432 that the high lignin and C:N content of near pines fuels (Wardle et al. 2002) and location based

433 differences in microbial communities result in slower decomposition. While larger amounts of

434 Longleaf pine needles increased the intensity of 2016 prescribed fires, this did not affect microbial

435 decomposition rates following 2016 fires. Since microbial decomposition rates are slower near

436 pines, the greater suppression of decomposition following fire may contribute to natural fuel

437 accumulation that alters the likelihood or spread of future fires. At our study site, fires commonly

438 consume more than 60% of fuels (see appendix section "fuels"), and are primarily reliant on fuel

439 accumulated in the last year. This may be a key difference, for example, from fire suppressed

440 forests in the Western US, where the buildup of coarse woody debris (Brown 1983, Kalies and

441 Yocom Kent 2016), can create fires so severe that upper soil horizons are completely lost or

442 sterilized. Fire-driven decomposition differences then may depend on the fuels accumulated since

443 the last fire, and in fire-frequented systems fuel loads may have a strong seasonal relationship.

444   Linking fire regime and microbial function elucidates the largely unconsidered, but

445 important roles that microbes play in pyrophilic ecosystems. Historically, fire ecology has focused

446 on the interaction of fire with above ground communities (i.e. plants) and biogeochemistry

447 (Archibald et al. 2018), while rarely exploring microbial functions like decomposition. Our study

448 identified pathways through which fire history governs microbial decomposition of fuels, fire

449 characteristics, and soil nutrient availability. Short-term fire history's effects on microbial

450 decomposition should modify fine fuel loads, which could ultimately impact future fires. Other

451 microbial functions, however, may also contribute to (or mitigate) fire feedbacks. Fire regime

452 impacts on microbial mutualists, (i.e. mycorrhizae), could alter their benefits for post-fire plant

453 survival and fuel production (Peay et al. 2009, Glassman et al. 2016, Carson et al. 2019). Microbial

454 pathogen responses to fire history may also be important due to their role in plant productivity

455 (Schnitzer et al. 2011), with pathogen suppression by fire potentially allowing for greater post-fire

456 plant survival and faster fuel production. The indirect impact of microbe-plant symbioses on fuels

457 may counterbalance, or even exacerbate the positive fire feedbacks from microbial decomposition.

458 Future work can explore how fire-microbe interactions shape fire feedbacks through fuel load

459 alterations (as seen here) and plant-microbe interactions.

460　　　In conclusion, we demonstrated that short-term fire history and microbial decomposition

461 are closely connected through direct fire and fuel related pathways. Furthermore, we identified a

462 feedback mechanism through which increased numbers of fires may increase fine fuel

463 accumulation and the intensity of future fires. Understanding how different fire histories impact

464 microbial decomposers and associated fine fuels is critical to our knowledge and maintenance of

465 pyrophilic ecosystems, many of which are endangered (Bowman et al. 2009). Furthermore, our

466 study system may provide a conservative model for predicting the effects of increasing fire

467 frequencies in other ecosystems. Fire helps maintain more than 50% of terrestrial ecosystems, and

468 its occurrence is becoming increasingly frequent due to anthropogenic change (Archibald et al.

469 2018). Including foundational microbial processes like decomposition in fire models can improve

470 our understanding and management of fire-dependent and non-fire dependent ecosystems alike.

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692   **Fig. 1:** Hypothesized pathways by which short-term fire history modifies microbial decomposition

693   of fine fuels. Fire History shown in light red, nutrients in blue, fire characteristics in orange, fuel

694   traits in green, and decomposition in pink. Path A: Increasing recurrence of fire should slow

695   decomposition through repeated negative effects on microbes. Path B: Frequent fires should lessen

696   fire severity characteristics and the negative effect of fire on decomposition. Path C: Frequent

697   fires alter nutrient availability, which could lead to nutrient loss and slow decomposition. Path D:

698   Distinct from fires, increasing amounts of fine fuels should increase fire severity characteristics

699   and slow decomposition. citations for hypothesized pathways are detailed in table S1.5.

700   **Fig. 2:** Short-term fire history and pines proximity effects on microbial decomposition. Trend

701   lines represent microbial decomposition rate constants (k) calculated by fitting decomposition

702   measurements in each plot to a negative exponential decay function. Error bars and points

703   represent 95% confidence intervals and means for microbial decomposition rate constants (k).

704   ANOVA results are annotated in the figures, fire = fire history and prox = pine proximity. a) As

705   fires became more frequent, decomposition rate constants (k) were lower, and larger amounts of

706   plant fuels remained at the end of the experiment. b) Experimental sites located near pines had

707   lower decomposition rate constants (k) than sites located away from pines. Note that $* p \leq 0.05$.

708   **Fig. 3:** SEM model for short-term fire history's effect on microbial decomposition. Components

709   are group by color as in Figure 1. Coefficients are standardized regression coefficients. Red and

710   black paths denote negative and positive associations between linked variables respectively.

711   Increasingly frequent fires were associated with lower microbial decomposition rates, however

712   fire history related effects on fire characteristics and nutrients did not affect decomposition.

713   Additionally, sites located near pines had lower decomposition rates than sites located away from

714   pines.

Table 1

Table 1: Experimental field design for short-term fire history treatments (2014-2016) in plots located near and away from pines. For each year, 1 indicates groups of plots that were burned and 0 indicates groups of plots that were not burned. The design produced five short-term fire history treatments that involved 0-3 fires, both near & away from pines. Of the total of 60 plots, 48 were established in 2014. Fire maps were used to establish an additional 12 plots (marked with *) in 2016 that did not burn in the three previous years.

		Near Pines						Away from Pines				
Short-Term Fire History	2014	1	1	0	0	0		1	1	0	0	0
	2015	1	0	1	0	0		1	0	1	0	0
	2016	1	1	1	1	0*		1	1	1	1	0*
Number of Plots		6	6	6	6	6		6	6	6	6	6
Number of Fires		3	2	2	1	0		3	2	2	1	0

Table 2

Table 2: Final SEM pathway coefficients and justifications. For each pathway in the model, the table identifies the response variable(s), explanatory variable(s), standardized estimate (effect size), standard errors, P-values for significance, R-squared estimate for model pathway, and justification for inclusion in the final model.

Response Variable	Explanatory Variable	Stand. Estim.	Stand. Err.	P-value	R^2	Justification
Microbial Decomposition Rate Constant (k)	# of Fires	-0.517	0.123	**<0.001*****		Ficken and Wright 2017
	Pine Proximity	-0.268	0.236	**0.021****	0.325	pine fuels are more recalcitrant than non-pine fuels
	Surf. Dur. > 60	0.169	0.129	0.181		Peay et al. 2009
Maximum Instant Surface Temp. Increase (°C)	# of Fires	0.546	0.097	**<0.001*****		presence of fire = hotter temperatures
	Pine Needle Fuels	0.335	0.11	**0.002****	0.441	Ellair and Platt 2013
Maximum Instant Soil Temp. Increase (°C)	Max Inst. Surf. Inc.	0.645	0.091	**<0.001*****	0.416	Peay et al. 2009
Surface Fire Duration > 60°C (sec)	# of Fires	-0.223	0.067	**0.001*****		smaller fuel loads w/ increased fire frequencies
	Max Inst. Surf. Inc.	1.027	0.073	**<0.001*****	0.843	Bárcenas-Moreno and Bååth 2009
Total Soil Carbon (%)	# of Fires	-0.252	0.13	**0.08***		Czimczik et al. 2005
	Max Inst. Surf. Inc.	0.241	0.146	0.102	0.06	Johnson & Curtis 2000
	Surf. Dur. > 60	-0.279	0.121	**0.032****		Johnson & Curtis 2000
Total Soil Nitrogen (%)	# of Fires	-0.551	0.111	**<0.001*****	0.304	Christensen 1977

| Variable	Predictor					Reference/Note
Inorganic Soil Phosphorus (ppm)	# of Fires	-0.58	0.132	**<0.001*****		Butler et al. 2018
	Max Inst. Surf. Inc.	-0.494	0.304	**0.071***	0.519	Butler et al. 2018
	Surf. Dur. > 60	0.861	0.252	**<0.001*****		Butler et al. 2018
NO_3^- (ppm)	# of Fires	-0.368	0.15	**0.008****		Christensen 1977
	Max Inst. Surf. Inc.	-0.686	0.348	**0.02****	0.447	Raison 1979
	Surf. Dur. > 60	1.094	0.289	**<0.001*****		longer, low intensity fires release more N
NH_4^+ (ppm)	# of Fires	-0.774	0.1	**<0.001*****	0.526	Christensen 1977
	Surf. Dur. > 60	0.195	0.096	**0.041****		longer, low intensity fires release more N
Pine Needle Fuels (g)	Pine Proximity	0.53	0.217	**<0.001*****	0.281	more pine needles near pines
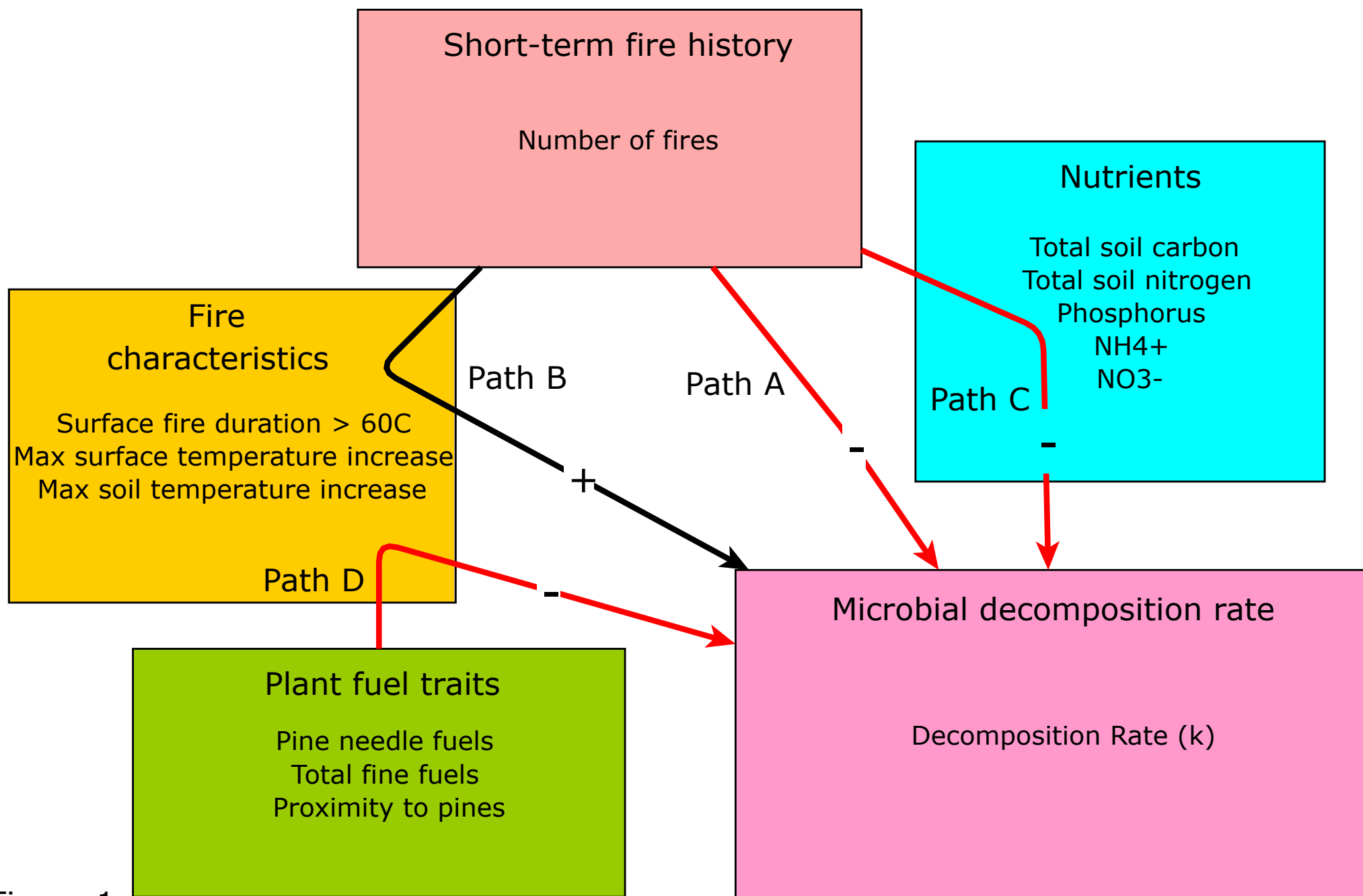
Figure 1
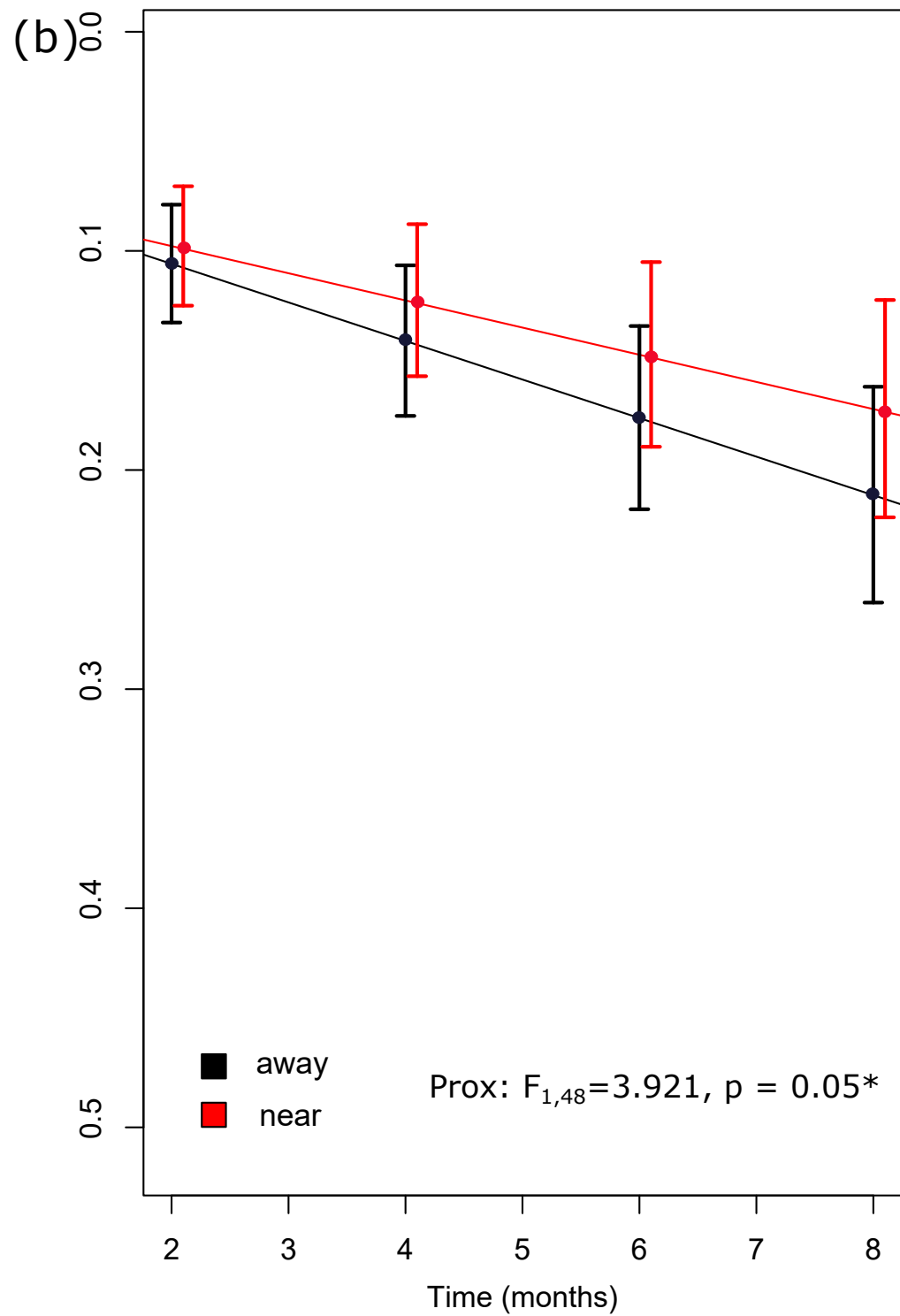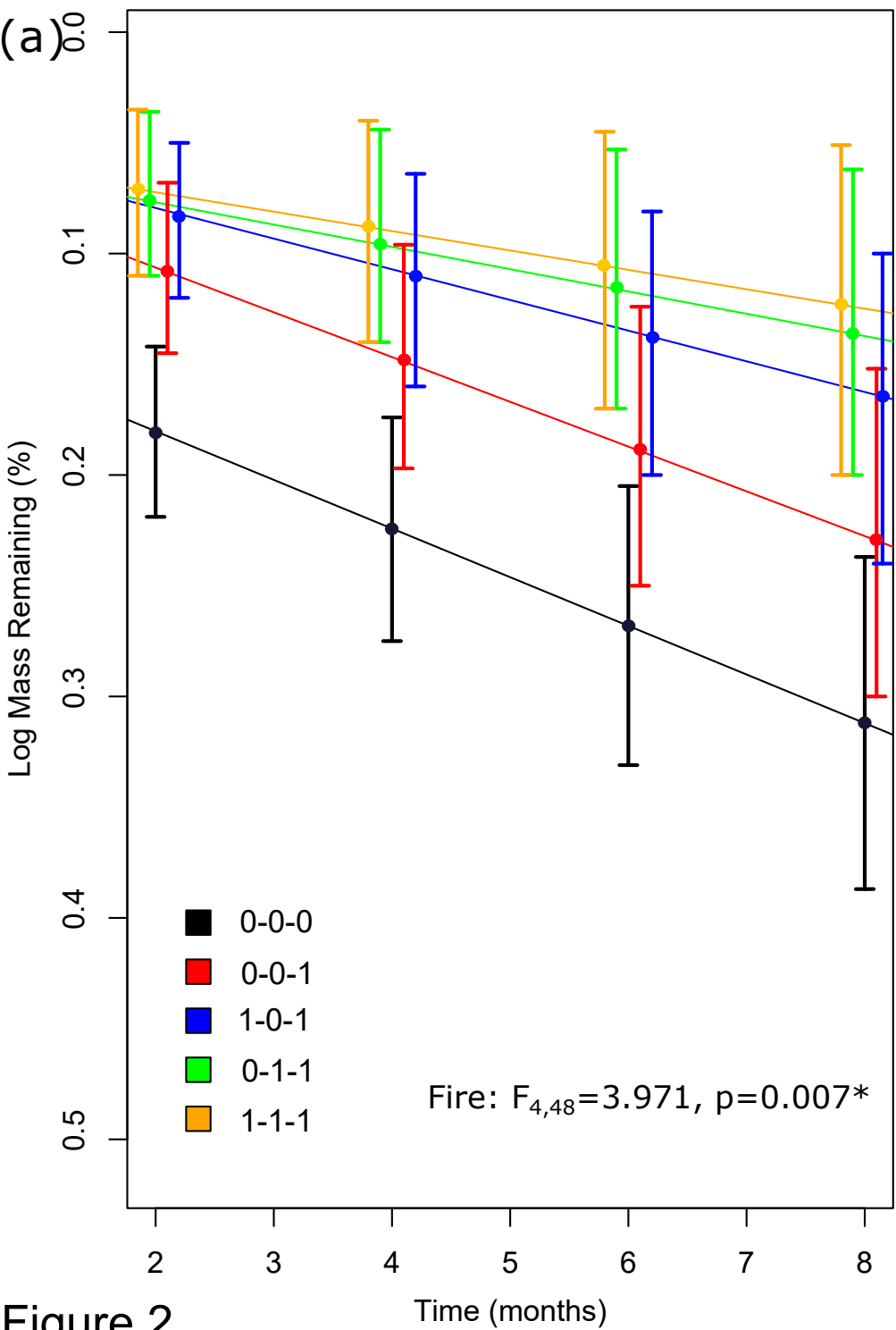
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Figure 1

Figure 2

Figure 2

Figure 3

Figure 3