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Soil nitrogen mineralisation and organic matter composition revealed by ^{13}C NMR spectroscopy under repeated prescribed burning in eucalypt forests of south-east Queensland

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Abstract

The effects of burning on *in situ* extractable nitrogen ($\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) and net N mineralisation following scheduled fuel reduction burns in repeatedly burnt dry and wet sclerophyll forest sites in south-east Queensland were assessed. In addition, soil organic matter composition in the wet sclerophyll site was assessed by ^{13}C NMR spectroscopy. The results showed that at the dry sclerophyll site, extractable N and net N mineralisation for 1 year were largely unaffected by burning, while at the wet sclerophyll site, these parameters decreased. ^{13}C NMR analysis of soil samples from the wet sclerophyll site revealed that there was a significant reduction in the proportion of O-alkyl (alkoxy/carbohydrate) C with increasing burning frequency. Statistically significant effects on the other chemical shift regions were not detected. The ratio of alkyl C to O-alkyl C, a proposed index of organic matter decomposition, increased with increasing burning frequency. A high ratio of alkyl C to O-alkyl C suggests low amounts of carbohydrates relative to waxes and cutins, which could in turn lead to slower mineralisation. The findings are in accord with this hypothesis. There were significant linear relationships between cumulative N mineralisation for 1 year and the proportions of alkyl C and O-alkyl C, and the ratio of alkyl C/O-alkyl C. Thus, in addition to reductions in substrate quantity (low organic C and total N for burnt soils), there was also an alteration of substrate quality as revealed by ^{13}C NMR spectroscopy which is reflected in low N mineralisation.

Additional keywords: fuel reduction burning, soil C pools, soil N availability.

Introduction

Fire, whether natural or human-caused, is a form of ecosystem disturbance that could lead to disproportionately high losses of nitrogen (N) (Vitousek and Howarth 1991). Since fire removes organic matter and nutrients from a site through volatilisation and ash transfer (Raison *et al.* 1985), one of the major effects of burning is to influence the quantity, form, and availability of soil N. Because of the key role of N in the nutrition and productivity of forest ecosystems, it is important to monitor long-term changes in soil N availability, particularly

in those forests subjected to low-intensity, repeated fuel reduction burning. Soil N mineralisation is one important biological process that could be a sensitive indicator of soil N fertility changes under repeated burning. Soil N availability may be greatly reduced by burning because a large proportion of the N cycling fraction is stored in the litter and understory vegetation, and this portion of the N pool is volatilised in a fire (Keith and Raison 1992). On the other hand, another major effect of fire is to convert the N (and other nutrients) tied up in organic matter to a soluble form that can be utilised by plants, so that N availability may actually increase following burning (Kimmins 1987). Both arguments are tenable because, as with other soil properties, documented effects of prescribed burning on N mineralisation are variable ranging from increases (e.g. Covington and Sackett 1986, 1992; Schoch and Binkley 1986; Knoepp and Swank 1993) to decreases (e.g. Vance and Henderson 1984; Bell and Binkley 1989; Keith and Raison 1992; Hossain *et al.* 1995; Monleon *et al.* 1997). It appears that the magnitude and direction of the responses are quite site-specific because of the spatial and temporal variability of the controlling factors that affect soil N mineralisation, which include soil moisture, temperature, and substrate quantity and quality. It should be noted, however, that most fire-induced increases reported in the literature are rather short-lived (lasting only a few months) and so increases need not necessarily be deemed as beneficial to the ecosystem and must be considered in terms of the losses due to burning and the effects on the long-term nutrient supplying capacity of the soil (Keith 1991).

Repeated fires may affect not only soil organic matter quantity but also quality with subsequent negative effects on N mineralisation and plant N uptake. Wells (1971), in a pot experiment, observed a tendency for loblolly pine (*Pinus taeda* L.) seedlings grown on soils burned annually for 20 years to take up less N, despite a slight increase in soil total N with burning, and suggested that the N in burnt soils was less available to plants than the N in unburnt soils. Vance and Henderson (1984) found decreases in soil-extractable and mineralisable N in an oak (*Quercus* spp.)–hickory (*Carya* spp.) forest burned annually for 30 years despite an increase in the soil's total N content. They hypothesised that repeated burning may have altered the N-containing substances in organic matter, rendering them less susceptible to microbial mineralisation. A later study by Eivazi and Bayan (1996) on this same forest site revealed that long-term burning reduced the activities of soil enzymes involved in the breakdown of organic forms of N, phosphorus (P), and sulfur (S).

The ^{13}C nuclear magnetic resonance (NMR) spectroscopic technique, applied to the study of soil organic matter composition (Preston 1996), has been employed in investigations of the effects of fire on whole soils (Golchin *et al.* 1997), soil humic substances (Almendros *et al.* 1992), and plant litter (Knicker *et al.* 1996). These studies have shown that fire-affected organic matter consists of rearranged macromolecular substances of increased aromaticity, which may contribute to increased resistance to microbiological degradation.

This study was conducted to assess the influence of repeated low-intensity fuel reduction burning on soil mineral N pools and N mineralisation rates *in situ* in 2 native eucalypt forest sites of south-east Queensland; and to determine, by using ^{13}C NMR spectroscopy, whether there are any changes in soil organic matter composition as a result of burning.

Materials and methods

Sites, fire treatments, and burn description

Sites and fire treatments are described in detail in Guinto *et al.* (1998). Briefly, dry and wet sclerophyll forest sites, subjected to particular prescribed burning frequencies, are being studied. The dry sclerophyll site is dominated by spotted gum (*Corymbia variegata* F. Muell., formerly *Eucalyptus maculata* Hook.) while the wet sclerophyll site is dominated by blackbutt (*Eucalyptus pilularis* Smith). At the dry sclerophyll site, there are 3 treatments, namely unburnt since 1946, annual burning since 1952, and periodic burning (every 2–3 years) since 1973. At the wet sclerophyll site, the 3 treatments are unburnt since 1969, quadrennial burning since 1972, and biennial burning since 1972. In 1994, scheduled fuel reduction burns were conducted on 2–3 August at the wet sclerophyll site and on 13–14 September at the dry sclerophyll site. During that year, all fire-treated plots were burned. A summary of the range of weather conditions and fire and fuel characteristics during the burns is given in Table 1. The burn intensities were quite variable but are typical of low-intensity (<500 kW/m) prescribed burns in eucalypt forests.

Table 1. Range of weather conditions and fire and fuel characteristics during the 1994 prescribed burns in two eucalypt forests of south-east Queensland

Parameter	Dry sclerophyll site	Wet sclerophyll site
<i>Weather conditions</i>		
Air temperature (°C)	19–30	15–19
Relative humidity (%)	47–82	29–53
Wind speed (m/s)	1.8–3.5	0.8–1.8
<i>Fire characteristics</i>		
Mean flame height (m)	0.1	0.05
Rate of spread (m/min)	0.1–1.5	0.1–1.6
Intensity (kW/m)	16–239	32–550
<i>Fuel</i>		
Pre-burn fine fuel ^A (t/ha)	5.1–14.1	10.7–17.4
Post-burn fine fuel ^A (t/ha)	2.4–3.5	0.1–11.4
Fine fuel reduction (%)	37–79	34–99

^AFine fuel refers to the total collection of all combustible materials (litter, grasses, shrubs) up to 1 cm maximum diameter, to a height of 2 m above ground.

Measurement of soil nitrogen mineralisation

At both sites, 6 topsoil samples (0–10 cm) were collected on each plot and bulked prior to and immediately after burning, and were analysed for NH_4^+ -N and NO_3^- -N. Subsequently, soil N transformations were determined *in situ* using a modified sequential coring technique (Adams and Attiwill 1986; Adams *et al.* 1989). Field incubations provide a measure of potential net production of NH_4^+ and NO_3^- as affected by substrate quality, temperature conditions, soil moisture content at the time of sampling, and soil disturbance during sampling and bulking, so that they are now becoming the preferred technique for obtaining an index of soil N availability in forest soils (Binkley and Hart 1989).

PVC tubes, 10 cm internal diameter, were cut into 15-cm lengths and were sharpened at the bottom for use as soil sampling cores. One-half of these cores were perforated near the bottom and near the middle. Four 1-cm holes were drilled (2 on each side of the core) in order to ensure equilibration with ambient soil temperature and moisture content. Also, the holes were drilled so that they are not opposite each other in the same diameter to reduce the loss of mineral N out of the cylinder through mass flow and ion diffusion during incubation. Except for the presence of perforations, this technique is essentially the same as that employed by Raison *et al.* (1987). In the field, a pair of cores 30 cm apart were hammered into the soil to a

depth of 10 cm after first removing the surface litter. The unperforated core containing intact soil was immediately removed, sealed in a plastic bag, placed in a styrofoam box containing ice bricks, and transported to the laboratory for initial or 'zero time' measurements of NH_4^+ -N and NO_3^- -N. This unincubated core represents the extractable or standing crop of mineral N during a given sampling. The remaining perforated core was incubated for 1 month and covered with an inverted cut bottom end of a 2-L plastic softdrink bottle to keep moisture out and prevent leaching of mineral N during rains. Masking tape was put around the tube to securely fasten the cover. The softdrink bottle cover also had an opening on each side (flaps) to allow evaporation to occur. After 1 month, the incubated core was removed, sealed in plastic, transported to the laboratory, and processed similarly to the 'zero time' core. New cylinder pairs were installed and this process was repeated until mid-autumn (April). After this, sampling was stopped because of anticipated low mineralisation rates during the winter period. This is true even on the more fertile hoop pine (*Araucaria cunninghamii* Aiton ex D. Don) soils of south-east Queensland (Bubb 1996). The last of the incubated samples were collected 1 year after burning.

Immediately after collection, the samples were stored in a cold room at 4°C until processed, usually within 1–2 days. The samples were then sieved to less than 2 mm and extracted for 1 h with 2 M KCl using a 1:10 soil:extractant ratio (5 g soil:50 mL KCl). Separate subsamples were weighed for the determination of moisture content by oven drying. The extracts were filtered using a Whatman No. 42 filter paper and the filtrates were analysed for NH_4^+ -N and NO_3^- -N colorimetrically using a LachatTM flow injection autoanalyser. The NH_4^+ -N was determined using the indophenol blue method, while NO_3^- -N was determined by using cadmium reduction and the Griess-Ilosvay method (Keeney and Nelson 1982). Extracts which could not be analysed immediately were frozen at -18°C until ready for analysis. The net rate of mineralisation during each sampling period was computed as the difference in the mineral N concentration of the incubated soil and the unincubated soil. All measurements were expressed on an oven-dried soil basis and converted to kg per ha using measured bulk densities.

Logistical constraints, differences in plot sizes and soil properties between sites, and the expected high spatial variability of mineral N concentrations called for slightly different approaches in sampling soils on each site. To reduce spatial variability of measured mineral N concentrations, repeated sampling within each plot was confined to a smaller net area (micro-plot) near the centre of the larger plot. At the dry sclerophyll site, plot sizes were large and only 9 plots were sampled. Consequently, a larger net area was allotted (10 m by 8 m) and 4 pairs of cores (unincubated and incubated) were collected. The samples were also sectioned into 0–5 and 5–10 cm depths. At the wet sclerophyll site, plot sizes are smaller and there were more plots (18) sampled. Thus, a smaller net area was allotted (8 m by 4 m) and only 2 pairs of cores were collected in each net plot. Due to the sandy nature of the soil and because its topsoil (0–10 cm) contains appreciable organic matter (especially the unburnt plots), further sectioning into 2 equal depths was not attempted. Also, a reduction in potentially mineralisable N by anaerobic incubation in the 0–10 cm depth of biennially burnt plots (Guinto *et al.* 1998) was demonstrated. Thus, it is felt that the effect of burning will still be demonstrable using this depth. Because of delays in fabricating sufficient mineralisation cores, we missed early N mineralisation measurements at both sites. Also, at the dry sclerophyll site, some cores used in the initial samplings were damaged because of the hardsetting nature of the soils and thus could not be re-used. Thus, we may have underestimated the actual cumulative or annual net soil N mineralisation.

¹³C NMR spectroscopy

To assess any changes in soil organic matter composition as a result of burning, surface soils (0–5 cm) were re-sampled only from the wet sclerophyll site 18 months after burning because it was shown earlier (Guinto *et al.* 1998) that potentially mineralisable N decreased with frequent burning. All plots were sampled, but because of the exploratory nature of the NMR investigation, only 3 plots per treatment were selected. Prior to ¹³C NMR analysis, each soil sample was ground to a fine powder using a ring grinder (RocklabsTM, NZ). At the time of sampling, quadrennially burnt plots had received a total of 8 burns while the biennially burnt plots had received a total of 11 burns since 1972.

Solid state cross-polarisation magic-angle spinning (CP/MAS) ^{13}C NMR spectra were obtained at ambient temperature on a Varian Unity-400 spectrometer at 100.593 MHz. Single contact times of 1 ms were used with a proton pulse of 5.2 μs , a proton decoupling field of 62 kHz, and a recycle delay time of 4 s. Each sample was packed in a silicon nitride rotor and spun at a speed of 5 kHz at the magic angle. A total of 18 000 transients were collected and transformed with an experimental line broadening value of 50 Hz. Chemical shift data were referenced to the aromatic signals of hexamethylbenzene at 132.1 ppm. This is equivalent to using tetramethylsilane referencing at 0 ppm. For each NMR spectrum, the areas of the 4 chemical shift regions [i.e. alkyl (0–50 ppm), O-alkyl (50–110 ppm), aromatic (110–160 ppm), and carboxyl (160–185 ppm)] were calculated by integration. These values were then used to compute the areas under each region as a proportion of the total area. The exact chemical shift limits of each type of C were not strictly followed resulting in some overlapping areas between adjacent chemical shift regions—a method similar to that described by Skjemstad *et al.* (1996). The ratio of alkyl C to O-alkyl C was also computed. This parameter has been proposed as an index of decomposition of forest floor and soil materials (Baldock and Preston 1995).

Statistical analysis

Guinto *et al.* (1998) found that differences in soil texture at the wet sclerophyll site could become a confounding factor in the interpretation of treatment effects on soil properties, so for this site we attempted to employ analysis of covariance of post-burn *in situ* mineral N using clay content as a covariate. The analyses showed that this covariate was not significant despite good correlations with mineral N. Clay content was also insignificant as a covariate for the various relative areas of ^{13}C NMR chemical shift regions. Therefore, plain analysis of variance (ANOVA) was employed on all data sets.

Due to the temporal response of N transformations, repeated measures ANOVA (von Ende 1993) was performed on the extractable mineral N of the unincubated soil and net N mineralisation. This method tests for an overall (mean or total) burning effect across all sampling dates, even though the treatment effect may not be significant at a given date. Thus, when the response variable is extractable mineral N, the results were interpreted as the effect of burning on mean extractable mineral N across all sampling dates. When the response variable is net N mineralised, the results were interpreted as the effect of burning on cumulative or annual net N mineralisation. The method also tests for the effects of sampling time and its interaction with the burning treatments. Burning effects were considered significant at $P = 0.10$. It should be noted that for both sites, the number of samples used for measuring N mineralisation was small and this must be considered when interpreting the results.

Results and discussion

Mineral N pools

Figure 1 shows the extractable mineral N on each sampling date at both sites. The NH_4^+ -N and NO_3^- -N concentrations were combined because *in situ* nitrification in these forests is negligible (NO_3^- -N concentrations typically <1 mg/kg) regardless of burning treatments. This is consistent with the observations of Richards and Charley (1983) and Richards *et al.* (1985) in other Australian east coast eucalypt forest soils. At both sites, mineral N levels were low during most sampling times (<10 kg N/ha). Since there were very few significant differences in mineral N among treatments in the 0–5 cm and 5–10 cm depths at the dry sclerophyll site, data for these 2 depths were summed to reflect the 0–10 cm depth. These could then be directly compared with the mineral N data of the wet sclerophyll site. The effect of sampling time on mineral N pools as well as net N mineralisation at both sites was significant ($P = 0.0001$), indicating that concentrations varied with season regardless of burning treatments.

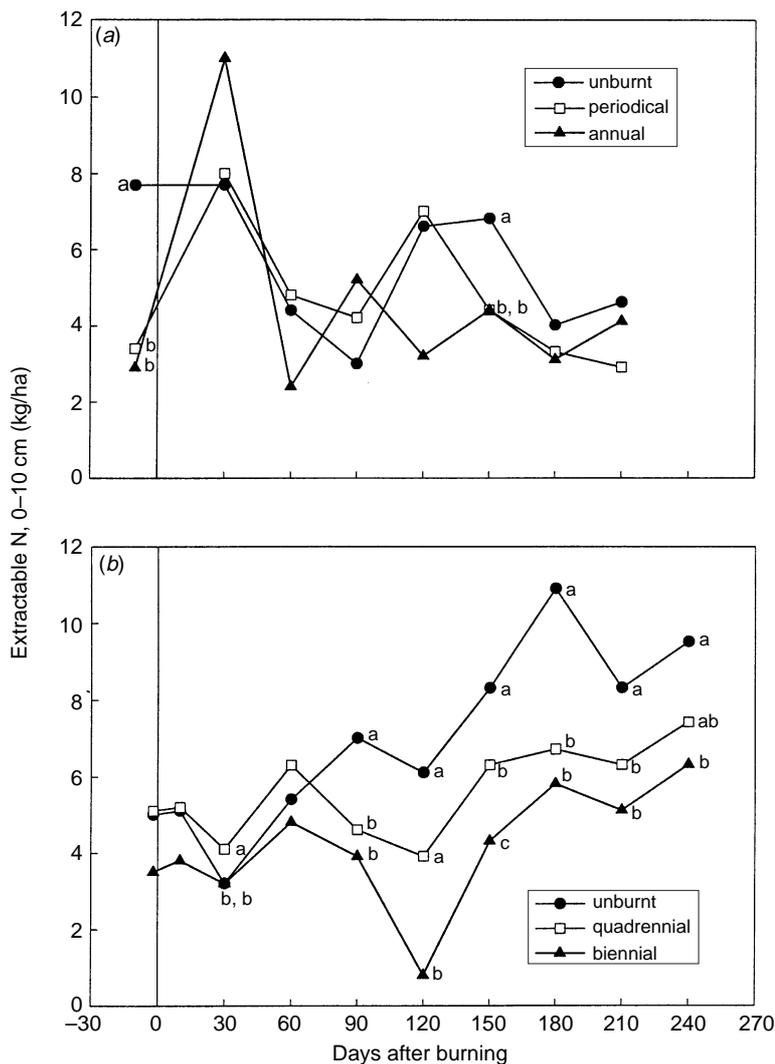


Fig. 1. Extractable soil mineral N (0–10 cm) in (a) dry and (b) wet sclerophyll forest sites in south-east Queensland as affected by prescribed burning treatments. Within a site and sampling date, treatment means with a common letter or no letter are not significantly different at $P = 0.10$.

At the dry sclerophyll site, pre-burn extractable mineral N was significantly greater in the control treatment than in the burnt treatments. One month after burning, a temporary elevation of mineral N levels mainly from an increase in NH_4^+ -N was observed in both the periodic and annually burnt treatments, although the increases were not statistically significant (Fig. 1a). Such short-term increases have been attributed to thermal decomposition of organic matter, death of microbial biomass, and small amounts of mineral N added in the ash (Raison 1979; Tolhurst *et al.* 1992). Subsequent samplings showed that mineral N responses to burning were variable and that, except for one sampling date where

burning reduced mineral N (in late summer when rainfall was highest), there were no significant differences among treatments. Averaged across all sampling dates, the effect of burning on mineral N pools at this site was insignificant.

At the wet sclerophyll site, the temporary increase in mineral N concentration immediately after burning was not observed. In fact, there was a slight depression 1 month after burning (Fig. 1*b*). There were no significant differences among treatments 2 months after burning. Subsequent samplings, however, showed that burning consistently lowered mineral N levels in both the quadrennially and

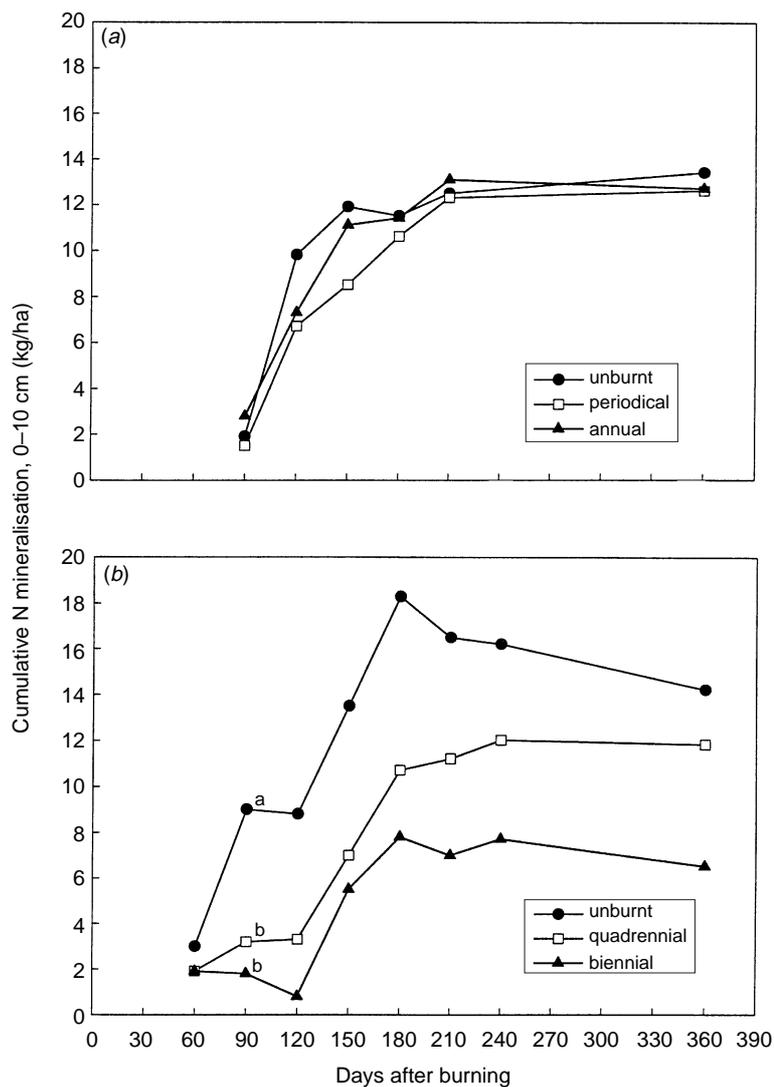


Fig. 2. Cumulative net soil N mineralisation (0–10 cm) in (a) dry and (b) wet sclerophyll forest sites in south-east Queensland as affected by prescribed burning treatments. Within a site and sampling date, treatment means with a common letter or no letter are not significantly different at $P = 0.10$.

biennially burnt plots. The overall analysis indicated that burning significantly reduced mineral N concentrations averaged across all sampling dates ($P = 0.021$).

Nitrogen mineralisation

At the dry sclerophyll site, the rate of N mineralisation was largely unaffected by burning during each sampling period (Fig. 2a). No significant differences in cumulative or annual net N mineralisation were observed at this site (unburnt 13.1 kg N/ha, periodic 12.4 kg N/ha, and annual 12.6 kg N/ha), which means that frequent burning had no deleterious effect on soil N availability. Net annual N mineralisation by the *in situ* core technique is highly correlated with annual N return in litterfall (Adams *et al.* 1989) and the latter parameter could be used as a surrogate for the former. These estimates of annual N mineralisation are comparable with annual leaf litterfall N (range 9.1–12.3 kg N/ha) measured by Lamb *et al.* (1980) on this same dry sclerophyll site.

At the wet sclerophyll site, N mineralisation rates were lower in the burnt treatments during many sampling dates (Fig. 2b). When each sampling date was analysed separately, significant differences among treatments ($P = 0.023$) were observed only during one sampling date, i.e. in late spring (3 months after burning) when mineralisation in the unburnt treatment was highest. Spatial variability of both mineral N and net N mineralisation were high and may have obscured the detection of real treatment differences. Nevertheless, the overall test showed that the effect of burning on cumulative net N mineralisation was still significant ($P = 0.089$). This indicates that the treatment differences in N mineralised during the other sampling dates were not sufficiently large themselves but they still contributed to an overall difference when all data were analysed simultaneously. The cumulative net N mineralised 1 year after burning was highest in the control (14.3 kg N/ha), followed by the quadrennial burn (11.8 kg N/ha), and lowest in the biennial burn treatment (6.4 kg N/ha). There was a 55% reduction in cumulative N mineralisation in the biennially burnt treatment relative to the control. This reflects the long-term negative influence of frequent burning on N mineralisation at this site and implies that less frequent burning would be necessary to allow time for natural processes (N inputs from rainfall and biological N fixation) to compensate for N losses and maintain soil N availability. It is also significant to note that there is evidence that fires in the biennially burnt plots are more intense than fires in the quadrennially burnt plots due to lower forest floor moisture in the former plots. For example, during these present burns, average fire intensity in biennially burnt plots was 315 kW/m compared with 177 kW/m in the quadrennial burnt plots. Interestingly, the above annual N mineralisation estimates are close to those reported by Hossain *et al.* (1995) who also found a 55% reduction in topsoil (0–10 cm) annual N mineralisation rate in the triennially burnt treatment (7.2 kg N/ha) relative to the unburnt treatment (16.1 kg N/ha) in a snowgum (*E. pauciflora*) forest soil near Canberra.

The above findings are in accord with the results of laboratory incubations (Guinto *et al.* 1998). There was a reduction in N mineralisation and hence the N supplying capacity of the biennially burnt soils at the wet sclerophyll site owing to reductions in total N and organic C, i.e. a reduction in substrate quantity. This was not the case for the frequently burnt soils of the dry sclerophyll site because total N and organic C were not adversely affected, despite more than 40

years of annual burning. Whether there are also changes in substrate quality in the wet sclerophyll forest soil as a result of prescribed burning is explored later.

Relationships between cumulative N mineralisation and selected soil properties

At the dry sclerophyll site, there were no significant relationships between *in situ* cumulative N mineralisation and selected topsoil (0–10 cm) properties [clay content (%), organic C (%), total N (%), and potentially mineralisable N by anaerobic incubation (mg/kg)] reported in Guinto *et al.* (1998). On the other hand, at the wet sclerophyll site, significant linear relationships between cumulative N mineralisation and the same topsoil properties were obtained and these properties could well be used to estimate cumulative N mineralisation (Table 2). Stepwise multiple regression employing all these soil properties, however, revealed that the best predictor of annual cumulative N mineralisation is the potentially mineralisable N by anaerobic incubation ($R^2 = 0.66$). Therefore, at this site, this biological index can be a useful diagnostic tool for ranking relative N availability in unburnt and prescribed burnt soils.

Table 2. Linear regression relationships between cumulative N mineralisation for one year (kg/ha) and selected soil properties at the wet sclerophyll site

Soil property	Intercept (a)	Slope (b)	R^2	P
Clay (%)	-11.30	1.04	0.58	0.0002
Organic C (%)	1.97	2.74	0.51	0.0009
Total N (%)	2.13	53.03	0.56	0.0003
Pot. mineralisable N (mg/kg)	-2.43	0.82	0.66	0.0000

Organic matter composition revealed by ^{13}C NMR spectroscopy

Figure 3 shows the raw ^{13}C spectra of surface (0–5 cm) soil samples from replicate plots of the wet sclerophyll site. Regardless of fire treatment, topsoils from replicate 3 have considerably lower C content than those in replicates 1 and 2 as evidenced by lower intensities (peaks) and areas under the curves. Also, biennially burnt soils in replicates 1 and 2 appear to have lower C content. These observations are consistent with the levels of organic C in these soils (Guinto *et al.* 1998). Measurement of the area under each of the chemical shift regions as a proportion of the total area under each curve and statistical analysis of these derived data revealed that there was a significant reduction ($P = 0.018$) in the proportion of O-alkyl (alkoxy/carbohydrate) C with increasing burning frequency (Table 3). Statistically significant effects on the other chemical shift regions were not detected. However, there was a trend of increasing alkyl C (cutins/waxes) and decreasing carboxyl C with increasing burning frequency. Aromatic C remained essentially unchanged with burning. The ratio of alkyl C to O-alkyl C increased significantly ($P = 0.073$) with increasing burning frequency. A high ratio of alkyl C to O-alkyl C suggests low amounts of carbohydrates relative to waxes and cutins, which could in turn lead to slower mineralisation (Prescott and Preston 1994). The findings are in accord with this hypothesis. There were significant linear relationships between cumulative N mineralisation

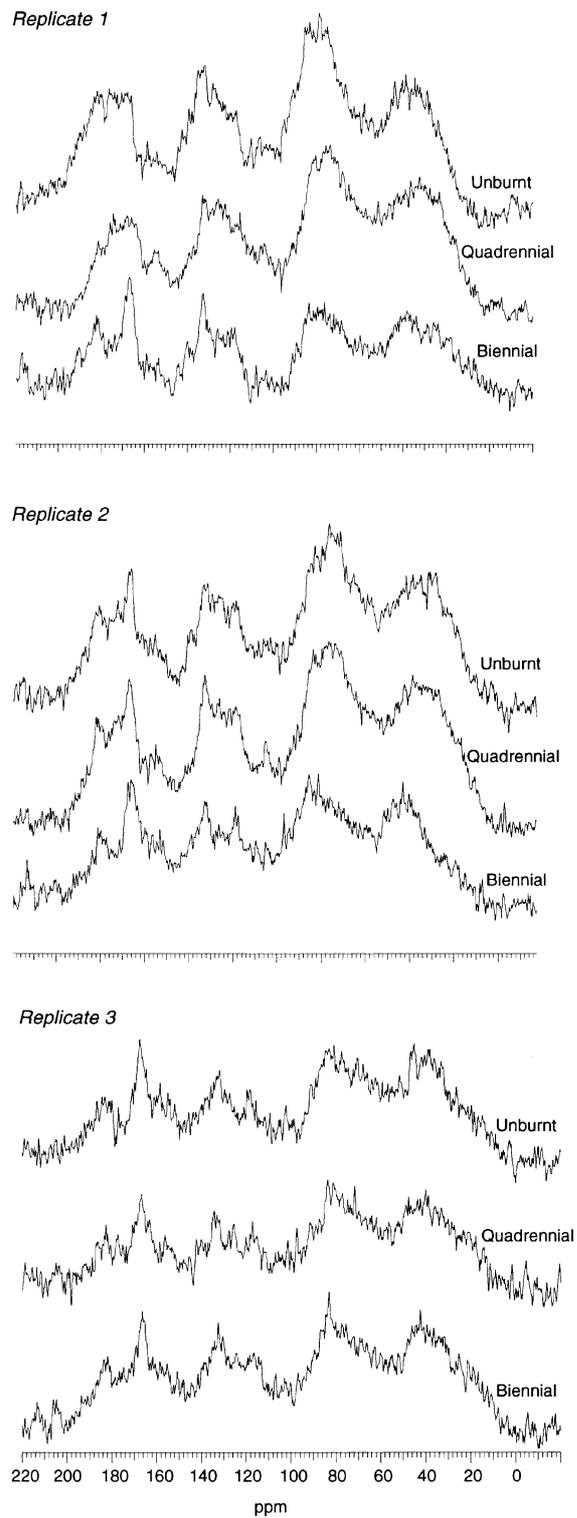


Fig. 3. ^{13}C NMR spectra of surface soil samples (0–5 cm) from the wet sclerophyll forest site as affected by prescribed burning treatments.

Table 3. Relative areas (%) of chemical shift regions in ^{13}C NMR spectra of surface soil samples (0–5 cm) from the wet sclerophyll forest site as affected by prescribed burning treatments

Mean of 3 replicates (\pm s.e.). Relative areas refer to areas under peaks within each of the chemical shift regions as a percentage of the total area under the shift. Within a column, values followed by the same letter are not significantly different at $P = 0.10$

Treatment and no. of burns	Chemical shift region (ppm)				
	Alkyl (0–50)	O-alkyl (50–110)	Aromatic (110–160)	Carboxyl (160–185)	Alkyl C/O-alkyl C
Unburnt	32.7a (3.9)	31.4a (2.6)	18.9a (0.6)	17.0a (2.3)	1.08a (0.22)
Quadrennial (8)	37.7a (3.4)	28.4a (1.7)	17.1a (1.3)	16.8a (1.0)	1.35a (0.21)
Biennial (11)	48.1a (6.0)	19.6b (0.7)	18.0a (4.4)	14.3a (3.8)	2.47b (0.36)

for 1 year and the proportions of alkyl C ($r = -0.84$, $P = 0.005$) and O-alkyl C ($r = 0.80$, $P = 0.009$), and the ratio of alkyl C to O-alkyl C ($r = -0.79$, $P = 0.011$). Thus, in addition to decreases in substrate quantity (low organic C and total N for burnt soils), there was also an alteration of substrate quality as revealed by ^{13}C NMR analysis which is reflected in low net N mineralisation.

As mentioned earlier, Vance and Henderson (1984) found decreases in soil extractable and mineralisable N in an oak–hickory forest repeatedly burnt for 30 years, despite an increase in the total N content of the soil. They explained that burning may have altered the N-containing substances in organic matter, rendering them less susceptible to microbial mineralisation. Such a hypothesis is supported by NMR studies which confirm that the humic substances in burnt soils could be more recalcitrant to subsequent mineralisation. For example, Golchin *et al.* (1997) found that, compared with soils from long unburnt forest sites, a grassland soil which had been burnt annually for several hundred years contains a higher proportion of aromatic C compounds which are considered more recalcitrant to mineralisation by virtue of their chemical structures. An increase in the proportion of aromatic C was not observed at our study site, possibly because of the low-intensity nature of the burns.

Given the exploratory nature of our work with the ^{13}C NMR technique, it is clear that more research is required using this tool to understand the effects of repeated fires on soil organic matter quality. This is because, as Almendros *et al.* (1992) emphasised, the simultaneous occurrence of a number of processes during forest fires, such as the selective destruction of humus fractions, chemical changes in soil organic matter, and external inputs of charcoal and charred lignocellulose, makes it extremely difficult to explain the origin of humus in burnt areas.

Conclusions

Annual burning in the dry sclerophyll site had no deleterious effect on soil N mineralisation. However, the reduction in cumulative soil N mineralisation and changes in soil organic matter composition, as revealed by ^{13}C NMR spectroscopy for the biennially burnt treatment at the wet sclerophyll site, reflect the long-term negative influence of burning on soil N fertility, which could have an adverse impact on the growth of the existing and future vegetation. This implies that, in this forest type, less frequent burning would be necessary to allow time for natural processes to compensate for N losses and maintain soil N availability.

^{13}C NMR spectroscopy is a useful tool in assessing organic matter composition in prescribed burnt forest soils and its full potential should be tapped in future studies on the effects of forest management practices on soil organic matter.

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