Temporal dynamics of carbon and nitrogen in the surface soil and forest floor under different prescribed burning regimes

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Keywords:

Prescribed fire regimes

Temporal variations

Soil carbon and nitrogen

Forest floor carbon and nitrogen

17 **ABSTRACT**

18 Prescribed burning has been widely used in the management of forests for reducing wildfire risk,
19 and can have significant effects on soil carbon (C) and nitrogen (N) pools and their temporal
20 changes. This study aimed to investigate the impacts of different burning frequency regimes on the
21 temporal dynamics of C and N in the topsoil and forest floors. The experimental site was a 39 year
22 old wet sclerophyll forest prescribed burning trial at Peachester, southeast Queensland, Australia,
23 with treatments of no burning (NB) since 1969, 2 yearly burning (2yrB) and 4 yearly burning
24 (4yrB) since 1972. Each of three burning treatments had four replications and these plots were
25 randomly distributed over an area of the forest with similar vegetation and soil characteristics. Soil
26 (0-10 cm) and forest floors were sampled monthly for 6 months prior to the next scheduled burning
27 for both burning treatments to minimize the effects of fire recency. Prescribed burning significantly
28 ($P<0.01$) affected most C and N variables in both soils and forest floors. The 2yrB treatment had
29 significantly lower ($P<0.01$) soil total C, total C:N ratio, microbial biomass C (MBC) and N
30 (MBN), MBC:MBN ratio, dissolved organic C (DOC) and N (DON), NO_3^- -N, inorganic N and L
31 layer total N, DON and NO_3^- -N, compared with the NB and 4yrB treatments. However, there were
32 no overall significant differences in these variables between the NB and 4yrB treatments. Sampling
33 month significantly ($P<0.01$) affected C and N variable in both soils and forest floors except for soil
34 total C and N and F layer MBC. Temporal dynamics of most of these labile C and N variables were
35 highly related to soil and forest floor moisture content, seven day mean air temperature (MAT) and
36 cumulative rainfall prior to sampling date. However, fire effects were independent of sampling
37 month, as there were no significant interactions between them for most response variables measured.
38 This study has clearly demonstrated that more frequent burning (2yr burning) had negative impact
39 on soil and forest floor C and nutrient pools. Insignificant differences observed in soil C and N and
40 forest floor N pools between less frequent burning (4yrB) and NB treatments highlighted that the
41 prescribed burning at four year interval gave sufficient time for recovery of these soil and forest
42 floor nutrients to pre-burn levels.

1. Introduction

Prescribed burning regimes can induce shifts in species composition, reduce number and average height of species (Fairfax et al., 2009; Lewis et al., 2012) and thus may alter ecosystem C and N balance over the long term. Repeated prescribed burning at different intervals may change the temporal dynamics of C and N cycling, possibly via immediate direct loss of C as CO₂ fluxes, N by volatilization and ash convection to the atmosphere (Caon et al., 2014) or indirectly by interacting with variations in rainfall and air temperature in different months which ultimately affects C fixation by vegetation, transfer of C and N from vegetation to the soil (affecting both quantity and chemistry of litters) and soil C and N cycling by changes in microbial population and activity. Variations of rainfall and temperature over different months of the year affect C and N cycling, either directly by influencing soil temperature and moisture, or indirectly by influencing plant production and uptake of water and nutrients (Rastetter et al., 2005; Borken et al., 2006). Significant relationships between soil moisture, rainfall, soil microbial biomass and soil C and N contents in forest ecosystems have been observed (Eaton, 2001; Saynes et al., 2005).

During fire, C and N are lost from the forest floor through volatilization, but the remaining N becomes more available after fire (Wan et al., 2001; Peay et al., 2009) due to thermal breakdown of proteins in the soil, yielding soluble N species such as NH₄⁺ (Moghaddas et al., 2007; Roaldson et al., 2014). With time, this available N is lost by leaching or utilized by plants and microbes and becomes less available gradually (Monleon et al., 1997). Most studies on the effects of wildfire and prescribed burning on the forest floor and soil have focused on the short-term effects following fire (Stephens and Finney, 2002; Hubbard et al., 2004; Centri, 2005; Murphy et al., 2006). Few studies have investigated the effects of repeated prescribed burning and the variation in environmental conditions with time to determine how these factors interact to shape the C and N dynamics of the forest floor and soil (Ponder et al., 2009; Zhou et al., 2009). Changing impacts of prescribed

68 burning with time of sampling are largely unknown, but are critical to understand C and N cycling
69 in forests managed with prescribed burning (Hubbard et al., 2004).

70 To explore the effects of burning regime and monthly variation, this study aimed to investigate:
71 (1) the long-term effects of prescribed fire frequency regimes on soil and forest floor C and N
72 pools; and (2) the temporal dynamics of these soil and forest floor C and N variables and
73 interactions of fire frequency and sampling month. A 39 year old prescribed burning trial in a wet
74 sclerophyll forest, with repeated burning treatments of different intervals was selected for this study.
75 It should be noted that the latest burning for burning treatments before sampling was conducted in
76 2005 (for 4 yearly burning plots) and 2007 (for 2 yearly burning plots). The time of soil and forest
77 floor sampling, 3.5 and 5.5 years after last fire event for the 2yrB treatment and 4yrB treatments,
78 respectively (6 months prior to the next fire event for both 2yrB and 4yrB treatments), was chosen
79 to minimize the fire recency's effects. Therefore, this study sought to examine the long-term
80 impacts of prescribed burning treatments on temporal dynamics of soil and forest floor C and N
81 pools. It was hypothesized that (1) prescribed burning would interact with sampling month and
82 consequently different burning regimes would affect soil and forest floor C and N pools differently
83 among different months of the year; and (2) prescribed burning would significantly affect soil and
84 forest floor C and N pools with tendency to have smallest pools under more frequent burning (2
85 yearly burning) treatment.

86

87 **2. Materials and Methods**

88

89 *2.1. Field site and experimental design*

90

91 This study used a long-term prescribed burning experimental forest site (Peachester State Forest;
92 lat. 26°50' S, long. 152°53' E) situated about 100 km north of Brisbane in the Sunshine Coast
93 hinterland, southeast Queensland, Australia, a region with a moist sub-tropical climate. The

94 vegetation at the site comprises tall native wet sclerophyll forest dominated by several species of
95 Myrtaceae, especially *Eucalyptus pilularis* (Blackbutt), with other canopy species including
96 *Corymbia intermedia* (bloodwood), *E. microcorys* (tallowwood), *E. resinifera* (red mahogany),
97 *Syncarpia glomulifera* (turpentine) and *Lophostemon confertus* (brush box). The topography is
98 undulating to rolling (2-16% slopes with an easterly aspect). The clay contents of this deep sandy
99 soil increased with soil depth, being 20-28%, 30-40% and 50%-60% in the 0-20 cm, 20-60 cm and
100 60-80% soil layers, respectively. The soil was classified as red to yellow Kandosols (Isbell, 1996)
101 that is equivalent to Alfisols in USDA soil taxonomy.

102 The prescribed burning experiment was established in 1972 and has treatments of: (1) 2 yearly
103 burning (2yrB); (2) 4 yearly burning (4yrB); and (3) no burning (NB) since 1969. Long unburnt
104 treatment had an understory of rainforest species while burnt treatments had a grassy understory
105 (Lewis et al., 2012). Prescribed fires were carried out in winter and are generally of lower intensity
106 ($<2500 \text{ kW m}^{-1}$) and light severity consuming forest floor and understory species during burning
107 but fire not spreading to the tree crown (Lewis et al., 2012; Toberman et al., 2014). The prescribed
108 burning was patchy in nature since small parts of the plot might not be burnt due to topographical
109 conditions (Toberman et al., 2014). There have been no wildfires at the site since 1969, no logging
110 since the 1950s, and no fertilizer application or other silvicultural practices have been applied since
111 the establishment of the burning experiment (Lewis et al., 2012). There are 4 replicate plots (ca.
112 0.08 ha) for each of the three treatments with a total of 12 plots and these plots are randomly
113 distributed over the experimental area with similar vegetation and soil characteristics (Lewis et al.,
114 2012).

115 The last prescribed burns prior to sampling were in 2007 in the 2yrB treatment and 2005 in the
116 4yrB treatment. Scheduled burns for 2009 were not carried out as a result of adverse weather
117 conditions (wind speed, wind direction, air temperature and relative humidity etc). As of 2011, the
118 2yrB treatment had been successfully burnt 17 times and the 4yrB treatment had been burnt 8 times.

119

120 2.2. Rainfall and temperature.

121

122 The whole one year in southeast, Queensland (Australia) is categorized into December-February
123 (summer, average temperature 21-30 °C), March-May (autumn, 15-25 °C), June-August (winter,
124 11-21 °C), September-November (spring, 15-25 °C). We acknowledge that the six month sampling
125 only covers three seasons (summer, autumn and winter) and is unlikely to take into account all of
126 the seasonal variations occurring within a year. However, this choice of sampling months was made
127 in the light of minimizing fire recency's effects. February indicates late summer, March early
128 autumn, April mid-autumn, May late autumn, June early winter, and July mid-winter.

129 Meteorological data, including temperature and precipitation, were taken at Beerburrum State
130 Forest Australian Bureau of Meteorology weather station, which is in close proximity to the
131 Peachester site (approximately 14 km away). Seven day cumulative rainfall (CR) and mean
132 atmospheric temperature (MAT) were calculated for the period prior to each sampling. These
133 parameters may reflect the immediate effects of climatic conditions on the C and N cycling
134 processes in the soil and forest floors.

135

136 2.3. Soil, L and F layer sampling

137

138 Soil, L and F layer samples were collected monthly from the plots at Peachester site over a
139 period from February to July 2011. Soil and forest floor samples were taken on six occasions prior
140 to the next burning event (August 2011) for all three fire treatments (no burning, 2yrB and 4yrB
141 treatments): 9th February, 9th March, 12nd April, 16th May, 15th June and 12nd July. This would
142 minimise the effects of fire recency (e.g. 3.5 and 5.5 years after last fire event for the 2yrB
143 treatment and 4yrB treatments, respectively, as scheduled burns for 2009 were not carried out as a
144 result of adverse weather conditions). This enabled the study to primarily look into the long-term
145 effects of prescribed fire and their interactions with the sampling month. At each sampling time,

146 fifteen surface soil cores (0-10 cm) (Hopmans et al., 2005; Wilson et al., 2002) were collected
147 randomly from each treatment plot using a 7 cm in diameter corer and bulked to produce one
148 composite sample. Field moist soil was sieved (<2 mm) immediately after sampling and stored at
149 field moisture contents in plastic bags at 4°C prior to measurements of microbial biomass C (MBC)
150 and N (MBN) and dissolved organic C (DOC) and N (DON) and inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$). A
151 subsample was dried at 105 °C for 24 h, to determine gravimetric soil moisture and all results were
152 expressed on a percentage oven-dry basis. Another subsample was air dried, and finely ground
153 (<150 µm) prior to determination of soil total C and N.

154 Forest floor materials (L and F layers) were randomly sampled from four different points (same
155 points used for soil sampling later) within each plot using a quadrat (25 ×25 cm) and bulked to one
156 composite sample. A subsample was cut into small square pieces (ca. 1 × 1 cm) with scissors,
157 thoroughly mixed and stored in plastic bags at 4 °C, for the measurements of MBC, MBN, DOC,
158 DON and inorganic N ($\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$). Another subsample of the forest floor material was oven
159 dried at 65 °C for one week for determination of moisture content. This subsample was then finely
160 ground (<150 µm) for the determination of total C and N. All results were expressed on an oven dry
161 basis.

162

163 *2.4. Soil and forest floor analysis*

164

165 Total C and N in soil and forest floor samples were measured using an isotope ratio mass
166 spectrometer with an Eurovector Elemental Analyser (Isoprime-EuroEA 3000, Milan, Italy). Soil
167 and forest floor MBC and MBN were determined using fumigation-extraction method on the fresh
168 samples as described by Chen et al. (2003b). A Shimadzu TOC- VCPH/CPN analyzer (fitted with total
169 N unit) was used to determine DOC and total soluble N (TSN) in the fumigated and non-fumigated
170 samples (Chen et al. 2005). The initial C and N results obtained for non-fumigated samples were
171 used as DOC and TSN, while differences in concentrations of DOC and TSN between the

172 fumigated and non-fumigated samples were used to calculate MBC and MBN respectively.
173 Concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in non-fumigated samples (0.5M K_2SO_4 extracts) of soil and
174 forest floors were used as indicators of inorganic N and measured using a Discrete Chemical
175 Analyser (DCA). The difference between TSN and inorganic N was calculated as DON.

176

177 *2.5. Statistical analysis*

178

179 Two-way analysis of variance (ANOVA) with repeated measures was used to determine the
180 effects of fire treatment, sampling month and their interaction on the different soil and forest floor
181 variables measured. The normality of all data and homogeneity of variance was checked and met
182 before ANOVA. Where the interaction term was not significant, further one-way ANOVA was used
183 to evaluate the effect of fire treatment at each sampling time. Where the effect of fire treatment was
184 significant in any month, the differences among different burning treatments were determined,
185 using a Tukey HSD test to separate means at the 5% level. Pearson correlation analysis was
186 conducted to determine the relationships between soil, forest floor and environmental variables,
187 using all data over six months. Statistical analyses were performed using Statistix for Window,
188 version 8 (Analytical Software, Tallahassee, FL, USA). All graphs were made using the Sigma Plot
189 version 10 (Jandel Scientific).

190

191 **3. Results**

192

193 *3.1. Air temperature, rainfall and soil and forest floor moisture*

194

195 Mean atmospheric temperature (MAT) declined from February to July (Fig. 1a). Maximum
196 MAT (26°C) was recorded in February and the maximum CR (65.4 mm) was recorded in March,
197 while minimum MAT (12°C) and CR (0 mm) were recorded in July (Fig. 1a). Prescribed burning

significantly affected soil moisture but had no effect on forest floor moisture (Fig. 1 and Table 1). Soil moisture was significantly lower in the 2yrB treatment relative to the NB and 4yrB treatments. Generally the forest floor also had lower moisture in the 2yrB treatment relative to the NB and 4yrB treatments but differences were not statistically significant. There was significant monthly variation in soil and forest floor moisture contents (Fig. 1 and Table 1). Variations in moisture contents were similar over time in all fire treatments in all layers, which coincided well with the fluctuations in 7-day accumulative rainfall prior to sampling (Fig. 1a). Fluctuations in moisture contents across the sampling months were greatest for the L layer and least for the soil (Fig. 1b, d).

206

207 *3.2. Dynamics of soil and forest floor total C, N and C:N ratio*

208

Prescribed burning significantly affected total C, N and C:N ratio in soil, L and F layers with the exception of F layer total C (Fig. 2 and Table 1). The 2yrB treatment had lower soil total C (35-54 g kg⁻¹) and N (1.27-1.63 g kg⁻¹) compared with the NB (total C 60-70 g kg⁻¹; total N 2.77-3.08 g kg⁻¹) and 4yrB (61-75 g kg⁻¹; total N 2.66-3.37 g kg⁻¹) treatments across all sampling times, while the 4yrB and NB treatments did not differ significantly (Fig. 2g). For the L layer the 2yrB treatment had higher total C (539-566 g kg⁻¹) compared with the NB (528-566 g kg⁻¹) treatment and lower total N (5.37-6.47 g kg⁻¹) compared with both the 4yrB (6.53-7.23 g kg⁻¹) and NB (6.46-7.81 g kg⁻¹) treatments, while no significant differences were detected between the 4yrB and NB treatments (Fig. 2a, b). The 2yrB treatment had a lower F layer total N content (5.09-6.36 g kg⁻¹) than the 4yrB (6.33-7.48 g kg⁻¹) and NB (6.59-7.94 g kg⁻¹) treatments, while no significant differences were detected between 4yrB and NB treatments across all times (Fig. 2e). The 2yrB treatment had a higher total C:N ratio in the L layer (85-100), F layer (61-75) and soil (27-33) relative to the other two treatments (Fig. 2c, f, i).

There was significant monthly variation in total C, N and C:N ratio in the L and F layers but no monthly effect on these parameters in soil (Fig. 2 and Table 1). Total C, N and C:N ratios were higher in the forest floor components than in the soil (Fig. 2a, b, c, d, e, f, g, h, i). Interactions

225 between prescribed burning and sampling month were significant only for L layer total C and soil
226 total C:N ratio (Fig. 2a, i and Table 1). Soil total C:N ratio varied more in the 2yrB treatment, with
227 a peak in early summer, but showed less fluctuation in 4yrB and NB treatments across all months
228 (Fig. 2i).

230 3.3. Dynamics of soil and forest floor DOC, DON and DOC:DON ratio

232 Prescribed burning significantly affected soil DOC, soil and L layer DON and L layer
233 DOC:DON ratio, but treatment effects were not significant in the F layer (Fig. 3 and Table 2). The
234 2yrB treatment had lower soil DOC and DON concentrations (24-87 and 0-15 mg kg⁻¹ respectively)
235 compared with both the 4yrB (88-147 and 0.60-21 mg kg⁻¹ respectively) and NB (99-185 and 0.40-
236 19 mg kg⁻¹ respectively) treatments (Fig. 3g, h), while no significant differences were detected
237 between the 4yrB and NB treatments. Across all times, L layer DON was lower in the 2yrB
238 treatment (35-138 mg kg⁻¹) compared with the NB (52-193 mg kg⁻¹) treatment (Fig. 3b), while no
239 significant differences were detected between the 4yrB and NB or the 4yrB and 2yrB treatments.

240 Month of sampling significantly affected soil, L and F layer DOC, DON and DOC:DON ratio
241 (Fig. 3 and Table 2). Soil DOC and DON significantly declined in all fire treatments in May (Fig.
242 3g, h). Two peaks were observed for L layer DOC in April and June (Fig. 3a). The F layer DOC
243 and DON also showed minimum values in May (Fig. 3d, e). The interaction between prescribed
244 burning and sampling month was significant only for soil DOC:DON ratio (Fig. 3i and Table 2).
245 Overall the L layer had highest values for DOC and DON followed by the F layer and lowest values
246 were observed in soil. Overall, the soil and forest floor dissolved pools were positively correlated
247 with their respective moisture contents (Table A2). The DON in all layers was positively correlated
248 with MAT (Table A2), while no such relationships were observed for DOC.

250 3.4. Dynamics of soil and forest floor MBC, MBN and MBC:MBN ratio

251

252 Prescribed burning significantly affected soil MBC and L layer MBC:MBN ratio but did not
253 influence any parameters in the F layer (Fig. 4 and Table 3). The 2yrB treatment had a lower soil
254 MBC (264-407 mg kg⁻¹) than both the 4yrB (422-715 mg kg⁻¹) and NB (422-672 mg kg⁻¹)
255 treatments (Fig. 4g), while no significant differences were detected between 4yrB and NB
256 treatments. The L layer MBC:MBN ratio was significantly higher in the 2yrB treatment relative to
257 the NB treatment (Fig. 4c).

258 Monthly variations were significant for all MBC and MBN variables with the exception of MBC
259 in the F layer (Table 3, Fig. 4). The L layer MBC and MBN peaked in June (Fig. 4a, b). Monthly
260 variations were generally greatest in the L and F layers, compared to the soil MBC, MBN and
261 MBC:MBN (Fig. 4). By comparing the three layers, all microbial parameters had highest values in
262 the L layer followed by the F layer and the soil. There was a significant interaction between
263 prescribed fire and time of sampling for F layer MBN (Table 3). Both soil and L layer microbial
264 pools were positively correlated with their respective moisture contents, while L layer MBC and
265 MBN was also positively correlated with CR (Table A2). The forest floor MBC and MBN were
266 generally positively related to MAT (Table A2), while soil MBC was negatively related to MAT.

267

268 3.5. Dynamics of soil and forest floor NO₃⁻-N, NH₄⁺-N and inorganic N

269

270 Prescribed burning significantly affected NO₃⁻-N in both the soil and L layer and influenced
271 inorganic N in the soil (Fig. 5 and Table 4). The 2yrB treatment had lower concentrations of soil
272 NO₃⁻-N compared with the NB treatment and lower concentrations of L layer NO₃⁻-N compared
273 with both the 4yrB and NB treatments across all sampling times, while no significant differences
274 were detected between 4yrB and NB treatments (Fig. 5b, h). Prescribed burning tends to decrease
275 concentrations of soil NH₄⁺-N compared with the NB treatment, but the difference was not
276 statistically significant (Table 5, Fig. 4g).

277 Soil, L and F layer NO_3^- -N, NH_4^+ -N and inorganic N varied significantly between months (Fig. 5
278 and Table 4). Overall, soil NO_3^- -N, NH_4^+ -N and inorganic N declined with sampling time from late
279 summer (Feb) to winter (Jul) across all different burning treatments (Figs. 5 g, h, i). Concentrations
280 of NO_3^- -N for soil in the 2yrB treatment showed less variation over time (Fig. 5h). When comparing
281 between layers NO_3^- -N and inorganic N were the highest in the soil and the lowest in the L layer.
282 There were significant interactions between sampling month and fire treatments for soil and L layer
283 NO_3^- -N (Table 4). Soil NH_4^+ -N and NO_3^- -N were positively correlated with soil moisture (Table
284 A2). The forest floor NO_3^- -N was negatively correlated with MAT, while forest floor NH_4^+ -N was
285 negatively correlated with CR (Table A2).

286

287 **4.4. Discussion**

288

289 *4.1. Comparison of soil and forest floor total C and N fluctuations*

290

291 The significantly lower levels of soil total C, N and significantly higher total C:N ratio in the
292 2yrB treatment compared with the 4yrB and NB treatments (Fig. 2g, h, i) are aligned well with the
293 results of previous studies at the same Peachester site (Guinto et al., 2001; Artz et al., 2009; Liu et
294 al., 2013; Toberman et al., 2014) and in a ponderosa pine forest (Wright and Hart, 1997). In contrast,
295 Murphy et al. (2006) in a Sierra Nevada forest after a single prescribed fire and Neill et al. (2007) in
296 a Cape Cod oak-pine forest over a 12 year period found that annual burning had no significant
297 effect on mineral soil total C and N. The discrepancy in the fire effects on soil and forest floor C
298 and N dynamics may be related to the fire frequency (Murphy et al. 2006) and forest type (Neil et al.
299 2007). Total C and N contents in soil varied little and similarly from late summer to mid-winter
300 (Fig. 4.2g, h). Similar findings were given by Chen et al. (2003a) for a forest in New Zealand for a
301 study period from July 1998 to July 1999.

302 Similar to our results, no significant effect of fire on F layer total C was reported by Hubbard et
303 al. (2004). Although, significant increase observed for L layer total C in the 2yrB treatment in the

304 present study is in contrast with results given by Hubbard et al. (2004), who reported that burning
305 significantly reduced C in the litter layer. Major N reductions in the forest floor pools can be
306 attributed to N losses via volatilization during fire. Lower forest floor total N contents as a result of
307 more frequent prescribed burning (2yrB treatment) are consistent with previous studies at the same
308 site (Guinto et al., 2001) and at other sites (Bell and Binkley, 1989; Hernandez and Hobbie, 2008).
309 Williams et al. (2012) reported similar findings in that biennial burning reduced litter N by 21% and
310 increased litter C:N ratio by 28% compared to the unburned controls in an upland oak forest.
311 Increased C:N ratios indicating slower rates of decomposition in litters produced in frequently burnt
312 sites may reduce N availability in forest ecosystems, further decelerating rates of N cycling (Wright
313 and Hart, 1997; Hernandez and Hobbie, 2008). In May, the 2yrB treatment had lower F layer total
314 C and N (Fig. 2d, e). This is well aligned with sudden decline in MAT and CR (Fig. 1a) and
315 moisture contents in both L and F layers in the 2yrB treatments (Fig. 1b, c) in late autumn (May)
316 leading to reduced microbial population, decomposition rate and dissolved organic C and N (Figs.
317 3a, b; 4d, e) leached from litter layer to underlying F layer in May. Forest floor total C, N and C:N
318 ratio were higher than in the underlying mineral soil and the F layer total C and N showed relatively
319 greater seasonal fluctuations than the L layer and soil layer (Fig. 2). Chen et al. (2003a) similarly
320 reported no significant seasonal variations in total C and N for the L layer but significant seasonal
321 variation in the F layer in a forest in New Zealand.

322

323 *4.2. Comparison of soil and forest floor labile organic C and N fluctuations*

324

325 The lower labile C and N concentrations (DOC, DON, MBC, MBN) observed in soil for the
326 2yrB treatment across sampling times are in accordance with the soil total C and N results and are
327 consistent with previous finding at the same site (Liu et al., 2013). As litter and humus layers are
328 the most important dissolved nutrient sources in soil and the release of dissolved C, N from forest
329 floors depends on substrate quality (e.g. C:N ratio) (Kalbitz et al., 2000). It is speculated that high

330 forest floor C:N ratios in the 2yrB treatment in this study could be responsible for lower release of
331 N in solution compared with other less frequent fire treatments. This was clearly evident from the
332 lower DON levels in the forest floor pools in the 2yrB treatment. This was further confirmed by
333 positive correlations found among soil and L layer DOC, DON, MBC and MBN (Table 5). The
334 similar levels of labile C and N for the 4yrB and NB treatments suggests ecosystem C and N
335 recovery during the longer fire-return interval, while minimizing the effects of fire recency
336 (maximum time since last burn in 4yrB treatment in 2005).

337 Monthly variation in labile C and N is important due to its influence on decomposition, nutrient
338 availability and ultimately plant nutrition (Schmidt et al., 2007). The gradual decrease in soil DON
339 from late summer to early winter may be related to less microbial activity at low temperatures,
340 resulting in lower decomposition and release of N in solution. This is supported by the positive
341 correlation between soil DON and MAT ($P < 0.05$; Table A2). Soil MBC tended to be slightly
342 higher in winter (May, June) than in summer and there was a significant negative correlation
343 ($P < 0.05$; Table A2) between soil MBC and MAT. This is consistent with previous findings (Chen
344 et al., 2003b). Both soil MBC and MBN followed the temporal patterns in soil MC and there were
345 highly significant correlations ($P < 0.01$; Table A2) between soil MBC, MBN and soil MC indicating
346 the dependency of soil microbes on soil moisture. The DOC in forest floors followed their
347 respective MC patterns over time and significant correlations were found between L and F layer
348 DOC and their respective MC ($P < 0.05$; Table A2). The high F layer MBC in May may be related
349 to low temperature, low microbial activity and immobilization of C in microbe bodies. These results
350 show direct or indirect regulation of forest floor MBC and MBN through dissolved nutrients,
351 brought about by a combination of environmental factors (MC, CR and MAT).

352 The low soil labile C and N ratio (DOC:DON, MBC:MBN) in the 2yrB may be the result of N
353 immobilization which occurred in response to high total soil C:N ratios in the 2yrB treatment (Xie
354 and Steinberger., 2001) indicating slow decomposition and release of nutrients. The soil
355 MBC:MBN ratio in the present study (average of 6.8 over all sampling times) is within the

MBC:MBN ratio range reported by Martikainen and Palojarvi (1990) for various forest soils (6-9). The higher MBC:MBN ratios in L layer in the 2yrB treatment, is in agreement with results from a litter bag study reported by Toberman et al. (2014) at the same site. But in contrast to our results, they reported that there was no significant burning treatment effect on litter DOC:DON ratios. This difference can be attributed to the fact that the forest floor was being renewed every month in the present study by freshly fallen litter fall, but this was not the case in the litterbag study of Toberman et al. (2014). The fluctuation in labile C and N ratios across sampling times for both soils and forest floors were driven by labile N rather than labile C. Yang et al. (2010) also found that C and N ratio increases or decreases, with trends dependent on changes in system N.

4.3. Dynamics of inorganic nitrogen

Although prescribed burning tended to decrease concentrations of soil NH_4^+ -N compared with the NB treatment, but the difference was not statistically significant after a long-term fire treatment (Table 5, Fig. 4g). This is consistent with other studies (Murphy et al., 2006; Liu et al., 2013; William et al., 2012). The lower soil NO_3^- -N levels in the 2yrB treatment compared with the NB and 4yrB treatments are consistent with the lower total N and DON in the 2yrB treatment. These results are also confirmed by the finding of lower mineralisable N in the 2yrB treatment at the same site (Guinto et al., 2000). High C:N ratio and low decomposition rate in the 2yrB treatment could be a reason for the lower mineralization. The main mechanism for loss of NO_3^- -N in the 2yrB is likely to be leaching (Monleon et al., 1997) rather than denitrification, as significantly lower N_2O fluxes were reported in the 2yrB treatment plots compared with the 4yrB and NB treatments at this site (Liu et al., 2013). Significant effect of prescribed fire on soil inorganic N with the lowest concentration in the 2yrB treatment is contradictory with Murphy et al. (2006) who found no significant effect of prescribed fire on soil inorganic N. Moreover, Wan et al. (2001) found increases in soil NH_4^+ -N and NO_3^- -N after prescribed fire, which may be attributed to immediate

382 fire effects. Generally lower concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and inorganic-N observed in F
383 layer in the 2yrB treatment are consistent with the other N variables results in the 2yrB treatment.

384 Highly significant correlations between soil inorganic N and MAT suggests that atmospheric
385 temperature was also responsible for temporal fluctuations in soil inorganic N concentrations (Table
386 A2). Temperature dependency of inorganic N is understandable as microbes drive mineralization.
387 The microbes carrying mineralization get energy from dissolved nutrients pool. In this study a trend
388 of decreasing soil dissolved organic N (Fig. 3h) coupled with low temperatures (Fig. 1a) may be the
389 reason for decreasing soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ and inorganic N in winter. Huang and Schoenau
390 (1998) also reported high mineralization in summer, which can raise inorganic N. This assumption
391 was further supported by highly significant correlations ($P<0.01$; Table A1) between soil DOC and
392 soil $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. The forest floor $\text{NO}_3^-\text{-N}$ below detection limit (Fig. 5b, e) could be due to
393 immobilization of $\text{NO}_3^-\text{-N}$ by forest floor microbes as indicated in previous studies (e.g. Qualls,
394 1991). Excessive leaching of $\text{NO}_3^-\text{-N}$ through the soil from the L and F layers in months receiving
395 high CR (Fig. 1a) prior to sampling could be another reason. This result is further supported by
396 significant negative correlations between forest floor $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and CR (Table A2). The
397 decrease in F layer $\text{NH}_4^+\text{-N}$ and inorganic N from February to lowest levels in June could be related
398 to decreasing temperature (Fig. 1a, Fig. 5d, f).

399 As discussed above, differences in soil C and N pools among the fire treatments were observed
400 at the 0-10 cm depth in present study (Figs. 2-5, Tables 1-4), while other studies at the same site the
401 impacts of fire could be observed at the 10-20 cm depth, but to a lesser extent (e.g. Liu et al. 2013).
402 Some other studies have also used this sampling depth (0-10 cm) to investigate the effects of fires
403 on soil properties (Wilson et al., 2002; Hopmans et al., 2005; Lewis et al., 2012; Hari Ram
404 Shrestha, 2015). However, Badia-Villas et al. (2014) found that direct effects of prescribed burning
405 were often limited to the top 1-2 cm soil layer. This may be due to an accumulative effects of
406 repeated prescribed fire on soils in this long-term fire study. On the other hand, Badia-Villas et al.
407 (2014) studied only the fire-induced changes in soil properties related to soil erodibility as water

408 repellency, aggregation and organic matter of a mollic horizon and the burning was carried out
409 under the laboratory condition. These may contribute to the different responses of soil properties
410 from different depths to fire.

411

412 **5. Conclusions**

413

414 Fire effects were independent of sampling month as there were no significant interactions
415 between them for most response variables. Thus the first hypothesis was unsupported. In agreement
416 with the second hypothesis, the 2yrB treatment generally resulted in lowest moisture contents and
417 both C and N in the soil and lowest N in the forest floor. Overall results suggested that total C and
418 N in all burning treatments showed less temporal variations relative to other C and N pools in both
419 soils and forest floors. Along with CR and MC, temperature played an additional role in the sharp
420 fluctuations in dissolved organic C and N, microbial biomass C and N and inorganic N pools in
421 both soils and forest floors. Burning every two years depleted the forest floor N and surface mineral
422 soil C and N pools. It is concluded that more frequent burning might not allow sufficient time for
423 soils and forest floors to fully recover between burns. The results strongly suggested that tradeoffs
424 between management goals and C and N storage should be carefully considered. Future studies
425 should take into account potential changes in annual net primary productivity resulting from plant
426 community changes induced by different prescribed burning regimes that could contribute to soil
427 organic matter.

428

429 **Acknowledgments**

430

431 We would like to thank Marijka Heenan, for her assistance in the field sampling. We would also
432 like to thank the Department of Agriculture, Forestry and Fishery, Queensland for permission to

433 access to the Peachester fire experimental site. This research was supported under the Australian
434 Research Council's Future Fellowship funding scheme (project number FT0990547).

435

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550

Response to Reviewers' Comments

Reviewer #1: Dear authors,

While you have, in my opinion, now substantially improved the paper, I would like to insist on the inclusion of some more information about the site. e.g. the soil information is minimal ("with clay content" means how much clay?), nothing is said about slope angle (while that could be important in terms of post-burning losses of sediment, organic matter, carbon and nitrogen by runoff), and the location of the plots is not specified (or shown on a map). Also, I believe that it would be good if you could add some more information about the severity of the prescribed burns, at least of the last ones.

Responses: Thanks for the further comments. We have added all information about the site as requested (see below) (also see lines 90-92, 96-99, 104-106);

Lines 90-92: This study used a long-term prescribed burning experimental forest site (Peachester State Forest; lat. 26°50' S, long. 152°53' E) situated about 100 km north of Brisbane in the Sunshine Coast hinterland, southeast Queensland, Australia,

Lines 96-99: The topography is undulating to rolling (2-16% slopes with an easterly aspect). The clay contents of this deep sandy soil increased with soil depth, being 20-28%, 30-40% and 50%-60% in the 0-20 cm, 20-60 cm and 60-80% soil layers, respectively.

Lines 104-106: Prescribed fires are carried out in winter and are generally of lower intensity ($<2500 \text{ kW m}^{-1}$) and light severity consuming forest floor and understory species during burning but fire not spreading to the tree crown.

Also, I would like to suggest the order of the 3 sampling layers in Figs 2 to 4 to the order used in Fig. 1, i.e. L, F, S, for the obvious reason.

Response: We have changed the order of the 3 sampling layers in Figs. 2-5 and Tables 1-4 and revised the text and the figure captions accordingly;

Furthermore, I would appreciate that you add some discussion on the fact that you find differences at 0-10 cm while direct effects of prescribed burning are often limited to the top 1-2 cm's (see papers by Badia and colleagues).

Responses: We have added a session discuss this at the end of Discussion (see lines 396-405) (see below as well).

Lines 396-405: As discussed above, differences in soil C and N pools among the fire treatments were observed at the 0-10 cm depth in present study (Figs. 2-5, Tables 1-4), while other studies at the same site the impacts of fire could be observed at the 10-20 cm depth, but to a lesser extent (e.g. Liu et al. 2013). Some other studies have also used this sampling depth (0-10 cm) to investigate the effects of fires on soil properties (Wilson et al., 2002; Hopmans et al., 2005; Lewis et al., 2012; Hari Ram Shrestha, 2015). However, Badia-Villas et al. (2014) found that direct effects of prescribed burning were often limited to the top 1-2 cm soil layer. This may be due to an accumulative effects of repeated prescribed fire on soils in this long-term fire study. On the other hand, Badia-Villas et al. (2014) studied only the fire-induced changes in soil properties related to soil erodibility as water repellency, aggregation and organic matter of a mollic horizon and the fire was carried out under the laboratory condition.

Finally, I believe that it would be good to carefully check the English of the ms.

Responses: We have carefully checked through the English grammar of the manuscript and corrected where needed.

Reviewer #2: Will need some editing by technical editor for grammar, but I am recommending acceptance on a scientific basis.

Responses: Thank you for your comments and recommendation. We have carefully checked through the grammar.

Temporal dynamics of carbon and nitrogen in the surface soil and forest floor under different prescribed burning regimes

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Highlights

- ✓ Temporal dynamics of forest soil C and N under different fire regimes were studied;
- ✓ All fire frequency treatments show similar temporal patterns for total C and N;
- ✓ Rainfall and temperature govern the temporal dynamics of labile organic C and N;
- ✓ High frequent fire (2 year interval) depletes the forest floor N and soil C and N.

Table 1

F values from Two-Way ANOVA (with repeated measures) of the effects of prescribed burning (B), sampling month (S) and their interaction (B×S) on litter, fermentation and soil layer moisture content, total C, N and C:N ratio over the period February 2011-July 2011 in a wet sclerophyll forest, southeast Queensland.

Factor	MC _L	TC _L	TN _L	C:N _L	MC _F	TC _F	TN _F	C:N _F	MC _S	TC _S	TN _S	C:N _S
B	3.15	7.77 ^{**}	13.50 ^{**}	17.41 ^{***}	2.53	1.45	11.37 ^{**}	20.12 ^{***}	12.09 ^{**}	11.89 ^{**}	16.84 ^{***}	23.22 ^{***}
S	54.84 ^{***}	5.13 ^{***}	3.38 ^{**}	3.87 ^{**}	13.38 ^{***}	8.09 ^{***}	6.78 ^{***}	8.34 ^{***}	6.35 ^{***}	1.78	1.39	1.80
B×S	1.80	3.00 ^{**}	0.85	0.89	0.75	1.61	1.45	1.31	0.61	0.88	0.65	2.20 [*]

Abbreviations: MC_L, MC_F and MC_S indicate moisture content for litter, fermentation and soil layers, respectively; TC_L, TC_F and TC_S indicate total carbon for litter, fermentation and soil layers, respectively; TN_L, TN_F and TN_S indicate total nitrogen for litter, fermentation and soil layers, respectively; C:N_L, C:N_F and C:N_S indicate total C and N ratio for litter, fermentation and soil layers, respectively. Bold values with *, ** and *** indicate significant differences at P<0.05, P<0.01 and P<0.001, respectively.

Table 2

F values from Two-Way ANOVA (with repeated measures) of the effects of prescribed burning (B), sampling month (S) and their interaction (B×S) on litter, fermentation and soil layer DOC, DON and DOC:DON ratio over the period February 2011-July 2011 in a wet sclerophyll forest, southeast Queensland.

Factor	DOC _L	DON _L	DOC:N _L	DOC _F	DON _F	DOC:N _F	DOC _S	DON _S	DOC:N _S
B	0.93	9.43 ^{**}	11.14 ^{**}	0.85	3.93	3.23	62.50 ^{***}	12.88 ^{**}	3.37
S	15.12 ^{**}	56.24 ^{***}	61.30 ^{***}	5.89 ^{***}	76.79 ^{***}	73.91 ^{***}	25.58 ^{***}	80.77 ^{***}	3.27 ^{**}
B×S	0.37	1.2	0.37	0.87	1.07	1.69	0.75	1.53	3.42 ^{**}

Abbreviations: DOC_L, DOC_F and DOC_S indicate dissolved organic carbon for litter, fermentation and soil layers, respectively; DON_L, DON_F and DON_S indicate dissolved organic nitrogen for litter, fermentation and soil layers, respectively; DOC:N_L, DOC:N_F and DOC:N_S indicate dissolved organic C and N ratio for litter, fermentation and soil layers, respectively.

Bold values with *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Table 3

F values from Two-Way ANOVA (with repeated measures) of the effects of prescribed burning (B), sampling month (S) and their interaction (B×S) on litter, fermentation and soil layer MBC, MBN and MBC:MBN ratio over the period February 2011-July 2011 in a wet sclerophyll forest, southeast Queensland.

Factor	MBC _L	MBN _L	MBC:N _L	MBC _F	MBN _F	MBC:N _F	MBC _S	MBN _S	MBC:N _S
B	0.22	1.25	7.62 ^{**}	0.35	0.6	0.04	5.77 [*]	3.09	4.05
S	63.98 ^{***}	51.32 ^{***}	11.13 ^{***}	1.64	13.70 ^{***}	27.05 ^{***}	8.47 ^{***}	5.27 ^{***}	9.92 ^{***}
B×S	1.53	0.56	0.6	1.01	2.08 [*]	1.17	0.6	0.87	0.77

Abbreviations: MBC_L, MBC_F and MBC_S indicate microbial biomass carbon for litter, fermentation and soil layers, respectively; MBN_L, MBN_F and MBN_S indicate microbial biomass nitrogen for litter, fermentation and soil layers, respectively; MBC:N_L, MBC:N_F and MBC:N_S indicate microbial biomass C and N ratio for litter, fermentation and soil layers, respectively.

Bold values *, ** and *** indicate significant differences at P<0.05, P<0.01 and P<0.001, respectively.

Table 4

F values from Two-Way ANOVA (with repeated measures) of the effects of prescribed burning (B), month (S) and their interaction (B×S) on litter, fermentation and soil layer NH_4^+ -N, NO_3^- -N and inorganic-N over the period February 2011-July 2011 in a wet sclerophyll forest, southeast Queensland.

Factor	NO_3^- -N _L	NH_4^+ -N _L	Inorganic-N _L	NO_3^- -N _F	NH_4^+ -N _F	Inorganic-N _F	NO_3^- -N _S	NH_4^+ -N _S	Inorganic-N _S
B	29.05 ***	0.67	0.74	3.28	3.54	3.25	11.29 **	2.35	15.69 ***
S	36.35 ***	21.37 ***	20.79 ***	10.59 ***	5.24 ***	5.24 ***	15.32 ***	5.34 ***	13.04 ***
B×S	9.42 ***	0.27	0.32	1.28	0.69	0.73	3.10 **	0.57	0.84

Abbreviations: NO_3^- -N_L, NO_3^- -N_F and NO_3^- -N_S indicate nitrate nitrogen for litter, fermentation and soil layers, respectively;

NH_4^+ -N_L, NH_4^+ -N_F and NH_4^+ -N_S indicate ammonium nitrogen for litter, fermentation and soil layers, respectively.

Bold value with *, ** and *** indicate significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

1 **Figure captions**

2

3 **Fig. 1.** Seven day mean air temperature (MAT) and cumulative rainfall (CR) prior to sampling date
4 (a), and % moisture contents of the L, F layers and soil (b, c, d respectively) over the period
5 February 2011 to July 2011 in a wet sclerophyll forest, southeast Queensland. Observed mean
6 values and standard errors are shown (n=4).

7

8 **Fig. 2.** Changes in L layer (a, b, c), F layer (d, e, f) and soil (g, h, i) total C, N and C:N ratio over
9 the period February 2011 to July 2011 in a wet sclerophyll forest, southeast Queensland. Observed
10 mean values and standard errors are shown (n=4).

11

12 **Fig. 3.** Changes in L layer (a, b, c), F layer (d, e, f), and soil (g, h, i) dissolved organic C (DOC), N
13 (DON) and C:N ratio (DOC:DON) over the period February 2011 to July 2011 in a wet sclerophyll
14 forest, southeast Queensland. Observed mean values and standard errors are shown (n=4).

15

16 **Fig. 4.** Changes in L layer (a, b, c), F layer (d, e, f) and soil (g, h, i) microbial biomass C (MBC), N
17 (MBN) and microbial biomass C:N ratio (MBC:MBN) over the period February 2011 to July 2011
18 in a wet sclerophyll forest, southeast Queensland. Observed mean values and standard errors are
19 shown (n=4).

20

21 **Fig. 5.** Changes in L layer (a, b, c), F layer (d, e, f) and soil (g, h, i) $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and inorganic
22 N over the period February 2011 to July 2011 in a wet sclerophyll forest, southeast Queensland.
23 Observed mean values and standard errors are shown (n=4).

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5