High-frequency fire alters C : N : P stoichiometry in forest litter

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Abstract

Fire is a major driver of ecosystem change and can disproportionately affect the cycling of different nutrients. Thus, a stoichiometric approach to investigate the relationships between nutrient availability and microbial resource use during decomposition is likely to provide insight into the effects of fire on ecosystem functioning. We conducted a field litter bag experiment to investigate the long-term impact of repeated fire on the stoichiometry of leaf litter C, N and P pools, and nutrient-acquiring enzyme activities during decomposition in a wet sclerophyll eucalypt forest in Queensland, Australia. Fire frequency treatments have been maintained since 1972, including burning every 2 years (2yrB), burning every 4 years (4yrB) and no burning (NB). C : N ratios in freshly fallen litter were 29–42% higher and C : P ratios were 6–25% lower for 2yrB than NB during decomposition, with correspondingly lower 2yrB N : P ratios (27–32) than for NB (34–49). Trends in litter soluble and microbial N : P ratios were similar to the overall litter N : P ratios across fire treatments. Consistent with these, the ratio of activities for N-acquiring to P-acquiring enzymes in litter was higher for 2yrB than NB, whereas 4yrB was generally intermediate between 2yrB and NB. Decomposition rates of freshly fallen litter were significantly lower for 2yrB (72 ± 2% mass remaining at the end of experiment) than for 4yrB (59 ± 3%) and NB (62 ± 3%), a difference that may be related to effects of N limitation, lower moisture content, and/or litter C quality. Results for older mixed-age litter were similar to those for freshly fallen litter although treatment differences were less pronounced. Overall, these findings show that frequent fire (2yrB) decoupled N and P cycling, as manifested in litter C : N : P stoichiometry and in microbial biomass N : P ratio and enzymatic activities. Furthermore, these data indicate that fire induced a transient shift to N-limited ecosystem conditions during the postfire recovery phase.

Keywords: enzyme activity, litter decomposition, microbial biomass, nitrogen limitation, phosphorus limitation, prescribed fires, stoichiometry

Received 3 August 2012 and accepted 17 September 2013

Introduction

Fire is a major driver of ecosystem change across many regions worldwide (Whelan, 1995; Schultz *et al.*, 2008). It is predicted that wildfire frequency and intensity will increase under the current changing climate (Westerling *et al.*, 2006) alongside continued widespread use of prescribed burning as a management tool to reduce fuel loads and subsequently reduce the risk of wildfire occurrence and intensity. Therefore, understanding fire impacts upon ecosystem functioning is critical for predicting fire-related ecosystem interactions with climate process as well as for guiding the development of sustainable fire management practises.

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The nature of nutrient cycling pathways through ecosystem compartments is a major determinant of ecosystem status (DeAngelis, 1980; Barot *et al.*, 2007). The interrelationships among carbon (C), nitrogen (N) and phosphorus (P) are of particular importance because primary production and all ensuing ecosystem pathways are frequently limited by N and/or P (Vitousek & Howarth, 1991; Elser *et al.*, 2007) and because C : N : P stoichiometric ratios can influence important ecosystem processes such as detrital turnover and trophic dynamics in food webs (Sterner & Elser, 2002). Thus, because fire has marked and often disproportionate impacts on C, N and P pools, repeated fire may substantially affect ecosystem nutrient status.

Fire results in volatile losses of C, N, and to a lesser extent P (Certini, 2005; Schultz *et al.*, 2008), chemical modification in remaining compounds (González-Péreza *et al.*, 2004; Certini, 2005; Knicker, 2007), and release of inorganic N and P (Cade-Menun *et al.*, 2000;

Prieto-Fernández *et al.*, 2004). Ash and charred particulate run-off may also drive nutrient loss (Bayley *et al.*, 1992; Lane *et al.*, 2008). In turn, fire frequency will determine the degree to which ecosystem processes are able to restore N and labile P losses, with more frequent fire increasing the likelihood of long-term nutrient decline. Numerous studies have reported the depletion of soil N with long-term repeated forest burning (Wright & Hart, 1997; Newland and DeLuca, 2000; Bastias *et al.*, 2006; Hernandez & Hobbie, 2008). Consistent with this, reduced leaf litter N contents have also been reported with repeated burning (Brennan *et al.*, 2009; Williams *et al.*, 2012). Effects of repeated fire on soil P, however, have been mixed (Binkley *et al.*, 1992; Eivazi & Bayan, 1996; Guinto *et al.*, 2001). Fire can also affect other ecosystem processes. For example, shifts in the structure of fire-affected plant communities can decrease canopy cover, increasing evaporation from soil/litter layers (e.g., Neary *et al.*, 1999). This, together with fire-induced reductions in soil organic matter, can reduce soil moisture content and thus also influence soil/litter decomposition and nutrient cycling (Neary *et al.*, 1999; Guinto *et al.*, 2001). Indeed, repeated forest fire has previously been reported to lower leaf litter mass loss rates (Hernandez & Hobbie, 2008; Kay *et al.*, 2008; Brennan *et al.*, 2009), which was attributed to changes in litter N content or moisture regime. The majority of research regarding fire effects on nutrient cycling, however, has been focussed on soil, with a paucity of work regarding litter. Thus, in this study, our primary focus is on how fire frequency influences the stoichiometric coupling of C, N and P in forest litter processing by microbes, a critical control point in ecosystem nutrient cycling (Bardgett *et al.*, 2008; van Der Heijden *et al.*, 2008).

As discussed above, repeated fire may affect nutrient cycling in forests differentially for different nutrients (e.g. N, P), requiring a multidimensional framework to guide the analysis. Ecological stoichiometry seeks to understand the coupling of multiple chemical elements in ecological interactions and to evaluate how this coupling affects ecological processes (Elser *et al.*, 2000; Sterner & Elser, 2002; Allen & Gillooly, 2009). For example, large-scale global patterns in the C : N : P stoichiometry of plant biomass have been found to be associated with key environmental variables as well as with plant traits (McGroddy *et al.*, 2004; Reich & Oleksyn, 2004; Agren, 2008). Similar stoichiometric relationships have also been identified for soil microbial biomass (Cleveland & Townsend, 2006; Cleveland & Liptzin, 2007; Aponte *et al.*, 2010; Manzoni *et al.*, 2010), and, while data are scarce, it is likely that such stoichiometric coupling also plays a role for litter microbial decomposers. For example,

litter C : N : P ratios are key contributors to variability in litter mass loss (Enriquez *et al.*, 1993; Cotaeux *et al.*, 1995; Guesewell & Gessner, 2009; Zhou *et al.*, 2008) and litter nutrient mobilization (Parton *et al.*, 2007; Manzoni *et al.*, 2010). Few studies, however, have measured both the resource stoichiometry of decomposing litter and that of the decomposer microbial biomass driving decomposition so that the implications of their relationship for litter nutrient recycling can be deduced, and none have considered how fire frequency shapes these relationships.

The aim of our study was to use a long-term prescribed burning experiment in Queensland, Australia to investigate the long-term impact of repeated fire on stoichiometric relationships during litter decomposition. To our knowledge, this is the first study to use a stoichiometric approach to examine the impacts of fire on nutrient recycling in forest ecosystems via litter decomposition and among the first to consider the interrelationships between litter and microbial biomass stoichiometry as mediated by shifts in environmental nutrient supply. As noted above, we expect that fire decouples N and P cycling because fire volatilizes ecosystem N pools but leaves P largely in place for reuptake by vegetation and microbes. Indeed, previous sampling at our research site has indicated that high-frequency (once every 2 years) burned sites had lower soil N : P ratio relative to unburned and low-frequency (once every 4 years) burned sites (soil total N : P ca. 11 : 1 by mass vs. ca. 27 : 1 by mass, Chen *et al.*, 2009). Based on this reasoning and the hypothesis that soil nutrient conditions affect litter stoichiometry and microbial nutrient status, we predicted that high fire frequency would:

- alter litter C : N : P stoichiometry, lowering litter C : P and N : P ratios,
- shift the nutrient status of litter microbes toward greater N-limitation relative to P (as indexed by their C : N : P ratios and enzyme activities) and
- contribute to lower litter decomposition rate.

Materials and methods

Study site and fire treatments

This study used a long-term prescribed burning experiment located at Peachester State Forest, southeast Queensland, Australia (26°52′S, 152°51′E), a region with a subtropical climate. The vegetation at the site comprises tall forest dominated by *Eucalyptus pilularis* (Blackbutt), with other canopy species including *Corymbia intermedia*, *Eucalyptus microcorys*, *Eucalyptus resinifera*, *Syncarpia glomulifera* and *Lophostemon confertus*. Soils at the site are classified as Alfisols (USDA classification).

The prescribed burning experiment was established in 1972 and consists of the following treatments: (i) burning once every 2 years (2yrB); (ii) burning once every 4 years (4yrB) and (iii) no burning (NB). Prescribed fires are carried out in the winter and are generally of low intensity (<2500 kW m^{-1}) and are patchy in nature. There have been no wildfires, no logging (the last logging at the site took place between 1950 and 1960) and no silvicultural treatments since establishment of the experiment. There were four replicate plots (30 × 27 m) of each treatment randomly distributed over an area of the forest with similar vegetation and soil characteristics (Lewis *et al.*, 2012).

Litter sampling and decomposition experiment

Tree leaf litter was collected from plots in December 2010, at which time it had been 3.5 and 5.5 years since the last burns in the 2yrB and 4yrB treatments respectively. Scheduled burns for 2009 were not carried out as a result of adverse weather and the next burn at both fire frequencies was scheduled for winter 2011. Collection at this time, therefore, provided litter material from as long as possible since the immediate ecosystem effects of the last burn, while allowing time to run a 5-month decomposition experiment before the plots were burned again. To investigate the effects of the burning treatments on both early and later stages of litter decomposition within the short time window for the experiment, two types of mixed species tree leaf litter were collected: (i) 'freshly fallen' litter consisting of recently fallen leaves with no obvious signs of decay; and (ii) 'older mixed-age' litter consisting of surface litter of whole leaves but at progressive stages of decomposition as indicated by browning, structural collapse and visual signs of microbial colonization. Freshly fallen litter samples were collected in litter traps (1.5 m × 1.5 m) and harvested 1 month after installing the traps; four traps were arranged randomly in each plot and all litter materials from the same plot were bulked for the litter bag experiment. Older mixed-age litter was collected directly from the forest floor. Between litter collection and field installation of the litter bags (6 weeks), the field-moist litter was stored in paper bags at 4 °C to slow decomposition but to avoid biochemical alteration by air drying.

Individually labelled litter bags (20 × 15 cm) were constructed of 1 mm nylon mesh sewn with nylon thread. The two litter types were installed in the field in separate litter bags: (i) freshly fallen leaf litter bags (8 g fresh weight); and (ii) older mixed-age leaf litter bags (10 g fresh weight). Subsamples of each litter type from each plot were taken for determination of bag dry weight contents and starting point chemical and microbial parameters. Litter was placed back into the same plots from which it came, with pairs of bags consisting of one freshly fallen leaf litter and one older mixed-age leaf litter bag placed next to one another on the surface of the *in situ* litter. Bags were secured with stainless steel pins at regular intervals along an 8 m transect through each plot. Sufficient litter bags for each plot were prepared for retrieval of three pairs of bags (randomly selected) at approximately

monthly intervals over a 155-day field incubation period conducted from 09 February 2011 until 12 July 2011. Meteorological data, including temperature and precipitation, were taken at Beerburrum State Forest Australian Bureau of Meteorology weather station (26°96′S 152°96′E), which is in close proximity to the site (ca. 5 km away in a similar topographical position and elevation). Daily maximum and minimum air temperatures and rainfall ranged from 12 to 35 °C (mean: 25 °C), 4 to 23 °C (mean: 14 °C) and 0 to 79 mm day^{-1} (mean: 4 mm day^{-1}) over the field incubation period.

Litter was carefully removed from each bag following retrieval, and visually obvious extraneous material (e.g. dirt, debris and invertebrates) was removed before weighing. Following weighing, litter was cut into small squares for thorough mixing and laboratory analysis, and a subsample from each bag dried (at 65 °C) for determination of bag-specific dry weight conversions for mass loss determination. Litter from the three bags for each plot was bulked to give one composite sample of each litter type per plot. This pooled sample was then analysed for enzyme activity (last three bag retrieval dates only) within 1 week and microbial biomass and soluble chemical parameters within 2 weeks of collection, and a subsample was dried at 65 °C and ground (<150 μm) for total chemical determination.

Laboratory analyses of litter C, N and P pools

Litter total C and N contents (as percentage of dry mass) were analysed using an isotope ratio mass spectrometer with a Eurovector Elemental Analyser (Isoprime-EuroEA 3000; Isoprime, Milan, Italy). Litter total P content (as percentage of dry mass) was measured using nitric acid–perchloric acid digestion (Kuo, 1996) followed by determination of digest P content by the molybdate blue colorimetric method (Murphy & Riley, 1962).

Litter microbial biomass C, N and P contents (as percentage of dry mass) were determined by the chloroform fumigation method using conversion factors of 2.64 (Vance *et al.*, 1987) and 2.22 (Brookes *et al.*, 1985) for C and N, and 0.4 for microbial biomass P (Brookes *et al.*, 1982) alongside correction for sorbed inorganic P during fumigation. Soluble organic C and total soluble N in the fumigated and unfumigated litter in 2 M KCl extracts were measured by the high-temperature catalytic oxidation method using a Shimadzu TOC-VCPH/CPN analyser (Shimadzu Scientific Instruments, Sydney, Australia) (fitted with a TN unit) as described by Chen *et al.* (2005). Soluble inorganic P in the fumigated and unfumigated litter in 0.5 M NaHCO$_3$ (pH 8.5) extracts was determined by the molybdate blue colorimetric method after precipitation of organic matter by acidification. The unfumigated results were also used as litter concentrations of soluble C, N, and P. Data on C, N, and P contents (per cent of dry mass) from paired samples were then used to calculate C : N, C : P and N : P ratios of various litter pools, expressed as mass ratios.

Potential activities of β-glucosidase (BG), NAGase (*N*-acetyl-β-D-glucosaminidase) (NAG) and acid phosphatase (AP) in litter were measured as an indication of the microbial acquisition of C, N and P respectively (Sinsabaugh *et al.*,

2009). Enzyme activities were measured colorimetrically using the methods of Eivazi & Tatabai (1988), Parham & Deng (2000), and Eivazi & Tatabai (1977) for β-glucosidase, NAGase and acid phosphatase respectively (Sigma product numbers for enzyme substrates used = N7006, N9376 and P4744 respectively).

Statistical analyses

A residual maximum likelihood model for repeated measures (RM) ANOVA in SAS Enterprise Guide 4.3 (SAS Institute Inc., Cary, NC, USA) (including the model's default *post hoc* analysis) was used to test for overall treatment effect and intertreatment effects of the burning regimes on the litter parameters over the field incubation period. Plot number and litter bag retrieval date were incorporated as 'subjects tested' and 'time points' in the model to accurately reflect the repeated sampling of plots allocated to the three treatments. Separate analyses were carried out for the freshly fallen leaf and older mixed-age leaf litter samples. The starting point litter data were included graphically as 'Day 0' but, to discount any effects of *ex situ* storage that may have most strongly affected the data from the 'Day 0' samples, these data were not included in the statistical analysis of treatment effects over the incubation period.

Results

The magnitude of many of the litter parameters measured fluctuated over the course of the litter bag field incubation. However, for the majority there was no significant interaction between treatment and time in RM ANOVA, reflecting the similar trajectories of change over time across treatments illustrated in the figures. Treatment effects on the litter parameters are therefore discussed in terms of overall magnitude over the course of the field incubation.

Litter microbial biomass and C : N : P ratios during decomposition

Microbial biomass C in freshly fallen leaf litter was significantly higher for 2yrB (8.1–14.5 mg g^{-1}) than for NB (6.3–14.2 mg g^{-1}) (Fig. 1; Table 1); the difference between 2yrB and NB was most pronounced for the sampling times with lowest microbial biomass C. There was no significant difference in microbial biomass C among treatments for the older mixed-age leaf litter (Fig. 1).

Overall, results from this study have indicated that the 2yrB litter was stoichiometrically distinct from the NB and 4yrB litter (Figs 2–4). Total C : N ratio in freshly fallen leaf litter increased significantly with fire frequency: 2yrB (69–84) > 4yrB (53–76) > NB (49–62) and was significantly higher for 2yrB (79–102) than NB
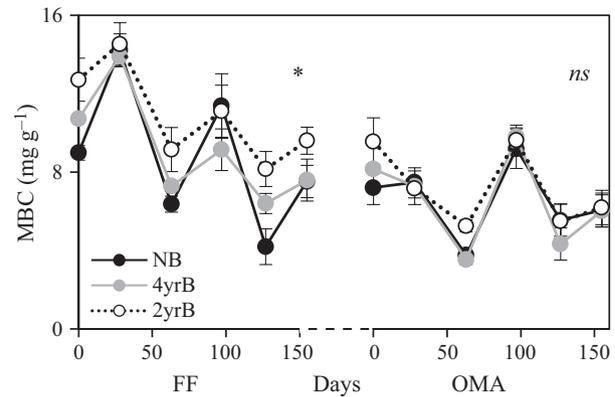

Fig. 1 Microbial biomass carbon (MBC) content of the freshly fallen (FF) and older mixed-age (OMA) leaf litter over the field incubation period for the three fire treatments (data are treatment means (n = 4) ± SE for each litter bag retrieval date). 2yrB = burning every 2 years; 4yrB = burning every 4 years and NB = no burning. Overall treatment effect for each litter type in RM ANOVA indicated by asterisks (see Fig. 2 for definitions).

(71–95) for the older mixed-age litter (Fig. 2a; Table 1). 2yrB litter microbial biomass C : N ratio (13–36 for the freshly fallen litter; 12–29 for the older mixed-age litter) was also significantly higher than NB for both litter types (5–23 for the freshly fallen litter; 10–27 for the older mixed-age litter). 2yrB litter microbial biomass C : N ratio for the freshly fallen leaf litter was also significantly higher than 4yrB (9–27) (Fig. 2c; Table 1), but there was no significant difference between 4yrB and NB microbial biomass C : N. There was no significant treatment effect on soluble C : N (Fig. 2b; Table 1) for either litter type.

Total, soluble and microbial biomass C : P ratios in older mixed-age leaf litter were significantly lower for 2yrB than 4yrB and NB (Fig. 3a–c; Table 1). Soluble C : P in freshly fallen leaf litter was also significantly lower for 2yrB than NB, although there were no significant differences among treatments for freshly fallen leaf litter total or microbial biomass C : P (P < 0.05). Soluble C : P ratios in freshly fallen leaf litter for 4yrB were significantly lower than for NB, whereas there was no significant difference between 4yrB and NB for either litter type for the remainder of the C : P ratios.

2yrB total, soluble and microbial biomass N : P ratios (freshly fallen leaf litter: 27–32, 10–57 and 3.0–4.7; older mixed-age leaf litter: 31–36, 16–282 and 2.9–6.2) were all significantly lower than NB (freshly fallen leaf litter: 35–49, 23–170 and 3.6–8.0; older mixed-age leaf litter: 51–56, 23–367 and 5.3–10.8) for both litter types (Figs 4a–c; Table 1). 2yrB total N : P was also significantly lower than 4yrB for both litter types, as was microbial biomass N : P for the older mixed-age leaf litter. Total, soluble and microbial biomass N : P ratios

Table 1 Probability values for intertreatment comparisons in RM ANOVA of treatment effects on mass loss and C, N and P pool concentrations and ratios for the freshly fallen (FF) and older mixed-age litter (OMA) overall over the field incubation period ($n = 60$).

	Treatment effect FF			Treatment effect OMA		
	2 vs.N	4 vs.N	2 vs.4	2 vs.N	4 vs.N	2 vs.4
Mass loss	**0.012 (<)**	0.507	**0.004 (<)**	**0.037 (<)**	0.974	**0.035 (<)**
Total C	0.445	0.626	0.775	0.459	0.412	0.933
N	**<.001 (<)**	0.200	**0.001 (<)**	0.059	0.311	0.346
P	0.094	0.710	0.170	**<.001 (>)**	0.563	**<.001 (>)**
C : N	**<.001 (>)**	**0.042 (>)**	**0.002 (>)**	**0.043 (>)**	0.301	0.239
C : P	0.109	0.677	0.210	**0.001 (<)**	0.494	**<.001 (<)**
N : P	**<.001 (<)**	**0.029 (<)**	**<.001 (<)**	**<.001 (<)**	0.715	**<.001 (<)**
Soluble C	**0.001 (<)**	**0.039 (<)**	0.085	**0.020 (<)**	0.667	**0.042 (<)**
N	**<.001 (<)**	0.153	**0.002 (<)**	**0.005 (<)**	0.385	**0.020 (<)**
P	**0.029 (>)**	**0.026 (>)**	0.946	**0.013 (>)**	0.823	**0.019 (>)**
C : N	0.382	0.389	0.101	0.229	0.870	0.291
C : P	**<.001 (<)**	**0.002 (<)**	0.230	**0.002 (<)**	0.559	**0.004 (<)**
N : P	**<.001 (<)**	**0.004 (<)**	0.133	**0.035 (<)**	0.250	0.240
Microbial C	**0.010 (>)**	0.283	0.064	0.364	0.796	0.507
N	**0.028 (<)**	0.457	0.098	0.152	0.368	0.553
P	0.233	0.215	0.955	**<.001 (>)**	0.919	**<.001 (>)**
C : N	**0.001 (>)**	0.232	**0.004 (>)**	**0.026 (>)**	0.348	0.129
C : P	0.721	0.449	0.276	**0.001 (<)**	0.948	**0.001 (<)**
N : P	**0.002 (<)**	**0.013 (<)**	0.201	**<.001 (<)**	0.328	**0.001 (<)**

P values ≤0.05 in bold. (>/<) indicate direction of significant effect with respect to the first treatment listed. Treatment abbreviations: 2 = burning every 2 years; 4 = burning every 4 years and N = no burning.

in freshly fallen leaf litter for 4yrB were significantly lower than for NB, whereas there were no significant differences between 4yrB and NB N : P ratios in older mixed-age leaf litter. Because litter C contents did not differ among treatments (Fig. S1), N and P content data are redundant with C : N and C : P ratio data, our main focus here; details on patterns in N and P contents can be found in (Fig. S2).

Litter extracellular enzyme activity

Activity of the phosphate-acquiring enzyme, AP, declined with increasing fire frequency for both litter types (Fig. 5c) and activity of the C-acquiring enzyme, BG, declined with fire frequency for the freshly fallen leaf litter (Fig. 5a). There was no significant treatment effect for the N-acquiring enzyme, NAG (Fig. 5b). Ratios of litter BG, NAG and AP activity were calculated as an indication of the comparative activities of microbial acquisition of C, N and P respectively. The ratios of BG : AP (i.e. C : P acquisition) (Fig. 6b) and NAG : AP (i.e. N : P acquisition) (Fig. 6c) were significantly higher for the 2yrB treatment than for 4yrB and NB for both litter types, but there was no significant difference for either ratio between 4yrB and NB. There was no significant difference in BG : NAG (i.e. C : N

acquisition) activity among treatments for either litter type (Fig. 6a). Overall, these patterns are consistent with N limitation of microbial activity in 2yrB litter but P limitation in 4yrB and unburned plots.

Litter mass loss

Litter mass loss was significantly greater for the freshly fallen leaf litter than the older mixed-age leaf litter, with 59–72% and 75–80% mass remaining at the end of experiment respectively (Fig. 7). For both litter types, mass loss was significantly lower for the 2yrB treatment (72% and 80% mass remaining for freshly fallen leaf and older mixed-age leaf litters at the end of experiment respectively) than for the 4yrB (59% and 77%) and NB (62% and 75%) treatments, whereas there was no significant difference between 4yrB and NB (Table 1; Fig. 7).

Discussion

Our findings reveal that, consistent with our initial reasoning, the differential effects of fire on ecosystem N and P availabilities can be traced into the decompositional environment as a marked alteration in the stoichiometry of leaf litter as well as that of microbial
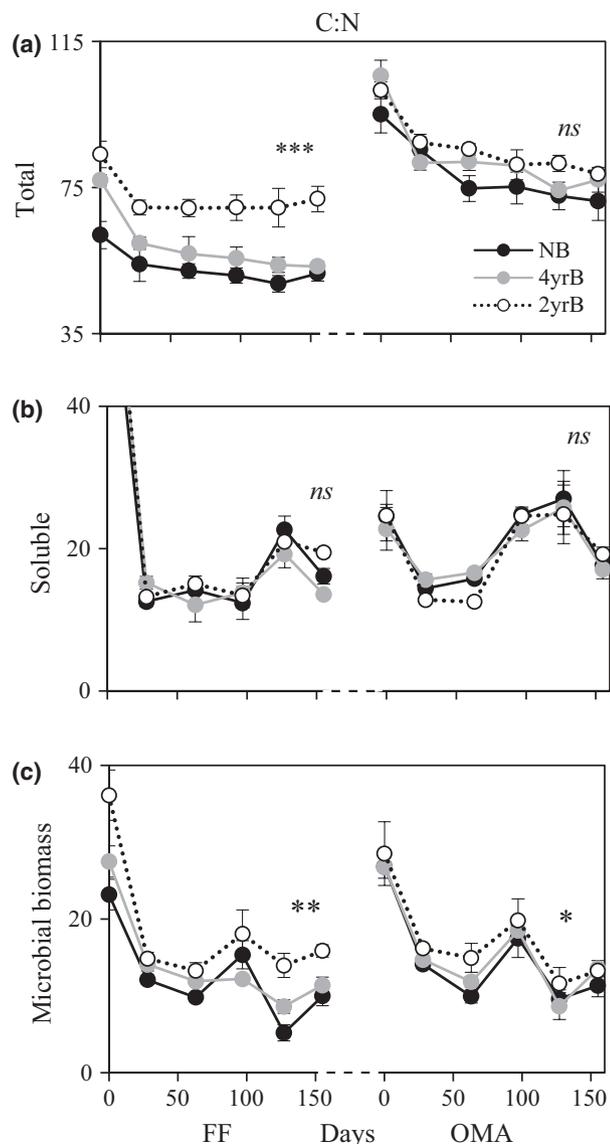
Fig. 2 Total (a), soluble (b) and microbial biomass (c) C : N ratios of the freshly fallen (FF) and older mixed-age (OMA) leaf litter over the field incubation period for the three fire treatments (data are treatment means (n = 4) ± SE for each litter bag retrieval date). 2yrB = burning every 2 years; 4yrB = burning every 4 years and NB = no burning. Overall treatment effect for each litter type in RM ANOVA indicated by asterisks: ***P < 0.001, **P ≤ 0.1, *P ≤ 0.5, ns = not significant.

Fig. 3 Total (a), soluble (b) and microbial biomass (c) C : P ratios of the freshly fallen (FF) and older mixed-age (OMA) leaf litter over the field incubation period for the three fire treatments (data are treatment means (n = 4) ± SE for each litter bag retrieval date). 2yrB = burning every 2 years; 4yrB = burning every 4 years and NB = no burning. Overall treatment effect for each litter type in RM ANOVA indicated by asterisks (see Fig. 2 for definitions).

biomass and investments in extracellular enzyme activity. These findings, therefore, make a significant contribution to our understanding of the long-term impacts of repeated fire on forest nutrient cycling and to the growing body of research applying perspectives of ecological stoichiometry to characterize ecosystem responses to environmental change (Elser *et al.*, 2000; Cleveland & Liptzin, 2007; Allen & Gillooly, 2009; Sistla & Schimel, 2012).
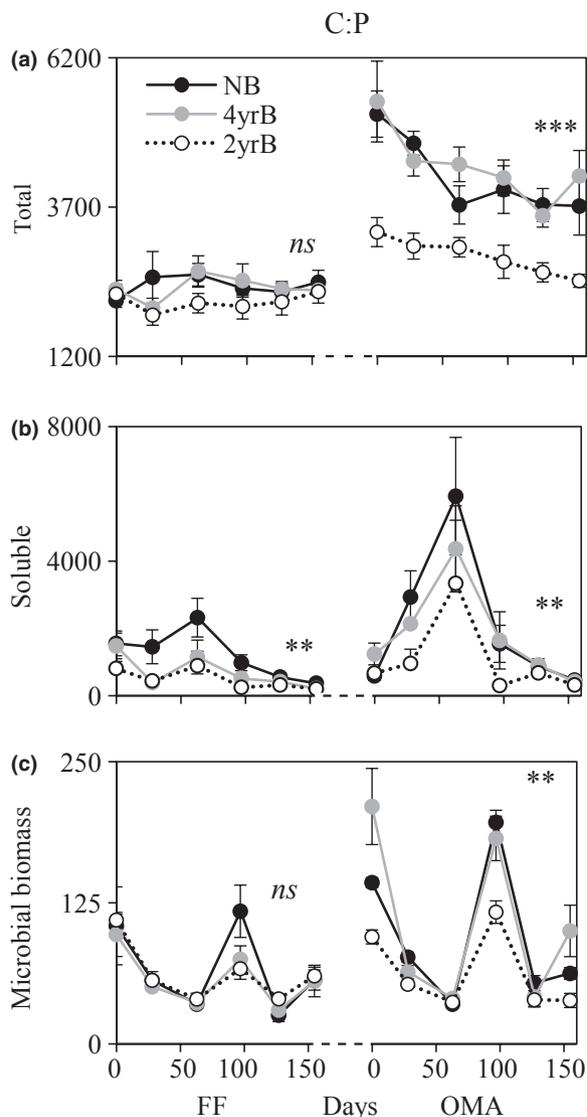
Prediction 1: Repeated burning effects on litter C : N : P stoichiometry

The effects of repeated burning on total N and P contents of leaf litter aligned well with previously documented reductions in the N : P ratio of soil nutrient pools at the site (Chen *et al.*, 2009): repeated burning increased C : N ratios for both the 2- and 4-year fire return intervals and lowered C : P ratios for the 2-year
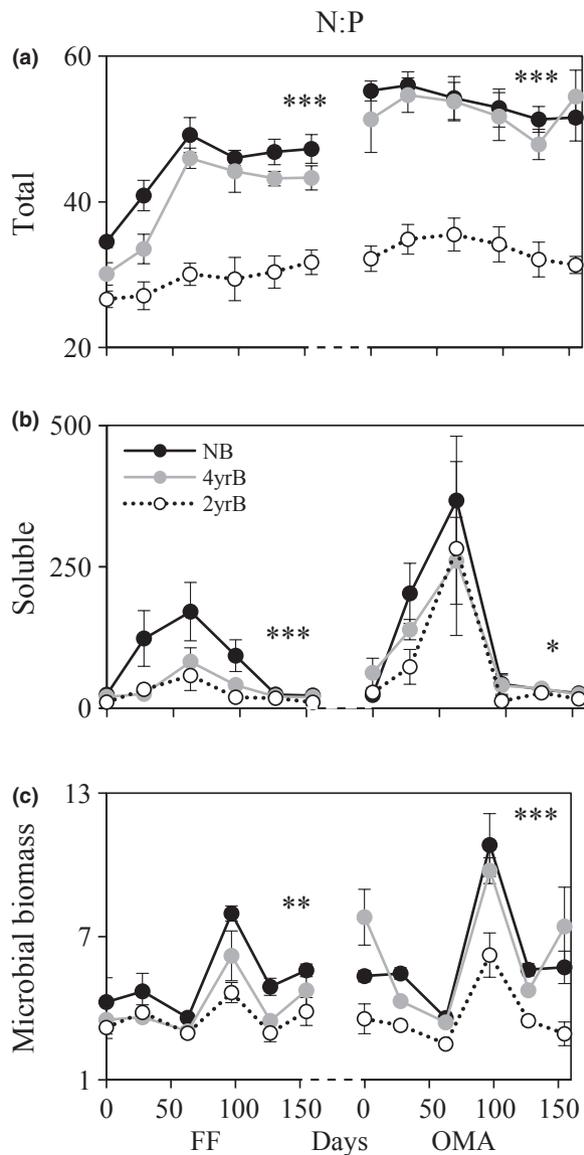
Fig. 4 Total (a), soluble (b) and microbial biomass (c) N : P ratios of the freshly fallen (FF) and older mixed-age (OMA) leaf litter over the field incubation period for the three fire treatments (data are treatment means (*n* = 4) ± SE for each litter bag retrieval date). 2yrB = burning every 2 years; 4yrB = burning every 4 years and NB = no burning. Overall treatment effect for each litter type in RM ANOVA indicated by asterisks (see Fig. 2 for definitions).

return interval, with corresponding lower N : P total ratios relative to unburned conditions (Figs 2–4). These shifts in litter stoichiometry are either due to stoichiometric changes in corresponding living plant biomass (McGroddy *et al.*, 2004; Agren, 2008) and/or to stoichiometric shifts in N and P reabsorption prior to leaf abscision (Aerts, 1996; Wright & Westoby, 2003). Future studies of foliar C : N : P stoichiometry and reabsorption are needed to evaluate these alternatives.

Nevertheless, greater plant return of P compared to N to the soil via litter flux in burned plots may be among the mechanisms driving the long-term elevation of soil P in the most frequently burned treatment (2yrB) (Chen *et al.*, 2009). Overall, the soil and litter chemistry data indicate that the high-frequency fire-affected stands at Peachester are not able to restore fire N losses to volatilization in the period between burns, consistent with ecosystem N declines in response to repeated fire reported for other forest systems (Wright & Hart, 1997; Hernandez & Hobbie, 2008).

Soluble N : P ratios in the decomposing litter also corresponded to litter total N : P ratios among the fire treatments. Lower soluble N : P ratios (Fig. 4b) in the most frequently burned treatment during litter decomposition demonstrates a distinct stoichiometric shift in nutrient availability for litter microbial activity. Thus, it is likely that this fire-induced shift to lower soluble N : P represents an increase in N limitation relative to P for the litter microbial community (Hobbie & Vitousek, 2000; Cleveland & Townsend, 2006). This has been supported by a laboratory nutrient manipulation experiment with soils from the site, which has confirmed that soil microbial production of CO_2 is N-limited in the most frequently burned treatment but is P-limited in the long-unburned (NB) treatment (C.R. Chen, unpublished data; Fig. S3). The weaker effects of fire on litter N and P for the longer fire return interval (4yrB) suggest the onset of ecosystem N recovery during the longer interburn period.

Prediction 2: Microbial response to fire-driven shifts in resource stoichiometry

The higher C : N, lower C : P and corresponding lowered N : P ratios observed across total, soluble and microbial biomass pools of litters in the most frequently burned treatment relative to unburned conditions (Fig. 4) indicate a shift in the biomass stoichiometry of the litter microbial community in response to the fire effects on resource availability during decomposition. Although a product of both lowered biomass N and elevated biomass C, it is likely that the shift to higher litter microbial biomass C : N in the most frequently burned treatment reflects lowered N resource availability. The shift to lower microbial biomass C : P observed in the most frequently burned older mixed-age leaf litter aligned with the shift in total C : P in this later phase of decomposition. In accordance with litter resource stoichiometry, fire effects on microbial biomass stoichiometry were weaker for the 4-year fire return interval. As microbial taxa likely exhibit a relatively strong degree of physiological regulation of their C : N : P stoichiometry (strong 'stoichiometric homeo-
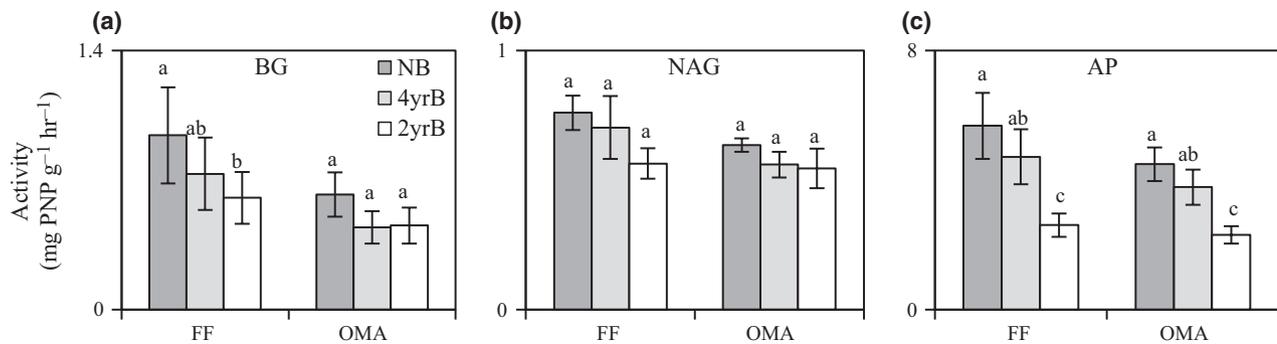
Fig. 5 Mean activity of (a) β-glucosidase (BG), (b) NAGase (NAG) and (c) acid phosphatase (AP) enzymes in the freshly fallen (FF) and older mixed-age (OMA) leaf litter over the latter half of the field incubation period for the three fire treatments (data are treatment means ($n = 4$) $\pm$ SE for each litter bag retrieval date). 2yrB = burning every 2 years; 4yrB = burning every 4 years and NB = no burning. Different letters denote overall significant differences among treatments ($P < 0.05$) in RM ANOVA of plot means for each litter bag retrieval date ($n = 36$).
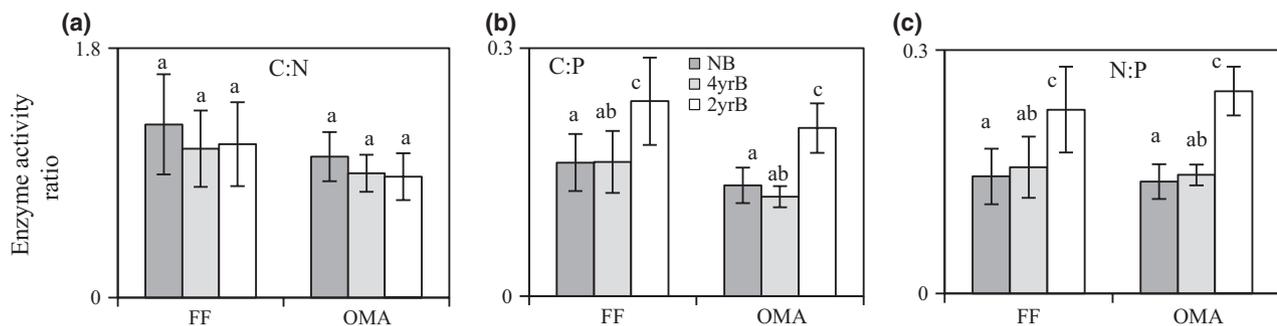

Fig. 6 C : N (a), C : P (b) and N : P (c) ratios of mean C-, N- and P-acquiring enzyme activity (as indicated by β-glucosidase, NAGase and acid phosphatase respectively) in the freshly fallen (FF) and older mixed-age (OMA) leaf litter over the latter half of the field incubation period for the three fire treatments (data are treatment means ($n = 4$) $\pm$ SE for each litter bag retrieval date). 2yrB = burning every 2 years; 4yrB = burning every 4 years and NB = no burning. Different letters denote overall significant differences among treatments ($P < 0.05$) in RM ANOVA of plot means for each litter bag retrieval date ($n = 36$).

stasis', Makino et al., 2003), it is likely that the observed shifts in litter microbial biomass stoichiometry in the fire-affected treatments reflect shifts in the relative abundances of stoichiometrically distinct microbial species making up the microbial communities. Indeed, soil microbial diversity differs considerably among the fire treatment regimes at this site (Bastias et al., 2006; Campbell et al., 2008).

The stoichiometry of microbial extracelluar enzyme activity may also be indicative of the stoichiometry of resource conditions and associated nutrient status of the microbial assemblage (Olander & Vitousek, 2000; Sinsabaugh et al., 2009). While we only measured a limited suite of litter extracellular enzymes, their relative activities were consistent with the observed fire effects on litter resource availability, as the N : P ratio of nutrient-acquiring enzyme activity in litter was substantially higher in the most frequently burned treatment than in the less frequently burned and long-unburned treatments (Fig. 6c). This aligns with the effects of the

repeated burning on soil N : P availability that are transmitted to litter N and P resource availability and then to the microbial community itself such that the microbial community in the most frequently burned treatment is more N-limited relative to P than in the less frequently burned and unburned treatments. In some ways, then, differential N/P recycling by fire, in which P is made disproportionately more available than N and promotes N limitation of microbiota, resembles the differential nutrient recycling produced by high N : P aquatic zooplankton that retain N and regenerate P, promoting N limitation in lakes (Sterner et al., 1992).

Prediction 3: Repeated burning impacts on litter decomposition rates

Slower litter mass loss (higher mass remaining) in the more frequently burned treatment (2yrB) than in the unburned treatment (Fig. 7) aligns with findings of
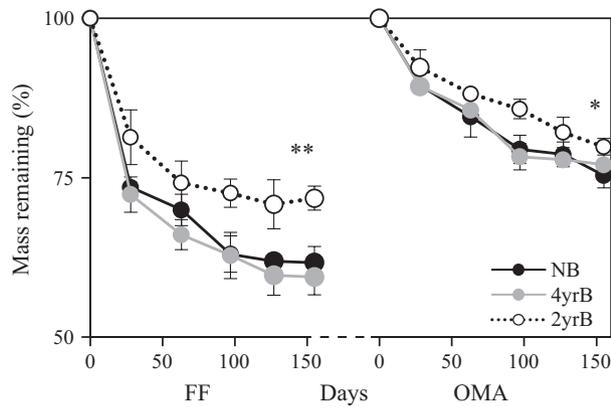
Fig. 7 Mass of freshly fallen (FF) and older mixed-age (OMA) leaf litter remaining over the field incubation period for the three fire treatments (data are treatment means ($n = 4$) ± SE for each litter bag retrieval date). 2yrB = burning every 2 years; 4yrB = burning every 4 years and NB = no burning. Overall treatment effect for each litter type in RM ANOVA indicated by asterisks (see Fig. 2 for definitions).

reduced litter decomposition rates with repeated burning at other forest sites (Raison *et al.*, 1986; Hernandez & Hobbie, 2008; Kay *et al.*, 2008; Brennan *et al.*, 2009). Comparable litter mass loss rate in the less frequently burned (4yrB) and unburned treatments also supports previous observations of reduced effects of fire at lower fire frequency (Hernandez & Hobbie, 2008).

Discerning the mechanisms for the slower decomposition rate in the high-frequency burn treatment is difficult with the available data. For example, lowered litter moisture at the highest burn frequency (Fig. S4), likely a result of reduced shade due to lower understory woody plant densities (Lewis *et al.*, 2012) and fire-induced reductions in soil organic matter (Neary *et al.*, 1999; Guinto *et al.*, 2001), may have resulted in moisture inhibition of litter enzyme activity (Sardans & Peñuelas, 2005; Toberman *et al.*, 2008) and contributed to the slower litter decomposition. However, in this study, there was no significant relationship between litter moisture content and litter decomposition rate in either freshly fallen ($R^2 = 0.0019$, $P > 0.05$) or older mixed age litter ($R^2 = 0.0559$, $P > 0.05$) (Fig. S5). It may be that N limitation, as indicated by lower litter N : P ratios (Fig. 4) and high N : P enzymatic ratios in the most frequently burned treatment (Fig. 6), played a key role in slowing the overall decomposition process. Litter C chemistry also affects microbial litter decomposition (Cotaeux *et al.*, 1995), and possible divergence in the recalcitrance of litter C associated with plant community impacts of repeated burning (Lewis *et al.*, 2012) may also have affected litter decomposition. Litter translocation experiments with targeted species are

required to assess the relative importance of litter chemistry, N limitation, and microclimate as drivers of the observed effects of fire on decomposition rates in these sites.

Given these uncertainties, it is nevertheless interesting to note that litter microbial biomass itself was not reduced by repeated burning (Fig. 1). This suggests that the slower litter decomposition in the most frequently burned treatment may be driven by differences in the functional characteristics of the litter microbial community in response to the fire-associated shifts in litter conditions, including resource stoichiometry. For example, in response to litter N depletion, the microbial community may contain a greater proportion of slower growing species with lower nutrient requirements and lower proportional resource C release to respiration, as for fungi relative to bacteria (Hodge *et al.*, 2000; Cross *et al.*, 2005). This would tend to lower litter mass loss rates (Enriquez *et al.*, 1993). Ongoing studies using genomic approaches are examining these potential shifts.

In summary, long-term burning at a 2-year frequency has led, relative to both unburned conditions and lower frequency burning, to the development of a more slowly cycling leaf litter environment with higher C : N but lower C : P and N : P ratios, alongside soil microbial activity that is more limited by N than by P. This was in-line with a lowered soil N : P ratio in the higher frequency burning (Chen *et al.*, 2009). Thus, our work has identified fire as a major driver of stoichiometric change in this forest ecosystem, providing a multidimensional view of the effects of fire on ecosystem functioning. Furthermore, our data demonstrating directional divergence in litter microbial biomass stoichiometry with altered soil nutrient conditions suggest that, as for soil (Cleveland & Liptzin, 2007), litter microbial biomass stoichiometry is closely indicative of ecosystem nutrient regimes.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Total carbon (a) and soluble organic carbon (SOC) (b) contents of the freshly fallen (FF) and older mixed-age (OMA) litter over the field incubation period for the three fire treatments (data are treatment means (n = 4) ±SE for each litter bag retrieval date; black, grey and white circles = NB, 4yrB and 2yrB respectively).

Figure S2. Total, soluble and microbial biomass nitrogen (a–c) and phosphorus (d–f) contents of the freshly fallen (FF) and older mixed-age (OMA) leaf litter over the field incubation period for the three fire treatments (data are treatment means (n = 4) ± SE for each litter bag retrieval date).

Figure S3. Soil microbial production of CO2 (over 35 days, n = 4) in response to addition of nitrogen (N) and phosphorus (P).

Figure S4. Overall litter moisture (freshly fallen and older mixed-age leaf litters combined) over the field incubation period for the three fire treatments (data are treatment means (n = 4) ± SE for each litter bag retrieval date; black, grey and white circles = NB, 4yrB and 2yrB respectively) and daily rainfall (black continuous line) for the Beerburrum State Forest Australian Bureau of Meteorology weather station (26°96′S 152°96′E).

Figure S5. Relationship between litter decomposition rate (as mass remaining %) and litter moisture content (%.). 2yrB, burning every 2 years; 4yrB, burning every 4 years; NB, no burning.