Biogeochemistry **29:** 3–24, 1995.

Changes in heated and autoclaved forest soils of S.E. Australia. I. Carbon and nitrogen

ISABEL SERRASOLSAS[1] and PARTAP K. KHANNA
CSIRO, Division of Forestry, P.O. Box 4008, A.C.T. 2600, Canberra, Australia; [1] *Present address: Department de Biologia Vegetal, Universitat Barcelona. Avda Diagonal 645, 0828 Barcelona, Spain*

Key words: microbial biomass-N, N mineralisation, nitrification, soil heating, soil autoclaving, soil respiration

Abstract. The effect of heating and autoclaving on extractable nitrogen, N mineralisation and C metabolism was studied by heating five forest soils in the laboratory, simulating the range of effects of heat due to bushfire. Top soil (0–5 cm) was heated to 60 °C, 120 °C and 250 °C for 30 minutes; unheated soil was taken as a control. Samples of the soil heated to 250 °C were also inoculated with fresh soil to accelerate the recovery of the microbial population. Soil autoclaving was carried out as another heat treatment (moist heat). Soils were analysed immediately after heating and 3 times during seven months of incubation to assess immediate and longer-term effects of heating.

Extractable N (organic and mineral forms) increased after heating to 120 °C, but decreased with further heating to 250 °C suggesting the volatilisation of N. N associated with microbial biomass diminished with heating and was barely detectable after the 250 °C treatment. Microbial biomass was an important source of soluble N in heated soils, and only partly recovered during subsequent long incubation. The amount of N mineralised during incubation depended on both soil and temperature. Nitrification did not occur when soils were heated to 250 °C (with or without inoculum), or after autoclaving, demonstrating the high sensitivity of nitrifiers to heat. At the beginning of soil incubation, respiration was enhanced in heated soils (250 °C, 250 °C inoculated) and autoclaved soils, but after 30 days of incubation respiration decreased to values either similar to or lower than those in control. This respiration pattern indicated that a fraction of labile C was released by heating, which was quickly mineralised within 30 days of incubation. These results demonstrate some effects of soil heating on C and N dynamics in forest soils.

Introduction

Fires are a common feature of the Australian forest landscape. In addition to wildfires, controlled fires are used as a management tool to reduce fuels and wildfire hazards. Fire is also used to burn logging slash, especially after clearcutting the native eucalypt forests. Fires affect soil properties relevant to plant nutrition in a number of ways and thus have been extensively studied in Australia. Nutrients and C are lost by volatilisation (Ellis & Graley 1983; Raison et al. 1985); ash produced during fire affects soil solution chemistry and exchangeable cations (Khanna & Raison 1986; Khanna et al. 1986;

Tomkins et al. 1991; Khanna et al. 1994); N is mineralised (Weston & Attiwill 1990; Polglase et al. 1992a; Bauhus et al. 1993), and soil P is transformed (Adams 1992; Polglase et al. 1992b).

Few studies (White et al. 1973; Khare et al. 1982; Kutiel & Shaviv 1989; Marion et al. 1991; Fritze et al. 1994; Khanna et al. 1994) have distinguished between the effects of ash additions and of heat on soils. In a heated soil, an increase in easily extractable C, N and other elements has been observed (Dunn et al. 1979; Kutiel & Shaviv 1989) probably for three reasons: (a) lysis of microbial cells (Jenkinson 1966; Sorensen 1974; Marumoto et al. 1982; Van Gestel et al. 1993). (b) Increase in the solubility of both non-microbial organic N and inorganic forms of N (Jenkinson & Powlson 1976; Kieft et al. 1987; Van Gestel et al. 1991). (c) Partial ashing of organically bound nutrients at high temperatures.

Microbial populations are sensitive to heat, but their sensitivity varies. For example, the activity of nitrifiers in heated soils is reduced to very low levels (Dunn et al. 1979; Kutiel & Shaviv 1989). Heating soils causes further changes in the available contents of N through drying (Sorensen 1974; Marumoto et al. 1982; Van Gestel et al. 1991). Drying of soils varies under field conditions depending upon the intensity and duration of heating.

The objective of the present study was to determine the immediate and longer-term changes in respiratory C and extractable N following heating to temperatures expected under field conditions during a bushfire, and during autoclaving of some forest soils. Autoclaving was included to compare dry heat with the moist heat (and high pressure). Changes in the longer-term biological activity of soils were assessed by measuring the release of C and N when heated and autoclaved soils were incubated under constant temperature. These processes would affect the amount of nutrients that would become available to plants after fire. A subsequent paper will document associated changes in soil P availability and phosphatase activity in heated and autoclaved forest soils.

Materials and methods

Soils

Five forest soils of different characteristics (Table 1) were collected from experimental sites in Victoria and in the Australian Capital Territory, ACT, (Australia). OUn, ODu and OGn soils from Orbost in Victoria (37 °42'S, 148 °43'E), were with principle profile forms of uniform, duplex and gradational soils respectively. These soils were collected under lowland sclerophyll forests of eucalypt, with *Eucalyptus sieberi*, *E. globoidea* and *E. baxteri* being

Table 1. Some relevant soil characteristics (0–5 cm). Soil groups follow the terminology of Stace et al. (1968).

Code location	Forest	Soil group	Sand %	Silt %	Clay %	pH (H_2O)	C %	N %	C/N
BFG Pierces Creek, ACT	Pinus radiata	Yellow podzolic	73	19	8	4.6	1.7	0.06	26.6
OUn Orbost, Victoria	Mixed eucalypt	Leached sand	98	2	0	3.6	8.9	0.22	39.9
ODu Orbost, Victoria	Mixed eucalypt	Yellow podzolic	72	17	11	4.2	3.2	0.08	42.6
OGn Orbost, Victoria	Mixed eucalypt	Yellow earth	65	23	12	4.0	6.2	0.12	51.4
PC Picadilly catch., ATC	Euc. pauciflora	Red earth	34	40	26	3.8	10.6	0.36	29.3

the dominant trees. Soils from ACT (35 °21′S, 148 °56′E) were under *Eucalyptus pauciflora* sub-alpine forest in Picadilly catchment (PC) and under *Pinus radiata* plantation at Pierces Creek, the site of the Biology of Forest Growth Experiment (BFG). BFG soil had a duplex profile and PC was a gradational soil. These sites were selected because they carried important forest resources and major nutrient cycling work was being undertaken on these soils (Khanna et al. 1986; Raison & Myers 1992; Falkiner et al. 1993; Khanna et al. 1994).

Bulk samples were taken from the surface (0–5 cm) of mineral soil at 10 randomly-located points in winter 1992. These samples were well mixed to form a composite sample and sieved (< 0.5 cm). The samples were stored at field moisture content at 4 °C until used. Some relevant soil characteristics are summarised in Table 1.

Treatment

Four sub-samples each of 400 g from the bulk sample were spread on aluminum foil to form a 2-cm thick layer and were heated for 30 minutes at 60 °C, 120 °C, 250 °C. Another four sub-samples of 400 g were placed in plastic containers and autoclaved for 30 minutes. Heat treatments were selected on the basis that surface soils will be subjected to temperatures of around 60 °C under low intensity forest fires and to temperatures higher than 200 °C under heaped slash burning (DeBano et al. 1979; Wells et al. 1979; Rundel 1981; Walker et al. 1986). The 120 °C treatment is intermediate, and the one which is used for autoclaving soils. Heat treatments were carried out in an oven (60 °C and 120 °C), in a muffle furnace (250 °C), and in an autoclave at 120 °C and 1.5 MPa pressure. During the heating, the oven and the muffle furnace were opened several times and soils were stirred to minimise both reducing conditions and heat gradients. The muffle furnace was difficult to adjust exactly to 250 °C, so the soils may have been exposed to temperatures ranging from 225 °C to 275 °C. In other cases the temperatures were probably slightly different among soils depending upon their characteristics (moisture content, organic matter, texture, etc). Change in the moisture content of soils during heating was, however, recorded. Unheated soils (20 °C) were taken as a control. Part of the soil heated to 250 °C was inoculated prior to the incubation by mixing with 0.5% (by weight) of respective fresh soil to accelerate recolonization by microbial populations.

Incubation of soils

Prior to incubation, soil samples were slowly rewetted to 80% of field capacity. Heated, autoclaved and control soils were incubated for 7 months at 25 °C

in the dark (4 replicates for each soil and treatment). Soils were placed in glass jars together with a container of water to avoid excessive moisture losses. Because of some drying, the soils were rewetted to initial moisture contents after 57 days of incubation. Jars were opened periodically (once every fortnight) for about 5 minutes to replace O_2. Mineral N, total soluble N and N associated with microbial biomass were measured in the soils before incubation and after 20, 60 and 160 days of incubation. Soil respiration was measured at 6, 18, 30, 57, 68, 80, 104, 126, 151 and 210 days of incubation by using a soda-lime absorption technique (Edwards 1982). Evolved CO_2 was absorbed in 10 g dried soda-lime, the weight of which was recorded before and after the absorption period. Ten blanks without soil were also monitored.

Soil analyses

Mineral N (NH_4-N and NO_3-N) was extracted by shaking subsamples of the soil for one hour with 0.5 M K_2SO_4 (1:5 weight:volume) and measured colorimetrically using continuous flow techniques (Technicon TRAACS 800). Total soluble N in the K_2SO_4 extract was determined colorimetrically (Technicon TRAACS 800) after acid digestion using H_2O_2 and H_2SO_4 (Hossain et al. 1993). N mineralisation was calculated as the difference between initial and final mineral N, following 160 days of incubation.

The estimate of N associated with microbial biomass, 'N-flush', was analysed by a fumigation-extraction method using hexanol as a biocide (McLaughlin et al. 1986; Hossain et al. 1990; Bauhus et al. 1993, Bauhus & Khanna 1994). Four ml of hexanol was added to 10 g of moist soil sample. After 24 hours of fumigation, soil was extracted by using 0.5 M K_2SO_4. Total soluble N was measured by the technique mentioned above. The N-flush was calculated as the difference in total soluble N in 0.5 M K_2SO_4 extract between the fumigated and unfumigated soil. N-flush was not corrected by using any K_N factor, because such factors are highly variable, and differ with moisture content, soil type, sampling season, microbial population or C:N ratio of micro organisms (Jenkinson 1988; Ross 1990) and should be determined for each soil and soil condition. Previous experiments (Hossain et al. 1990) demonstrated that non-microbial soil organic matter was solubilised using hexanol fumigation-extraction method, and Díaz-Raviña et al. (1992) reported similar results in heated soils (fumigant – chloroform, and extractant – K_2SO_4).

Soil pH was measured in water (1:2.5 ratio). Total C and N were determined with a Carlo Erba 1500 CHNS elemental analyser. Texture was determined by hydrometer method (Gee & Bauder 1986) in 2-mm sieved soil. All data are expressed on oven-dry (105 °C) basis.

Statistical analyses

Data from the four replicates for each treatment and soil were analysed using analysis of variance to compare heating and autoclave treatments and incubation time. The Duncan test was used to determine significant differences among treatments for each soil at a probability of <0.05. Results from different soils were not compared because temperature treatments were probably slightly different among soils. Regression analysis using all soils was also performed.

Results and discussion

Immediate effects of heating and autoclaving on nitrogen

With increase in heating temperature, moisture content of soils decreased until they were completely dry at 250 °C, except for the PC soil that still had 8.5% of moisture, probably due to high initial moisture and high organic matter contents. Loss of weight by ignition at 250 °C in all soils was very low (undetectable). Total soil carbon and nitrogen contents and pH did not change significantly with heat treatments (Table 2).

Total extractable N

Total extractable N was higher in soils heated to 120 °C and 250 °C than in the control soils. The highest amount of extractable N for OUn, OGn and PC soils was found at 120 °C, and for BFG and ODu soils at 250 °C (Fig. 1). A significant fraction of the total extractable N in these soils was organic in nature. The organic N fraction remaining in heated samples depended on temperature of heating. When compared to the control, samples heated to 120 °C had a lower fraction of total soluble N as organic N, suggesting that a fraction of organic N had mineralised, whereas at 250 °C an increase in soluble organic N of 1.6–5 fold was observed in all soils.

Total extractable N in autoclaved soils was slightly lower (PC), equal (ODu and OGn) or higher (BFG, OUn) than in 120 °C heated soil (Fig. 1). This variable pattern among soils suggested that soil properties such as organic matter content, initial moisture and clay content affected the results obtained on dry and moist heating of soils. However, autoclaving the soils resulted in a higher organic N content than either controls or heating soils to 120 °C (Fig. 1).

Mineral nitrogen

Mineral-N occurred as ammoniacal form in all the unheated and heated soils, and no nitrate was detected. Heating the soils to high temperatures increased

Table 2. Imediate effects of heating and autoclaving on moisture content, weight loss on ignition, pH, and total %C and %N.

Soil	Temperature °C	Moisture %	Loss ignition %	pH (H_2O)	C %	N %
BFG	20	7.6	–	n.d	1.7	0.06
	60	4.6	–	n.d	1.7	0.05
	120	1.4	–	n.d	1.8	0.05
	250	0.0	0.6	n.d	1.7	0.05
	auto	10.5	–	n.d	1.8	0.05
OUn	20	30.9	–	3.6	6.9	0.22
	60	26.1	–	3.5	6.6	0.12
	120	22.4	–	3.4	6.8	0.12
	250	0.0	0.1	3.1	n.d	n.d
	auto	30.0	–	n.d	6.2	0.18
ODu	20	17.5	–	4.2	3.2	0.08
	60	13.5	–	4.7	3.0	0.08
	120	7.6	–	4.6	2.9	0.07
	250	0.0	0.6	4.3	3.4	0.07
	auto	15.0	–	n.d	2.3	0.05
OGn	20	36.0	–	4.0	6.2	0.12
	60	31.7	–	4.7	6.3	0.12
	120	22.3	–	4.6	6.4	0.14
	250	0.0	0.1	4.1	6.3	0.17
	auto	32.0	–	n.d	6.2	0.17
PC	20	43.3	–	n.d	10.6	0.36
	60	38.5	–	n.d	10.4	0.37
	120	30.5	–	n.d	10.5	0.37
	250	8.5	–	n.d	10.9	0.39
	auto	32.5	–	n.d	10.6	0.34

n.d = not determined

NH_4-N concentration (Fig. 1). Similar results were reported by Dunn et al. (1979) and Kutiel & Shaviv (1989) who heated their soils to 250 °C and 300 °C. The concentration of mineral soil N has been generally observed to increase after fire or slash burning. Beside the increase in ammonium as a direct result of soil heating, as we found in this paper, N may also be added to the soil in ash; although the concentration of N in ash is generally low

compared to that of total soil nitrogen (Wilbur & Christensen 1985; Binkley & Christensen 1992).

Heating the soils to 60 °C had either no effect on or slightly increased (BFG and PC soils) the concentration of NH_4-N (Fig. 1). On the other hand, heating the soils to 120 °C increased the concentration of NH_4-N between 3- and 4-fold in OUn, ODu and OGn soils, and 12.5-fold in PC soils, but no increase was found in BFG soil. Increase in NH_4-N in 120 °C-heated soils was found to correlate positively with the initial organic C content ($r^2 = 0.90$, $P < 0.0001$, $n = 20$). The correlation suggested that NH_4-N produced by heating was coming from mineralisation of soil organic N (Kitur & Frye 1983; Giovaninni et al. 1990), including N from killed microorganisms. A similar relationship was also reported by Sertsu & Sánchez (1978).

Extractable NH_4-N increased in all soils after autoclaving (Fig. 1). For ODu, OGn and PC soils, the increase was lower than that observed for the 120 °C heating treatment, whereas for BFG and OUn soils the increase was higher. The differences between autoclaved and 120 °C-heated soils might be due to drying effects.

Soils heated to 250 °C showed a decrease in NH_4-N concentration when compared with 120 °C treatment in all soils, except BFG (Fig. 1). The decrease in NH_4-N was attributed to gas losses of N. BFG soil did not show this decrease, probably because solubilisation of N in this soil started at temperatures higher than 120 °C, and due to the increase in NH_4-N at 250 °C the gas losses of N were not evident. Gas losses of N at 250 °C in soils were calculated as the difference in N concentration (the highest value of NH_4-N or total soluble N) between 250 °C and 120 °C treatments. Values so obtained would however be low, as additional losses from other forms of N might occur (Walker et al. 1986). The estimated gas losses of N were 55, 25, 19 and 9 $\mu g\ N.g^{-1}$ soil for PC, OGn, OUn and ODu soils, respectively, which corresponded to 1–2% of total N and 33–57% of K_2SO_4 extractable total N in 120 °C soil. Gas losses of N from these soils were very low, as generally the case is for mineral soils affected by forest fires, because temperatures at 2.5 or 5 cm depth are not high enough to volatilise nitrogen.

Microbial Biomass N

Nitrogen associated with microbial biomass was high, ranging from 10 to 67 $\mu g\ g^{-1}$ in control soils. These values were double the amount of total soluble N in the unfumigated soils. During soil heating, especially at high temperatures, the soils dried. Both drying and heating would kill the microbial population and thereby decrease the N-flush, as shown in Fig. 1. Dunn et al. (1985) observed an exponential decrease in microbial populations with increasing soil temperatures. Fritze et al. (1994), working in a Scots pine forest, found

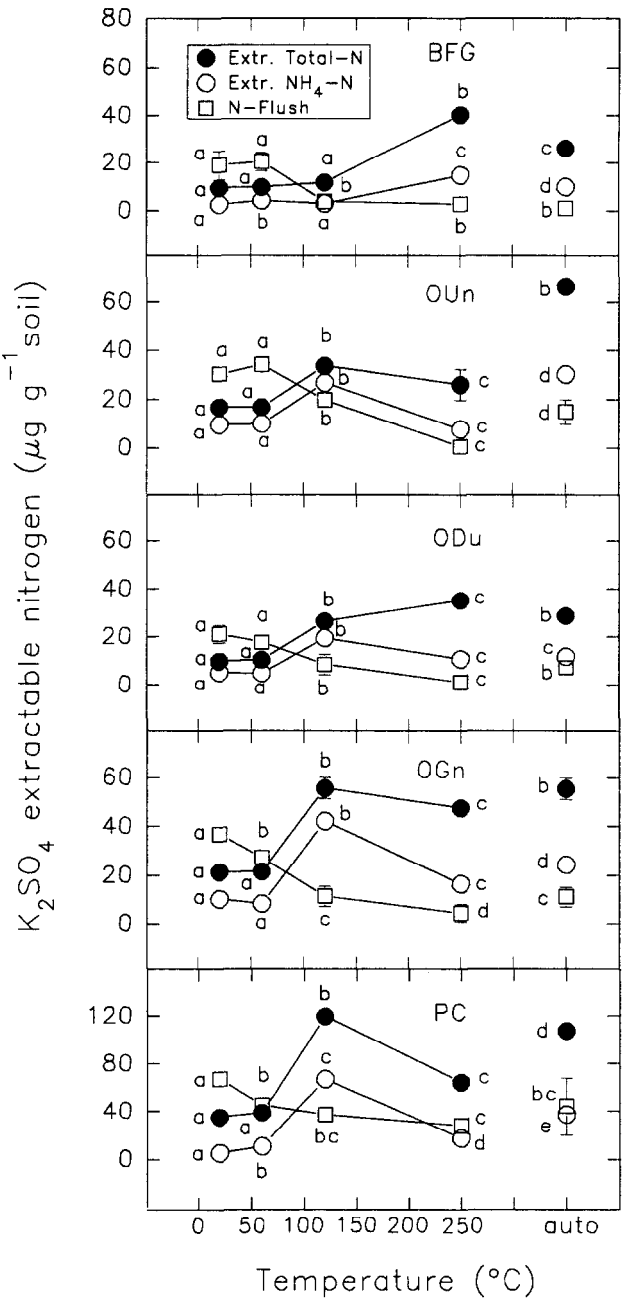

Fig. 1. Immediate effects of soil heating and autoclaving on K_2SO_4 extractable N: total N, NH_4-N and N-flush. Bars represent standard deviation. A different lowercase letter indicates a statistically significant significant difference at the $P = 0.05$ level. Note the different scale in PC soil.

Table 3. Contribution of N-flush (N-flush in control soil minus N-flush in heated soil) to the increase in total soluble N after heating. For 250 °C treatment the probable gas losses of N were taken into account for the calculation of total soluble N. Contribution of N-flush to increase of total extractable N after heating (%).

Soil	120 °C	250 °C	Autoclaving
BFG	0	53	100
OUn	62	100	31
ODu	77	59	73
OGn	74	63	75
PC	35	50	32

that burning caused a 50% decrease in the total microbial biomass, whereas addition of ash did not alter the soil microbial biomass. In our study, the decrease in microbial biomass depended mainly on the heating treatment, but was also related to soil properties such as organic matter content and texture. After heating to 120 °C, the N-flush decreased by 34–44% in soils rich in organic matter (OUn and PC), and by 60–80% in BFG, ODu and OGn soils. When soils were heated to 250 °C, the N-flush decreased by 85–99% in all soils (Fig. 1), except for PC (58%), which was rich in soil organic matter and clay suggesting that a significant part of the microbial biomass remained protected in PC soil. West et al. (1988) found that soil texture effects the rate of decline of microbial biomass C in air-dried soils, and Marshman & Marshall (1981) reported that clay content may protect microbial cells from desiccation and predation.

Microbial N is considered to be the main source of N mineralised after drying and heating in some soils (Marumoto et al. 1982; Okano 1990). In this study, we found a good relationship ($r^2 = 0.95$, $P < 0.0001$, $n = 18$) between N-flush of control soils and maximum ammonium increase recorded at either 120 °C or 250 °C, with the slope of a regression line equal to 1 (Fig. 2). When the decrease in N-flush with temperature was compared with the increase of total extractable N, it was found that between 35 and 77% of the total soluble N released by heating soils at 120 °C was derived from microbial biomass sources (Table 3). For the soils heated to 250 °C, its contribution was between 50–63% for BFG, ODu, OGn and PC soils and 100% for OUn soil (taking into account the probable gas losses of N). Marumoto et al. (1982) reported that, in oven-dried (70 °C, 24 hours) and air-dried soils, approximately 77 and 55% respectively, of the N mineralised after remoistening and incubating came from the freshly-killed biomass. Lower values (20% and 30%, respectively) were reported by Okano (1990). In contrast, Van Gestel et al. (1991) deduced

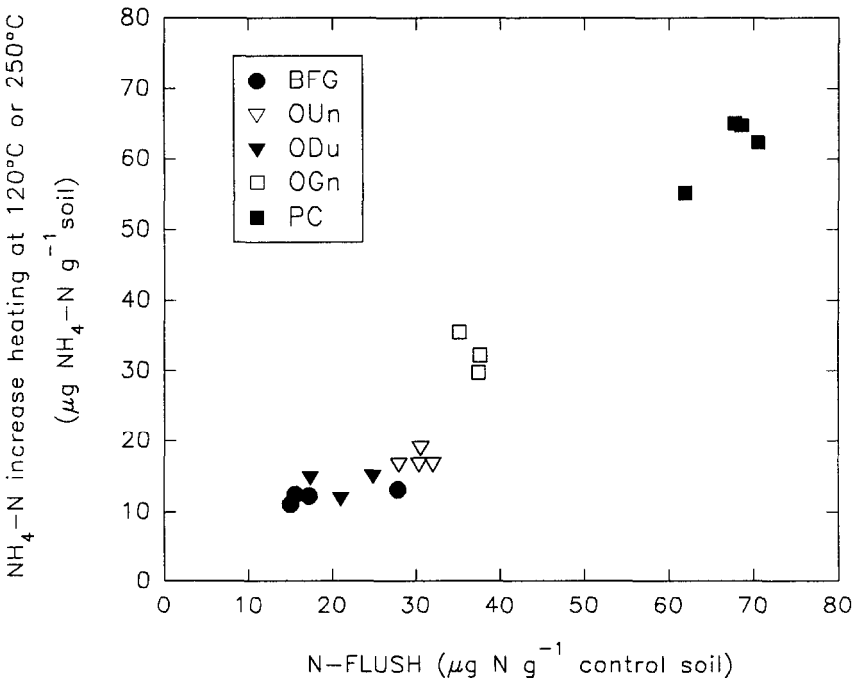

Fig. 2. The relationship between the maximum increase in NH_4-N after heating at either 120 °C or 250 °C and N-flush in control soil.

from their data that microbial cells killed by soil desiccation contributed only a minor amount to the C and N mineralisation on rewetting and incubation.

Microbial biomass decreased on autoclaving, and the values obtained were similar to those in soils heated to 120 °C (Fig. 1). The contribution of N-flush to total soluble N was similar (30–100%) in autoclaved and 120 °C treated soils, except for BFG and OUn soils (Table 2). A good relationship between total extractable N in fumigated control soils and unfumigated autoclaved soils was found ($r^2 = 0.90$, $P < 0.0001$, $n = 18$) for all soils, indicating that similar labile N (mineral N, labile organic N and microbial N) was extracted in both treatments. OUn had higher total extractable N in the autoclaved treatment than expected from the regression, suggesting that an additional amount of N may be solubilised from non-microbial sources in this soil, which has high organic matter content but almost no clay.

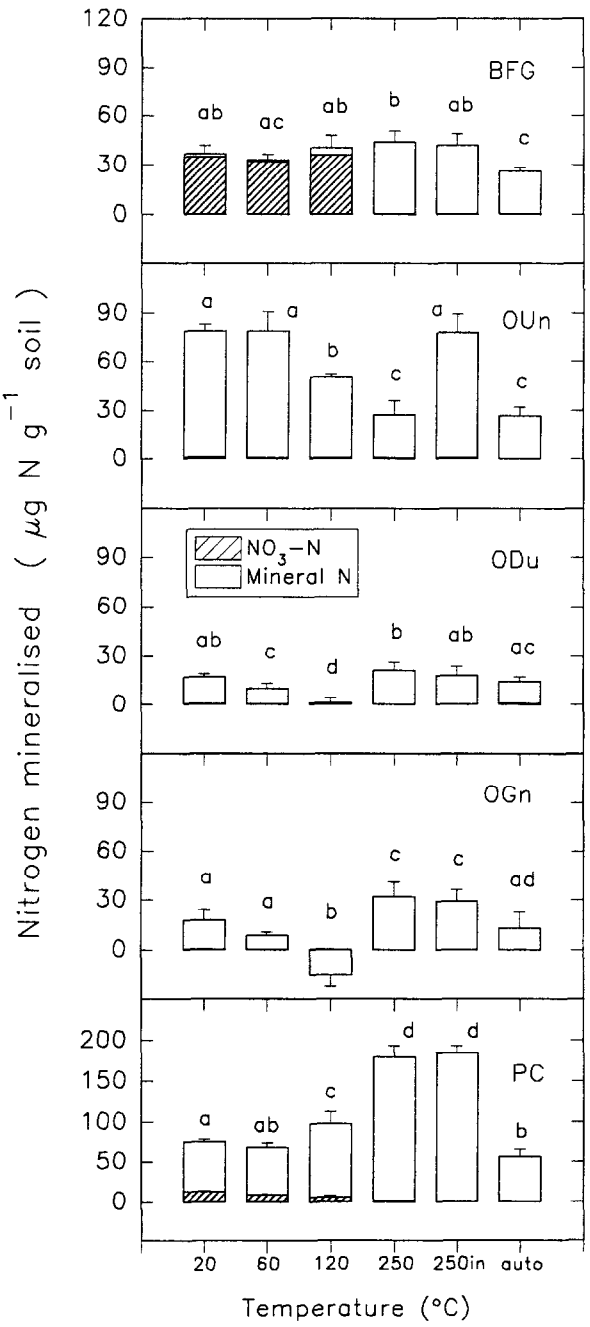

Fig. 3. Nitrogen mineralisation (total mineral nitrogen and NO_3-N) in heated and autoclaved soils during 160 days of incubation. Bars represent standard deviation. A different lowercase letter indicates a statistically significant difference at the $P = 0.05$ level. Note the different scale in PC soil.

Nitrogen mineralisation and Microbial Biomass N of heated and autoclaved soils during incubation

Nitrogen mineralisation

Mineral nitrogen increased during incubation in all soils for most of the treatments (Fig. 3). When compared with the control, soils heated to 60 °C had similar or slightly decreased (ODu) N mineralisation during 160 days of subsequent incubation. In OUn, ODu and OGn soils heated to 120 °C, the amount of N mineralised was lower than in the control, but equal or higher amounts were observed in BFG and PC soils, respectively (Fig. 3). OGn and PC soils, with high clay+silt content, showed increased N mineralisation in 250 °C heated soils compared with control, whereas BFG and ODu soils showed no differences, and a large decrease was observed in the leached sandy soil (OUn) (Fig. 3). An increase in mineralisation rate in heated soils suggests changes in the substrate quality and the fast recovery of the microbial populations. Several authors have reported increases in N mineralisation rate after fire or after drying and heating soils (Okano 1990; Singh et al. 1991; Fenn et al. 1993).

The decrease in N mineralisation observed in some heated soils (i.e., in OUn, ODu and OGn soils at 120 °C) could be due to a change of substrate quality and microbial activity, to an increase in N incorporation into humic substances, or to fixation by the mineral soil. Vázquez et al. (1993) found that, in the short term, fire produced a sharp increase in microbial numbers but affected the various groups studied differently. They attributed this increase to the favourable moisture, temperature and nutritional conditions and to the changes in the quality of the organic substrate. Studying N mineralisation in burnt eucalypt forests, Weston and Attiwill (1990), found that net mineralisation of N increased with increasing fire intensity, but within 100 days of the fire, some N immobilisation occurred, which was greater on sites of higher fire intensity.

Soils heated to 250 °C with inoculum showed similar N mineralisation as those without inoculum (Fig. 3), except for the leached sandy OUn soil, where the inoculum stimulated the mineralisation. This suggested that lack of protection to microorganisms had a major impact on both the microbial population (N-flush, Fig. 1) and N mineralisation (Fig. 3).

Autoclaved soils mineralised less than the control and 120 °C heated soils in BFG, OUn and PC soils, whereas in ODu and OGn soils the values were similar to those found in controls and higher than those of 120 °C heated soils (Fig. 3). Only two soils (BFG and PC) showed nitrification (Fig. 3). Nitrate was produced in the control and in the 60 °C heated BFG soil after 20 and 60 days, whereas in 120 °C heated soils, nitrification occurred 60 days later (data not presented), indicating a slow recovery of nitrifiers. After 160

days of incubation, most of the mineral N in these treatments of BFG soil was present as a nitrate (Fig. 3). Under conditions where all the NH_4-N is nitrified, the possibility of its loss through denitrification increases. Woodmansee & Wallach (1981) reported that denitrification occurred after intense burning, where the excess of mineralised NH_4-N was nitrified. Similar results to those in BFG soil were also obtained for the PC soil which, however, had a lower amount of nitrate (14% of the mineral N in control soil) and poorer recovery in 60 °C and 120 °C heated samples (9% and 3% of mineral soil as nitrate respectively). No nitrate was produced when nitrifying soils were heated to 250 °C or autoclaved, despite the accumulation of high amounts of NH_4-N (Fig. 3), suggesting high sensitivity of nitrifying organisms to heat (Ahlgren & Ahlgren 1965; Dunn et al. 1979).

Effect of autoclaving (moist heat) seems to be more drastic than that of dry heat on nitrifiers because soils subjected to dry heat of 120 °C showed nitrification in some cases (Fig. 3). Dunn et al. (1985) working in heated soils found that the different microbial populations varied in their sensitivity to high temperatures: fungi > nitrite oxidisers > heterotrophic bacteria. Marion et al. (1991) observed that in the burnt chaparral soils of California, the amount of nitrate decreased with increasing fire severity. However, Bauhus et al. (1993) and Khanna et al. (1994) incubating a range of burnt soils and soils mixed with ash, suggested that ash might create favourable conditions for nitrification after a bushfire. Thus soil heating and addition of ash, as both occur in a fire, may have opposing effects on nitrifier populations.

Microbial Biomass N

Nitrogen associated with microbial biomass recovered slightly during incubation in the heated and autoclaved soils. Soils heated to 60 °C had a similar N-flush at the control (Fig. 4). In 120 °C heated BFG and PC soils, the N-flush was not different from control, but in OUn, ODu and OGn soils its recovery was not complete (between 33% and 52% of the control value). Poor recovery of microbial biomass was recorded in all soils heated to 250 °C (50–56% recovery for BFG, OGn and PC soils, but only 19–23% for OUn and ODu sandy soils). Fritz et al. (1993) concluded that the microbial biomass C and N takes at least 10 years to recover after fire in coniferous forests. In contrast, in a short term after a bushfire, Vázquez et al. (1993) found rapid soil recolonization by microorganisms, which resembled control values one year after the fire.

Inoculation of 250 °C heated soils did not increase the N-flush, which was similar to values in the soil without inoculum. Díaz-Raviña et al. (1992) also found a slow microbial biomass recuperation in soils heated to 160 °C and 350 °C, and no recovery in soils heated to 600 °C, after they had been inoculated and incubated.

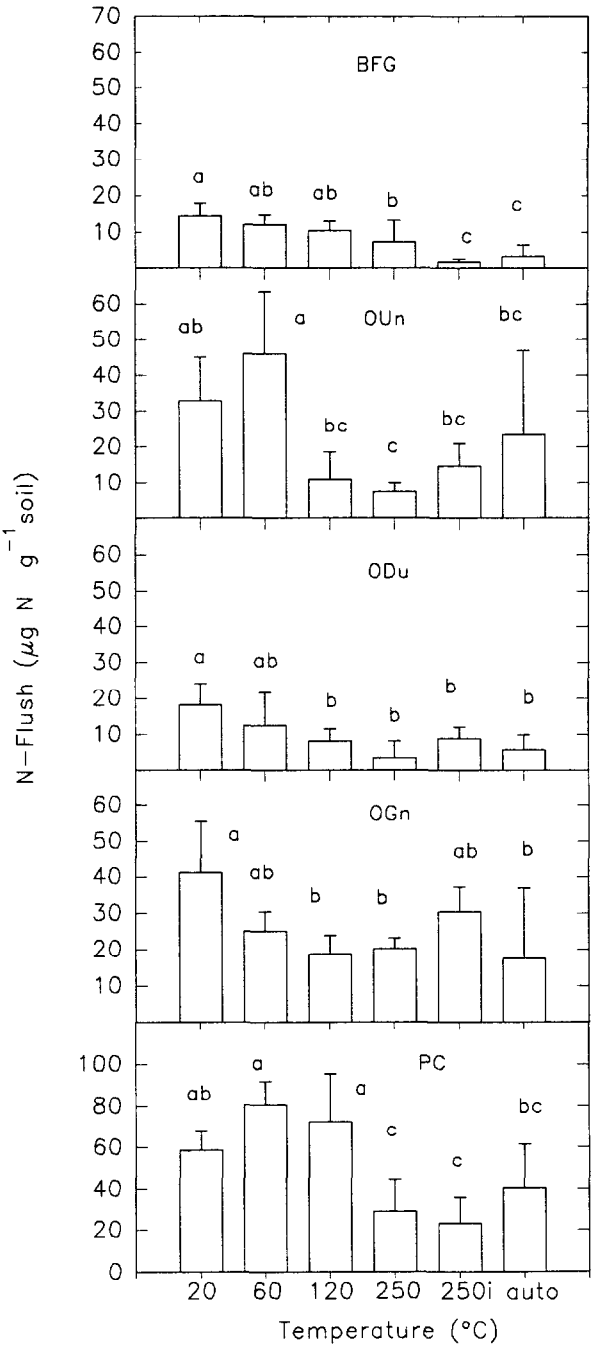

Fig. 4. N-flush in heated and autoclaved soils after 160 days of incubation. Bars represent standard deviation. A different lowercase letter indicates a statistically significant difference at the $P = 0.05$ level. Note the different scale in PC soil.

High variability in microbial biomass was exhibited among autoclaved soils, with recovery that was complete for OUn and PC soils, but 78, 68 and 51% lower than the control for BFG, ODu and OGn soils, respectively. In general, autoclaved soils showed similar microbial biomass as those subjected to 120 °C heating, except BFG and PC soils which had a lesser amount.

Soil respiration of heated and autoclaved soils during incubation

Soil respiration (mg $C\text{-}CO_2.g^{-1}$soil) was related to the soil C (%) in control soils ($r^2 = 0.89$, $P < 0.0001$, $n = 20$). PC soil had the highest respiration value (4.9 mg $C\text{-}CO_2.g^{-1}$), followed by OGn soil (4.2 mg $C\text{-}CO_2.g^{-1}$), and OUn, ODu and BFG soils with respective values of 3.7, 2.3 and 1.3 mg $C\text{-}CO_2.g^{-1}$ for 210 days of incubation.

Soils heated to 60 °C had respiration values similar to control, but soils heated to higher temperatures showed two distinct patterns in their respiration rates (Fig. 5). During the initial phase of 30 days, the respiration rate in soils heated to 250 °C was equal to (BFG, ODu and OUn) or higher than (OGn and PC) those heated to 120 °C and control. During the second phase (from 31 to 210 days) the respiration rate decreased for all treatments and the soils heated to 250 °C and 120 °C were equal to (BFG and PC) or lower than (OGn, ODu and OUn soils) control by 20% to 64%. Inoculated soils previously heated to 250 °C showed higher respiration rates in the first phase (BFG, OUn and PC) (Fig. 5), but equal in the second phase when compared with the non-inoculated soils. Autoclaved soils had higher respiratory activity at the beginning of incubation in OUn, OGn and PC soils (Fig. 5), although the rates decreased to values below those of the control in the second phase for all soils.

It was earlier pointed out that heating soils at high temperature killed microbial biomass and solubilised organic carbon. This carbon was easily mineralisable (Jenkinson 1966; White et al. 1973; Okano 1990) and was respired within 30 days of incubation. Inoculation of soils stimulated respiration without any time lag in the development of microbial populations, which had otherwise occurred in heated soils. Funke & Harris (1968) found an increase in respiration in soils heated to 80 °C, but when incubated soils were heated again these authors observed a dramatic decrease in respiration, suggesting that during the incubation the spores germinated and became labile to the second heat shock. West et al. (1992) found that only part of the increase in extractable C on drying soils could be accounted for by the decrease in microbial-C. In our study, the highest decrease in respiration rate with incubation for heated soils, compared to the control, indicated that the soluble carbon released by heating and incubation was rapidly depleted, leaving other sources of less labile carbon for further mineralisation. Similar

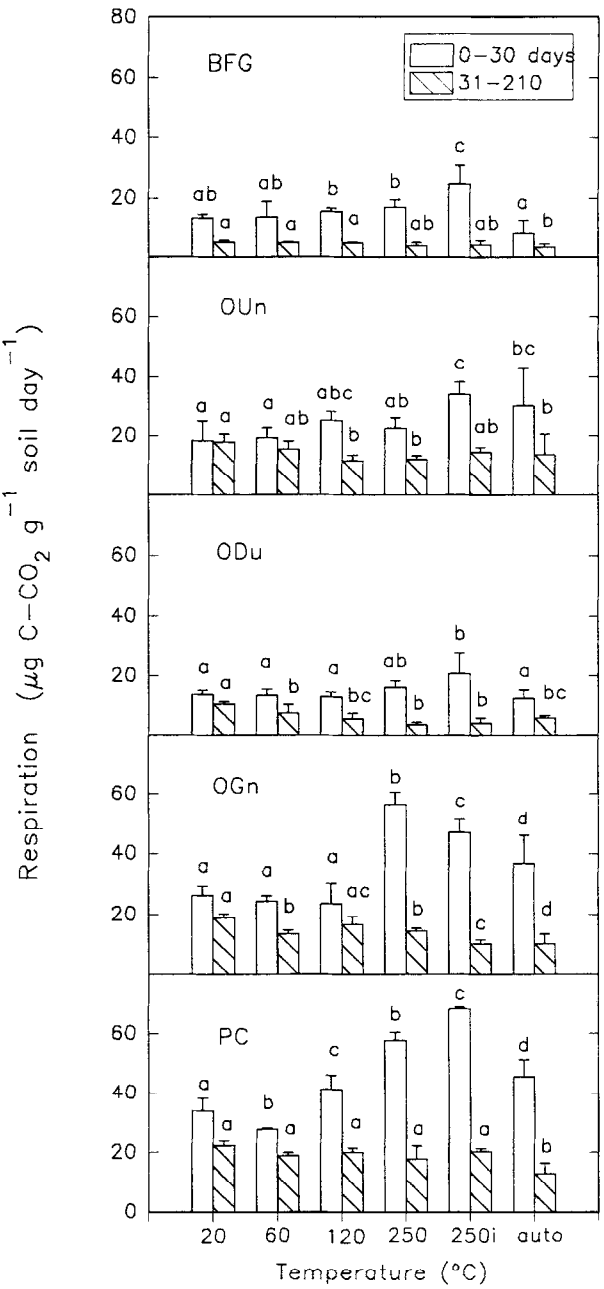

Fig. 5. Respiration rates in heated and autoclaved soils during incubation. Incubation period was split in two phases: the first 30 days and a second phase from 31 to 210 days. Bars represent standard deviation. A different lowercase letter among phases indicates a statistically significant difference at the $P = 0.05$ level.

results have been found for autoclaved soils. Bunt & Rovira (1955) and Raison & McGarity (1980) found that soil sterilisation (autoclaving) depressed both oxygen adsorption and CO_2 evolution. Several authors (Bowen & Rovira 1961; Salonius et al. 1967) have described inhibitory effects of autoclaving on plant and microbial growth.

Conclusions

Heating and autoclaving soils had immediate and long-term effects on nitrogen dynamics and on soil respiration patterns during 210 days of laboratory incubation. Treatments studied simulated temperatures observed in bushfires.

As generally observed after a bushfire, ammonium increased immediately after soil heating or autoclaving (dry- and moist-heating). A parallel decrease of microbial biomass on increasing temperature suggested that microbial N might be a source of soluble N in heated soils. Both microbial and non-microbial N were sources of soluble N in heated soils. Moreover, when soils were heated to 250 °C and higher, some N was lost by volatilisation. Consequently, labile mineralisable N content in heated soils would be a balance between organic nitrogen solubilisation and gas losses of N, and these processes were affected by soil characteristics (moisture content, organic matter content, clay content and microbial-N). Compared to the total soil N, gas losses of N were low, but they were high when compared to the labile N fraction, which is the easily mineralisable N in soils. Generally, in a bushfire, volatilisation losses of N are high because plant and soil litter are combusted by fire, but losses from mineral soil are usually confined to surface 5 cm depth and would depend upon the degree of combustion (temperatures reached). The gas N losses from the surface soil recorded in this experiment could be in the range occurring under natural conditions.

In the longer-term incubation, both heated and autoclaved soils partly recovered their biological activity. N mineralised during 160 days of incubation showed different patterns among soils indicating that soil N mineralisation depended on heating temperature and soil characteristics, such as clay and organic matter content, as well as the microbial populations. Nitrification was negligible after heating at 250 °C or on autoclaving soils, supporting the hypotheses that soil nitrifying bacteria are adversely affected by intense burning. Microbial biomass recovered well at mild heating, but poor recovery was recorded at high temperatures, even after reinoculating the 250 °C heated soils. Carbon solubilised by heating was quickly respired by the microbial population and, later on, respiration rate decreased to values similar to those of the control. In a similar way, after bushfires, microbial activity is generally

enhanced due to the enhanced C and nutrient availability caused by both heating and addition of ash, but revert quickly to prefire levels.

The complex effects observed in heated and autoclaved soils plus the significant gas losses of N estimated at 250 °C may affect the C and N status and their availability in these soils. The consequences of bushfires on soil N are more complex because, in addition to the effects of heat presented in this paper, losses of N occur during the fire and addition of ash, alone or in interaction with heat, would affect processes relating to N.

Acknowledgments

We would like to thank to KL Jacobsen, W Pienkowska, SJ Smith, JS Sweeney, AKMA Hossain & JS Holtzapffel for field and technical assistance; and VR Vallejo, J Romanyà, AM O'Connell and anonymous referees for constructive comments on the manuscript. Isabel Serrasolsas received a scholarship from the Spanish 'Ministerio de Educación y Ciencia' on the 'Programa de becas de Formación de Personal Investigador en el extranjero'.

References

Adams MA (1992) Phosphatase activity and phosphorus fractions in karri (*Eucalyptus diversicolor* F. Muell) forest soils. Biol. Fertil. Soils 14: 200–204

Ahlgren IF & Ahlgren CE (1965) Effects of prescribed burning on soil microorganisms in a Minnesota jack pine forest. Ecology 46: 304–310

Bauhus J, Khanna PK & Raison RJ (1993) The effect of fire on carbon and nitrogen mineralization and nitrification in an Australian forest soil. Aust. J. Soil Res. 31: 621–639

Bauhus J & Khanna PK (1994) Carbon and nitrogen turnover in two acid forest soils of southeast Australia as affected by phosphorus addition and drying and rewetting cycles. Biol. Fertil. Soils 17: 212–218

Binkley D & Christensen N (1992) The effects of canopy fire on nutrient cycles and plant productivity. In: Laven R & Omi P (Eds) Pattern and process in crown fire ecosystems. Princeton University Press, Princeton, New Jersey, USA

Bowen GD & Rovira AD (1961) Plant growth in irradiated soils. Nature 191: 936

Bunt JS & Rovira AD (1955) The effect of temperature and heat treatment on soil metabolism. J. Soil Sci. 6(1): 129–136

DeBano LF, Eberlein GE & Dunn PH (1979) Effects of burning on chaparral soils: I. Soil nitrogen. Soil Sci. Soc. Am. J. 43: 504–509

Díaz-Raviña M, Prieto A, Acea MJ & Carballas T (1992) Fumigation-extraction method to estimate microbial biomass in heated soils. Soil Biol. Biochem. 24: 254264

Dunn PH, DeBano LF & Eberlein GE (1979) Effects of burning on chaparral soils: II Soil microbes and nitrogen mineralization. Soil Sci. Soc. Am. J. 43: 27–52

Dunn PH, Barro SC & Poth M (1985) Soil moisture affects survival of microorganisms in heated chaparral soil. Soil Biol. Biochem. 17(2): 143–148

Edwards NT (1982) The use of soda lime for measuring respiration rates in terrestrial systems. Pedobiologia 23: 321–330

Ellis RC & Graley AM (1983) Gains and losses in soil nutrients associated with harvesting and burning eucalypt rainforest. Plant Soil 74: 437–450

Falkiner RA, Khanna PK & Raison RJ (1993) Effect of superphosphate addition on N mineralization in some Australian forest soils. Aust. J. Soil Res. 31: 285296

Fenn ME, Poth MA, Dun PH & Barro SC (1993) Microbial N and biomass, respiration and N- mineralization in soils beneath 2 chaparral species along a fire induced age gradient. Soil Biol. Biochem. 25: 457466

Fritze H, Pennanen T & Pietikainen J (1993) Recovery of soil microbial biomass and activity from prescribed burning. Can. J. For Res. 23: 1286–1290

Fritze H, Smolander A, Levula T, Kitunen V & Malkonen E (1994) Wood-ash fertilization and fire treatments in a scots pine forest stand-effects on the organic layer, microbial biomass, and microbial activity. Biol. and Fert. Soils 17: 57–63

Funke BR & Harris JO (1968) Early respiratory responses of soil treated by heat or drying. Plant Soil 28(1): 38–48

Gee GW & Bauder JW (1986) Particle-size analysis: Hydrometer method. In: Methods of soil analysis. Physical and mineralogical methods. Klute A (Ed) Agronomy 9. Part I. ASA.SSSA

Giovannini G, Lucchesi S & Giachetti M (1990) Beneficial and detrimental effects of heating on soil quality. In: Goldammer JG & Jenkins MJ (eds) Fire in ecosystem dynamics. Mediterranean and northern perspectives. The Hague: SPB Academic Publishing. The Netherlands. 95–102

Hossain AKMA, Khanna PK & Raison RJ (1990) A simple method of extracting microbial biomass in acid forest soils. In: Transactions of 14th International Congress of Soil Science, III, 240–241, Kyoto, Japan

Hossain AKMA, Khanna PK & Field JB (1993) Acid peroxide digestion procedure for determining total nitrogen in chloroform-fumigated and non-fumigated soil extracts. Soil Biol. Biochem. 25(7): 967–969

Jenkinson DS (1966) Studies on the decomposition of plant material in soil. II Partial sterilization of soil and the soil biomass. J. Soil Sci. 17: 280–302

Jenkinson DS & Powlson DS (1976) The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8: 209–213

Jenkinson DS (1988) Determination of microbial biomass carbon and nitrogen in soil. In: Wilson JR (Ed) Advances in nitrogen cycling in agricultural ecosystems. CAB International

Khanna PK & Raison RJ (1986) Effects of fire intensity on solution chemistry of surface soil under *Eucalyptus pauciflora* forest. Aust. J. Soil Res. 24: 423–434

Khanna PK, Raison RJ & Falkiner RA (1986) Exchange characteristics of some acid organic-rich forest soils. Aust. J. Soil Res. 24: 67–80

Khanna PK, Raison RJ & Falkiner RA (1994) Chemical properties of *Eucalyptus* litter ash and effects of ash on forest soils. For. Ecol. Manage. 66: 107–125

Khare DK, Hare PK & Mishra GP (1982) Effect of heat on nutrient release from soil under tropical dry deciduous forest. Jap. J. Ecol. 32: 107–110

Kieft LT, Soroker E & Firestone MK (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol. Biochem. 19: 119–126

Kitur BK & Frye WW (1983) Effects of heating on soil chemical properties and growth and nutrient composition of corn and millet. Soil Sci. Soc. Am. J. 47: 91–94

Kutiel P & Shaviv A (1989) Effect of simulated forest fire on the availability of N and P in Mediterranean soils. Plant Soil 120: 57–63

Marion GM, Moreno JM & Oechel WC (1991) Fire severity, ash deposition, and clipping effects on soil nutrients in chaparral. Soil Sci. Soc. Am. J. 55(1): 235–240

Marshman NA & Marshall KC (1981) Bacterial growth on protein in the presence of clay minerals. Soil Biol. Biochem. 13: 127–134

Marumoto T, Anderson JPE & Domsch KH (1982) Mineralization of nutrients from soil microbial biomass. Soil Biol. Biochem. 14: 469–475

McLaughlin MJ, Alston AM & Martin JK (1986) Measurement of phosphorus in the soil microbial biomass: a modified procedure for field soils. Soil Biol. Biochem. 18: 437–443

Okano S (1990) Availability of mineralized N from microbial biomass and organic matter after drying and heating of grassland soils. Plant Soil 129: 219225

Polglase PJ, Attiwill PM & Adams MA (1992a) Nitrogen and phosphorus cycling in relation to stand age of *Eucalytus regnans* F. Muell. II. N mineralization and nitrification. Plant Soil 142: 167–176

Polglase PJ, Attiwill PM & Adams MA (1992b) Nitrogen and phosphorus cycling in relation to stand age of *Eucalyptus regnans* F. Muell. III. Labile inorganic and organic P, phosphatase activity and P availability. Plant Soil 142: 177–185

Raison RJ & McGarity JW (1980) Effects of ash, heat and ash heat interaction on biological activities in two contrasting soils. I. Respiration rate. Plant Soil 55: 363–376

Raison RJ, Khanna PK & Woods PV (1985) Mechanisms of element transfer to the atmosphere during vegetation fires. Can. J. For. Res. 15: 132–140

Raison RJ & Myers BJ (1992) The biology of forest growth experiment: linking water and nitrogen availability to the growth of *Pinus radiata*. For. Ecol. Manage. 52: 279–308

Ross DM (1990) Influence of soil mineral nitrogen content on soil respiratory activity and measurements of microbial carbon and nitrogen by fumigation-incubation. Aust. J. Soil Res. 28: 311321

Rundel PW (1981) Fire as an ecological factor. In: Physiological plant ecology I. Lange, Nobel, Osmond & Ziegler (Eds) Springer-Verlag Berlin Heidelberg 12A: 501–538

Salonius PO, Robinson JB & Chase FE (1967) A comparison of autoclaved and gamma-irradiated soils as media for microbial colonization experiments. Plant Soil 27(2): 239–248

Sertsu SM & Sánchez PA (1978) Effects of heating on some changes in soil properties in relation to an Ethiopian land management practice. Soil Sci. Soc. Am. J. 42: 940–944

Singh RS, Raghubanshi AS & Singh JS (1991) Nitrogen mineralization in dry tropical savanna: effects of burning and grazing. Soil Biol. Biochem. 23: 269–273

Sorensen LH (1974) Rate of decomposition of organic matter in soil as influenced by repeated air drying-rewetting and repeated additions of organic material. Soil Biol. Biochem. 6: 287–292

Stace HCT, Hubble GD, Brewer R, Northcote KM, Slemman RJ, Mulcahy MJ & Hallsworth EG (1968) A handbook of Australian soils. Rellim Technical Publications: Glenside, South Australia

Tomkins IB, Kellas JD, Tolhurst KG & Oswin DA (1991) Effects of fire intensity on soil chemistry in a eucalypt forest. Aust. J. Soil Res. 29: 25–47

Van Gestel M, Ladd JN & Amato M (1991) Carbon and nitrogen mineralization from two soils of contrasting texture and microaggregate stability influence of sequential fumigation, drying and storage. Soil Biol. Biochem. 23: 313–322

Van Gestel M, Merckx R & Vlassak K (1993) Microbial biomass responses to soil drying and rewetting the fate of fast- and slow-growing microorganisms in soils from different climates. Soil Biol. Biochem. 25: 109–123

Vázquez FJ, Acea MJ & Carballas T (1993) Soil microbial populations after wildfire. FEMS Microbiol. Ecol. 13: 93–104

Walker J, Raison RJ & Khanna PK (1986) Fire. In: JS Russell & JS Isbell (Eds) Australian soils. The human impact. Univ. Queensland Press, Australia pp 185–216

Wells CG, Campbell RE, DeBano LF, Lewis CE, Fredriksen RL, Franklin EC, Froelich RC & Dunn PH (1979) Effects of fire on soil. A state-of-knowledge review. National fire effects workshop, Denver, Col., April 1978. USDA For. Serv. Gen. Tech. Rep. WO-7 34 pp

West AW, Sparling GP, Speir TW & Wood JM (1988) Dynamics of microbial C, N-flush and ATP, and enzyme activities of gradually dried soils from a climosequence. Aust. J. Soil Res. 26: 519–530

West AW, Sparling GP, Feltman CW & Reynolds J (1992) Microbial activity and survival in soils dried at different rates. Aust. J. Soil Res. 30: 209–222

Weston CJ, Attiwill PM (1990) Effects of fire and harvesting on nitrogen transformations and ionic mobility in soils of *Eucalyptus regnans* forests of south-eastern Australia. Oecologia 83: 20–26

White EM, Thompson WW & Gartner FR (1973) Heat effects on nutrient release from soils under ponderosa pine. J. Range Manage. 26(1): 22–24

Wilbur RB & Christensen NL (1985) Effects of fire on nutrient availability in a North Carolina coastal plain pocosin. Amer. Midland Nat. 110: 54–61

Woodmansee RG, Wallach LS (1981) Effects of fire regimes on biogeochemical cycles. In: Clark FE & Rosswall T. Terrestrial nitrogen cycles. Ecol. Bull. (Stockholm). 33: 649–669