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Volatile and water-soluble inhibitors of nitrogen mineralization and nitrification in a ponderosa pine ecosystem

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Summary. Bioassay experiments were performed to test for inhibition of the processes of nitrogen mineralization and nitrification by organics in the forest floor of a ponderosa pine ecosystem. Water-extractable organics in the forest floor were tested by applying filtered extracts to the assay soil. The extract decreased nitrate production by 17.0% and decreased net mineralization by 4.1%. Inhibition by volatile organics was tested by placing vials containing forest floor or selected terpenoids of ponderosa pine in sealed jars containing the assay soil. Nitrate production was inhibited by 87.4% and 100%, and net nitrogen mineralization was inhibited by 73.3% and 67.7% in the jars with forest floor and terpenoids, respectively. Organics which are partially water-soluble and are volatile (such as terpenoids) would be very effective inhibitors of nitrogen cycling processes.

Key words: Nitrogen mineralization – Nitrification – Water-soluble inhibitors – Allelochemic control

Allelochemic inhibition of the processes of nitrogen mineralization (Lamb 1975; Gosz 1981) and nitrification (Baldwin et al. 1983; Rice 1984) has been documented or suggested to occur in many ecosystems. It is generally thought that polyphenols, and particularly tannins, produced by the vegetation are the inhibitory agents. Ponderosa pine ecosystems exhibit potential allelochemic control of both nitrogen mineralization and nitrification processes (Lodhi Killingbeck 1980; Vitousek et al. 1982; White 1986; Gosz and White, in press). Lodhi and Killingbeck (1980) demonstrated that water extracts of ponderosa pine needles had the highest polyphenol content and the greatest toxic effects on laboratory cultures of nitrifying bacteria (a 93% reduction in the number of nitrifying bacetria). White (1986) reported significantly increased rates of nitrogen mineralization and nitrification in ponderosa pine forest floor and mineral soil horizons in plots treated by prescribed fire. He suggested that volatile organics (particularly terpenoids) may control the processes of nitrogen mineralization and nitrification in the unburned forest floor and soil.

The effects of volatile organics on the processes of nitrogen mineralization and nitrification are unknown. Volatile organics are known to inhibit specific microbial species and are commonly used as antibiotics (Stotzky and Schenck 1976). Resistance to bark beetle has been attributed to the reduced growth of the fungi associated with the beetle due to the inhibitory effects of volatiles and other constituents of coniferous tree resins (Cobb et al. 1968; Smith 1977; Sturgeon 1979; Raffa and Berryman 1982). However, a given terpenoid had different effects on the various species of fungi, inhibiting growth of some species and stimulating growth in others. Similarly, a given species of fungi was stimulated by some terpenoids and inhibited by others. Muller (1965) reported soil bacteria populations were smaller in areas void of other vegetation beneath *Salvia* than in nearby areas with grass vegetation. Muller suggested that volatiles may inhibit general mineral soil populations; how-

ever, he noted that the difference in bacteria populations may be the result of either inhibition by volatiles or stimulation of growth in the grass areas. Since nitrogen mineralization and nitrification processes are performed by a complex microbial community, the effects of terpenoids and other volatiles on these processes cannot be determined from the general literature.

The objectives of this research were to determine the effects of (1) water-soluble organics, (2) volatile organics in the forest floor of ponderosa pine, and (3) selected ponderosa pine resin terpenoids on the processes of nitrogen mineralization and nitrification. Bioassay experiments, under controlled conditions in the laboratory, were used to determine these effects.

Methods

White (this issue) identified immediate post-burn forest floor and mineral soil samples from a ponderosa pine ecosystem which demonstrated significantly increased rates of nitrogen mineralization and nitrification over unburned areas. Five months after the fire treatment, samples of forest floor and mineral soil were taken from the perimeter of the 4 × 9 m area of plot #7 (treated with prescribed fire) and plot #8 (a control plot) using the methods described below. The composite mineral soil from plot #7 was used as the assay soil in all bioassay experiments. The forest floor from plot #8 was used in both the water-soluble and volatile assay. The proportion of forest floor to mineral soil found in the field was maintained in all bioassay experiments.

Bioassay for effects of water-soluble organics. The forest floor composites from plot #7 and #8 (335 g and 909 g/0.25 m^2, respectively) were extracted with 6250 ml water. This amount of water simulated a 2.54-cm precipitation event leaching through the composited forest floor. After agitation by hand for 1 h followed by settling overnight, the forest floor solution was poured off and suction-filtered. Both extracts were diluted by 5 and by 25 to give X1 (no dilution), X1/5, and X1/25 concentrations.

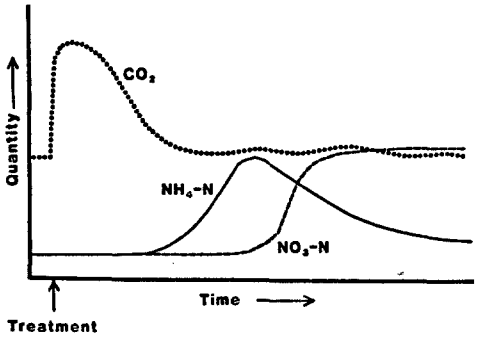

Fig. 1. Theoretical variation in respiration (CO_2) and inorganic nitrogen production following disturbance (treatment) of a soil. This pattern would be expected following any treatment that disturbs the soil (such as sample collection) and results in the creation of newly exposed (or available) resources to the heterotrophic decomposer community. Requirements of the decomposer community for nitrogen must be met prior to an increase in the available forms of nitrogen

The bioassay experiment consisted of the application of the extract solutions and a demineralized water blank to subsamples of the assay soil. The appropriate amount of an extract was added to the subsample to simulate the 2.54-cm precipitation event added to the top 10 cm mineral soil. After addition of the extract solution, the subsample was adjusted to a water content of -0.1 bars (see method below). Measurements of nitrogen mineralization potential and respiration were performed on each subsample using the methods below.

The application of the water extracts could cause a delay in ammonification and nitrification as a result of two very different factors which are related to the availability of the carbon substrate (Fig. 1 may aid in understanding the theoretical relationship between the rates of carbon and nitrogen mineralization). (1) Application of an inhibitor (depicted as treatment in Fig. 1) which affects the general decomposer community would lower the processing rates of both carbon (as measured by the rate of CO_2 evolution) and nitrogen (as measured by ammonium production). This would lengthen the time scale for the production of inorganic ammonium and effectively lower nitrogen mineralization rates. (2) Application of readily available organic substrate (treatment in Fig. 1) would stimulate growth in the microbial populations and would increase microbial respiration. This would result in the immobilization of nitrogen in the growing microbial biomass and would delay the production of inorganic ammonium until microbial growth requirements for nitrogen were satisfied. Thus, both measures of carbon mineralization (respiration) and nitrogen mineralization would be necessary to identify inhibition.

Bioassay for effects of volatile organics. The bioassay for volatile inhibitors used three amendments: (1) no treatment (control), (2) added forest floor, and (3) added simulated ponderosa pine resin terpenoids. The terpenoid treatment consisted of equal parts of limonene, myrcene, alpha-pinene and beta-pinene (Aldrich Chemical Company, Inc.), and beta-phellandrene (ICN Pharmaceutical, Inc., Plainview, NY). This mixture contained the major terpenoids found in ponderosa pine resin (Cobb et al. 1968). For each amendment, 50 g assay mineral soil was placed in 6 replicate jars. The forest floor material (2 g ash-free weight, 3.75 g fresh weight) or terpenoids (3 ml) were placed in small beakers and set inside the jar to avoid contact with the soil except by vapor. The concentrations of the volatile components in the jar atmosphere (although not measured directly) would be determined by their vapor pressures at the given incubation temperature. Three (3) of the replicates were moisture corrected, and the jars were sealed and placed in the dark at room temperature. The other 3 replicate jars were sealed, set in an oven at 60°C for 6 h (to achieve higher concentrations of volatiles at approximately maximum field temperature and under field moisture conditions), removed, and cooled. The soil moisture was adjusted and the jars were treated in the same manner as the first three replicates. All jars were opened weekly to replenish their atmosphere and maintain aerobic conditions. After 10 weeks, the soil was analyzed for nitrate and ammonium by the methods described below.

General methods. Forest floor samples contained all organic horizons (L, F, and H). The entire forest floor beneath a 100-cm^2 template was collected (Gosz et al. 1976). Five replicates were taken at 1-m intervals along a line transect. The five replicates were composited. In the laboratory, twigs and stones larger than 6.4 mm diameter were removed from forest floor collections.

Mineral soil samples were collected to a 10-cm depth with an auger 10 cm in diameter. The collection was made at the same point along the line transect where the forest floor was collected. The five replicates were composited in the field. Roots were

removed by hand sorting. Stones larger than 6.4 mm diameter were removed by sieving.

Subsamples of the forest floor and mineral soil composites were removed to perform other measurements. The water content at -0.1 bars was determined for the assay mineral soil by applying suction to a soil pressure plate holding a water-saturated subsample of the composite. Each subsample of the assay soil was then adjusted to -0.1 bars before analyses for nitrogen mineralization potential and potential respiration rates.

The method of Vitousek et al. (1982) was used to determine the potential rate of nitrogen mineralization. This method used aerobic incubation of samples at 20°C. A total of 35 subsamples of each composite were weighed into plastic cups. Five cups of each composite were extracted immediately with 100 ml 2 *N* KCl (containing 0.5 ppm phenylmercuric acetate). Five cups of each composite were extracted after incubations of 1, 2, 4, 6, 8, and 10 weeks. After settling, the clarified extracts were analyzed for inorganic ammonium by the modified Technicon AutoAnalyzer method of White and Gosz (1981) and for nitrate by the Technicon AutoAnalyzer cadmium reduction method.

Potential respiration rates were measured by the alkaline trap method. Fifty grams of the assay mineral soil was placed in 500-ml glass jars and the total weight recorded. An open scintillation vial containing 5 ml 1 *N* NaOH was placed in each jar and the sealed jar incubated at 20°C. The vials containing the NaOH were removed at the same intervals as the nitrogen mineralization cups and replaced with vials containing fresh NaOH. The materials in the jars were adjusted to their initial water content when needed. The removed NaOH was quantitatively transferred to a 150-ml beaker, 5 ml 1 *N* $BaCl_2$ was added to precipitate the diffused CO_2 as insoluble $BaCO_3$, and the NaOH was titrated to a pH of 8.3 with 0.1 *N* acid phthalate. A jar containing only the NaOH trap was treated in the same manner as the samples and provided an experimental blank to correct for absorbed atmospheric CO_2.

Moisture content was determined by weight loss upon heating at 105°C to constant weight. Total N and P were determined by a modified Kjeldahl digestion (Schuman et al. 1973) followed by analysis for ammonium and orthophosphate on a Technicon AutoAnalyzer. Ash content was determined by loss upon ignition at 500°C. Metal cations were analyzed by atomic absorption spectrophotometry using flame techniques. Calcium and magnesium were analyzed with lanthanum additions to prevent ionization in the flame.

Terpenoids were analyzed by gas chromatography. Approximately 1000 mg of frozen sample was ground under liquid nitrogen using a mortar and pestle. About 400 mg ground material was transferred to a tissue homogenizer and extracted 4 times with 0.5 ml diethyl ether. The extracts were filtered through silica gel. The filtrate and the filter washes were combined and spiked with fenchyl acetate as an internal standard. This mixture was brought up to 4.0 ml. After vortexing, a volume (usually 4.0 μl) was injected into a capillary gas chromatograph fitted with a bonded methylsilicone capillary column (Perkin-Elmer Corporation). The injector temperature was 200°C and the initial oven temperature was 60°C. The temperature gradient consisted of 60°–210°C at 4°/min and the final temperature was held for 10 min. Tentative identification of sample peaks was made by comparison with the retention times of known standards.

Relative tanning capacity was determined by a modified hemoglobin-binding method. This method measures the amount of protein left in a standard hemoglobin solution (bovine hemoglobin substrate powder type II; Sigma H-2625) after precipitation by the tannins in the sample (or standard tannin solution). A sample (1000 mg fresh weight) was ground under liquid nitrogen in a mortar and pestle. A 100-mg subsample was extracted by repeated grinding with 0.5 ml 50% aqueous methanol (v/v) in a tissue homogenizer. The solution was centrifuged and the solid discarded. The methanol was removed under a nitrogen atmosphere by heating in a 55°C shaker bath for 30–60 min. The solution was washed into a graduated cylinder and was brought to a final volume of 4.0 ml. If the solution was cloudy, it was centrifuged again. About 2.5–3.0 ml was filtered through a series of filters with the final pore size of 0.45 μm. A 1.0-ml portion of the filtered solution (either forest floor extract or the assay water-extract) was vortexed with 1.0 ml hemoglobin solution (buffered at pH 6.5 with 0.1 *M* phosphate buffer). After 15 min, the sample was centrifuged. A 1.0-ml portion of the supernatant (containing the unbound protein) was filtered through a column containing about 2.5 cm^3 hydrated Sephadex G 25–150 to remove phenolics. The filtered solution and washes were brought to a volume of 5.0 ml. In a spectrophotometer cuvette, 0.3 ml of the sample solution was mixed with 7.5 ml protein dye reagent (Bio-Rad Chemical Division 500–0006), vortexed, and the absorbance determined at 595 nm after 15 min. The relative tanning capacity was determined from a standard curve using Quebracho tannin (Pilar River Plate Corp., Newark, NJ) treated in the same manner as the samples.

Statistical methods. All statistical analyses were performed on SAS programs (Statistical Analysis System, SAS Institute Inc. 1982). MANOVA and Duncan's Multiple Range Test were used for most analyses. MANOVA analyses allowed the effect of treatment to be analyzed as a single factor. The weekly data were also analyzed by ANOVA for significant differences. This type of analysis allowed detection of significant treatment effects when none of the individual weekly analyses was significantly different.

Results

Bioassay for effects of water-soluble organics

The relative tanning capacities of the burned and control forest floor materials used in the assays were very low, with the burned forest floor having undetectable levels (Table 1). The water extracts were analyzed for major inorganic nutrients, pH, tanning capaci-

Table 1. Characteristics of the forest floor and the resultant water extracts collected from unburned plots (control) and plots managed with prescribed fire (burned)

Sample/characteristic	Control	Burned
Forest floor		
Total fresh weight (g/0.5 m^2)	1818	669
Tanning capacity (expressed as tannic acid equivalents, % fresh weight)	0.14	< 0.08
Water extracts		
Tanning capacity (expressed as tannic acid equivalents, mg/ml)	< 0.4	< 0.4
Relative absorbance (200–700 nm)	8210	5760
pH	4.31	7.67
Ca (μg per ml)	25.0	81.0
Mg (μg per ml)	5.0	12.5
Na (μg per ml)	2.00	5.05
K (μg per ml)	19.8	24.2
PO_4–P (μg per ml)	1.20	2.20
NH_4–N (μg per ml)	3.90	5.60
NO_3–N (μg per ml)	0.16	0.48

ty, and relative absorbance (Table 1). The tanning capacities of both extracts were below the detection limit. The relative absorbance of the extract from the burned forest floor was 70% of the absorbance of the extract from the control forest floor, although the total weight of the burned forest floor was only 36.8% of the control. This suggests the majority of the water-soluble fraction of the forest floor was contained in the lower forest floor horizons, which were the least consumed by the fire treatment. The humus layer of the forest floor typically contains large quantities of water-soluble "humic" compounds. Although the concentrations of inorganic nutrients varied between the two extracts, the addition of only 1.5 ml nondiluted extracts to 10 g (dry weight) mineral soil resulted in no detectable change in total soil nutrient levels or pH in any of the assay soils.

Respiration rates in the assay soils with the addition of the forest floor extracts were significantly lower than rates in the soil with the addition of demineralized water (Table 2). During a single 2-week period of the incubation, the pH of the water blank soil was about 0.5 units lower than all the soils which received extract additions. The lower pH in the control samples could have shifted the carbonic acid-bicarbonate equilibrium and released dissolved CO_2. Thus, some of the CO_2 could have been dissolved CO_2 which is exogenous to respiration sources. The pH of all the other soils were within 0.2 units of each other throughout the incubation period. Respiration rates for the undiluted extracts (X1 for burned and control) were nearly equal to each other. Similarly, respiration rates for all the dilutions were not significantly different from each other. This suggests that the undiluted extracts contained similar amounts of available carbon.

Table 2. Comparison of mean respiration rates during a 10-week incubation of amended mineral soil samples from a ponderosa pine ecosystem managed by prescribed fire. The amendments included the addition of: demineralized water (Water blank), water extracts of burned (Burned-1) and untreated (Control-1) forest floor samples, and the extracts diluted by a factor of 5 (Burned-5, Control-5) and by 25 (Burned-25, Control-25). Means followed by different letters are significantly different ($P < 0.05$)

Treatment	Respiration (mg CO_2/g/week) Mean	Grouping
Water blank	0.664	a
Control-1	0.570	b
Burned-1	0.563	b c
Control-5	0.524	c d
Control-25	0.513	d
Burned-5	0.506	d
Burned-25	0.502	d

The addition of the forest floor extracts significantly altered the nitrogen mineralization potential of the assay soil (Table 3). All of the extract additions resulted in an increase in mean ammonium levels in the assay soil relative to the demineralized water addition. Only the addition of the X1 and X1/5 extracts of the burned forest floor resulted in soil nitrate levels statistically equal to levels in the soil with the addition of the water blank. All other extract additions decreased nitrate levels. The most important effect of the extract additions occurred on the net nitrogen mineralization rates (the sum of ammonium and nitrate production rates). All extract additions, except the most dilute extracts, resulted in net nitrogen mineralization rates that were significantly different from the rates of the water blank addition. However, the rate of net nitrogen mineralization was increased with the addition of extracts from the burned forest floor and decreased with the addition of extracts from the unburned forest floor.

Bioassay for effects of volatile organics

Heating the jars and their contents had a significant effect on nitrogen mineralization and nitrification (Table 4). All the jars that were heated showed a

Table 3. Mean inorganic nitrogen levels during the incubation for nitrogen mineralization potentials on amended mineral soil samples from a ponderaosa pine ecosystem managed by prescribed fire. See Table 2 for explanation of treatments. For each analysis, means followed by different letters are significantly different ($P < 0.05$)

Ammonium (μg N/g)			Nitrate (μg N/g)			Ammonium + nitrate (μg N/g)		
Treatment	Mean	Grouping	Treatment	Mean	Grouping	Treatment	Mean	Grouping
Control-1	9.10	a	Burned-1	12.2	a	Burned-1	20.9	a
Control-25	8.86	a	Burned-5	12.2	a	Burned-5	20.6	a b
Burned-25	8.81	a b	Water blank	11.8	a	Burned-25	20.2	b c
Burned-1	8.75	a b	Burned-25	11.3	b	Water blank	19.7	c d
Burned-5	8.42	b c	Control-5	10.7	c	Control-25	19.5	d e
Control-5	8.28	c	Control-25	10.6	c	Control-5	19.0	e f
Water blank	7.88	d	Control-1	9.8	d	Control-1	18.9	f

Table 4. Inorganic nitrogen levels (μg N/g) in assay soils after 10-week incubation at 20°C. Vials containing either forest floor samples (FF), simulated ponderosa pine terpenoids (Terp), or nothing (Con) were placed in the jars with the assay soil and heated at room temperature (Rm) or 60°C for 6 h prior to incubation. For each analysis, means followed by different letters are significantly different ($P < 0.05$)

Ammonium		Nitrate		Ammonium + nitrate	
FF (60°C)	47.2 a	Con (Rm)	62.9 a	Con (Rm)	63.7 a
Con (60°C)	43.3 a	FF (Rm)	7.9 b	FF (60°C)	47.9 b
Terp (60°C)	27.7 b	Con (60°C)	2.5 c	Con (60°C)	45.8 b
Terp (Rm)	20.6 c	FF (60°C)	0.6 c	Terp (60°C)	27.7 c
FF (Rm)	9.1 d	Terp (Rm)	0.0 c	Terp (Rm)	20.6 c d
Con (Rm)	0.8 e	Terp (60°C)	0.0 c	FF (Rm)	17.0 d

Table 5. Retention times (RT), tentative identification (ID), relative concentration (expressed as parts per million of dry tissue) (ppm), and percentage of the pre-burn forest floor sample (%) for peaks on the gas chromatographs of pre-burn, immediate post-burn, and 5-months post-burn forest floor samples

ID	RT	Pre-burn (ppm)	Immediate post-burn (ppm)	(%)	5 month post-burn (ppm)	(%)
	6.4	0.0055		0		0
	6.9				0.0032	–
α-Pinene	7.26	0.0454	0.0035	7.7	0.0818	180.2
Camphene	7.65				0.0061	–
	8.25				0.0108	–
β-Pinene	8.53	0.0251	0.0017	6.8	0.0105	41.8
	9.06	0.0056		0		0
	9.76	0.0270		0	0.0205	75.9
Limonene	10.36	0.0135	0.0027	20.0	0.0055	40.7
	10.70	0.0036		0		0
	12.13				0.0029	–
	12.83	0.0098		0	0.0045	45.9
	13.01				0.0035	–
	13.55				0.0035	–
	14.13				0.0073	–
	14.28				0.0034	–
	14.54				0.0130	–
	16.32				0.0023	–
	16.70	0.0116		0	0.0087	75.0
	20.20				0.0050	–
	22.51				0.0097	–
	23.19				0.0046	–
	23.37	0.0099		0	0.0057	57.6
	23.61				0.0025	–
	23.77				0.0109	–
	24.27				0.0966	–

dramatic decrease in nitrate production. Nearly all of the mineralized nitrogen remained in the ammonium form in the heated soils. This indicates the nitrifying populations were particularly susceptible to heat effects. The heated soils exposed to volatile organics from the unburned forest floor were not significantly different from the control soils for ammmonium, nitrate (although lower than control levels), or net mineralized nitrogen levels. Exposure to the terpenoids resulted in significantly lower ammonium levels and net inorganic nitrogen levels. The terpenoids completely inhibited nitrification while the other heated soils had detectable amounts of nitrate.

The volatile organics had significant effects on the unheated soils (Table 4). The assay soil was efficient in converting ammonium to nitrate and had the highest net inorganic nitrogen production. The volatiles from the forest floor significantly reduced the net mineralization (the lowest of all samples). The forest floor did not completely inhibit nitrification and the nitrate levels were about equal to the ammonium levels in these samples. The terpenoids lowered net

mineralization relative to the control, but not as much as the forest floor. However, the terpenoids were 100% effective at inhibiting nitrate production (100% inhibition occurred at both temperature exposures).

The immediate pre-burn, immediate post-burn, and assay forest floor samples from plot #7 (collected 5 months after the fire treatment) were analyzed for their terpenoid content. The only compounds which were lower in both the post-burn and assay samples relative to the pre-burn sample were some of the compounds with retention times less than 24.3 min (compounds with lower boiling points, Table 5). Many compounds, including the compounds tentatively identified as alpha-pinene and camphene, were present in the assay forest floor sample in equal or greater concentration than the corresponding compound in the pre-burn sample. These compounds could not be responsible for inhibition since the post-burn samples demonstrated significantly higher rates of nitrogen mineralization and nitrification.

Discussion

The water extracts of the forest floor materials (burned and control) reflect the following effects of fire (Table 1): (1) an increase in pH, (2) an increase in base cations, and (3) an increase in water-soluble nutrients (particularly phosphorus). Small amounts of the extracts (at most 1.5 ml of the undiluted extracts) were added to subsamples of the assay soil but none of the additions significantly altered the assay soil characteristics. Thus, the effects of the water extracts cannot be attributed to changes in major soil parameters (such as pH).

Application of the undiluted extracts did increase respiration relative to the diluted extracts; thus, the extracts apparently added an available carbon source. Since the extracts were filtered to exclude microorganisms, the addition of the undiluted extracts should not have altered the initial composition of the microbial community in the assay soils. If immobilization of nitrogen by the microbial community during utilization of the added carbon source controls nitrogen mineralization, then both extracts should have the same immobilization capacity since similar concentrations of the extracts exhibited the same effect on respiration rates. However, the undiluted extracts had opposite effects on nitrogen mineralization and nitrification. The control forest floor extract significantly decreased net mineralization and nitrification while the burned forest floor extract signficantly increased ammonium production and net mineralization.

The nitrifying bacteria were very sensitive to the 60°C exposure. Belser (1979) cited studies where incubation of soils at 40°C completely inhibited nitrification and other studies where measured nitrification occurred in soils incubated at temperatures up to 60°C. The general conclusion was indigenous nitrifiers had temperature optima and maxima adapted to their climatic region. Ponderosa pine ecosystems are generally thought to be warm, dry forests. However, it is doubtful the mineral soil in ponderosa pine would ever be exposed to these temperatures (except where no forest floor exists) under normal field conditions. Soil temperatures may reach this level and hotter during prescribed fires and wildfires. The results may reflect the effect of the duration of exposure to elevated temperature (6 h) and the water content of the soil. Dunn and DeBano (1977) reported that *Nitrosomonas* and *Nitrobacter* bacteria were killed in dry soil at temperatures of 140°C, but at only 75° and 50°C, respectively, in wet soil. The assay soils were at field moisture content when they were heated, and perhaps more or less water in the soil would alter these results.

The other results from the trapped vapor experiment were particularly interesting from an ecological viewpoint. These data suggest: (1) the forest floor contains vapors which inhibit nitrogen mineralization processes in the soil and (2) the transfer of the vapor from the forest floor to the soil occurred at room temperature. No real thermal gradient was established since the forest floor and soil were at room temperature within the same jar.

A ponderosa pine stand in New Mexico was one of 17 forested sites studied by Vitousek et al. (1982). They identified processes having the potential to control the loss of nitrate after disturbance of forest ecosystems. Their research and the research performed by Gosz and White (in press) identified three factors which could account for the low nitrogen mineralization rates and the lag in nitrification found in the soil from their ponderosa pine site: (1) microbial immobilization of inorganic nitrogen (high C/N organic substrate), (2) refractory organic substrate, and (3) allelochemic inhibition. Vitousek et al. (1982) could not predict nitrogen mineralization rates from the C/N of the substrate in the soils using all of the 17 sites ($r^2 = 0.03$). This suggests that immobilization of nitrogen during processing of high C/N material did not control nitrogen mineralization across all the sites they studied. The results of the water-soluble bioassay also suggest immobilization of nitrogen during decomposition of available high C/N material does not control nitrogen mineralization processes in this soil since the rates of carbon processing (respiration) were the same in both undi-

luted extracts (Table 2). Also, the C/N of the assay soil was not different than prior to the fire treatment (White 1986). Thus, C/N and immobilization can be ruled out as factors which control nitrogen mineralization in this ponderosa pine ecosystem.

Refractory organics are probably not the main factor controlling nitrogen mineralization processes. If the refractory nature of the organic matter was the main factor, then the addition of relatively small amounts of refractory water-soluble material in the bioassay should have little effect on soil nitrogen mineralization and nitrification rates. This was not the case. Both processes of nitrogen mineralization and nitrification were significantly inhibited by the addition of relatively small amounts of water-soluble materials.

The role of water-soluble (completely or partially soluble) and volatile inhibitors in controlling the processes of nitrogen mineralization and nitrification is strongly supported by these results. Theories concerning allelochemic control of nitrogen mineralization processes have centered on the effects of tannins and polyphenols in coniferous ecosystems (Lamb 1975; Gosz 1981). The theory of inhibition of nitrification in this ecosystem and other "climax" ecosystems (Rice 1974 and 1984; Lodhi and Killingbeck 1980; Baldwin et al. 1983) also indicated tannins and polyphenols as the major inhibitors. However, neither the unburned forest floor not its extract had significant tanning capacities (Table 1). It is doubtful that polyphenols and/or tannins caused the changes in net mineralization in the bioassay soil or in the soil after the prescribed fire.

The residual forest floor collected within 24 h following a prescribed fire displayed increased ammonification and nitrification rates during incubation (White 1986). The immediate increase in nitrate means that the nitrifiers were present in the forest floor before the treatment, because it is unlikely they could have been introduced that quickly after the prescribed fire. If the tannins present before the fire were "toxic" and resulted in bacteria mortality (as suggested by the results of Lodhi and Killingbeck 1980), nitrifying bacteria would have been present in very low numbers or not at all.

It is unlikely that the majority of the polyphenols and the compounds which contribute to the tanning capacity would have been significantly altered in the soil by the fire treatments or the associated heat pulse. As reported by White (1986), the prescribed fire treatments were relatively light burns. The forest floor and mineral soil from plot #7 (used in these assays) demonstrated the greatest reduction in forest floor biomass and soil moisture after the burn treatments. This may indicate that the burn generated enough heat to reduce the concentrations of compounds with boiling points at or below that of water. Most polyphenols have boiling points much higher than that of water.

An alternative hypothesis to explain the above results is: Volatile organics (e.g., terpenoids) produced by ponderosa pine inhibit the processes of nitrogen mineralization and nitrification. The volatile materials are not directly toxic. The source of the volatiles would be the fresh litter and the forest floor. Transport of the volatile organics could be in the aqueous phase and in the volatile phase.

If terpenoids (and/or other volatiles) are responsible for the inhibition of nitrification and/or ammonification, this would be consistent with the results. Terpenoids have low boiling points and can form flammable volatile mixtures well in advance of the flame front in a fire (Chandler et al. 1983). Their removal would account for the immediate increase in nitrogen mineralization and nitrification in both the forest floor and mineral soil in plot #7 after the fire treatment. In addition, volatile organics could migrate along a thermal gradient from warm regions to cooler regions in the forest floor and/or mineral soil where condensation could occur. Assuming heating of the forest floor and soil occurs by solar insolation, such a thermal gradient within the forest floor and soil could be established on nearly a daily basis.

Many terpenoids are slightly water-soluble and could be transported in aqueous phase (Button 1984). Water draining a spruce-fir watershed in New Mexico was found to have 75% of the dissolved organics in the neutral fraction consisting of terpenoids (Rex G. Cates, personal communication). If the fire removed the inhibitory terpenoids and/or other volatiles, the water extract of the burned forest floor would not have inhibitory compounds (unless critical amounts of the inhibitor were added in litterfall or throughfall during the winter). Therefore, the addition of the burned forest floor extract could have a net fertilizer effect due to the dissolved nutrients and would stimulate nitrogen mineralization in the absence of inhibitors. The water extract of the unburned forest floor would contain any water-soluble inhibitor along with other organics. The addition of water-soluble inhibitors would account for the significantly reduced rates of net mineralization and nitrification in the assay soils seen in the unburned water extract addition. The extraction used in the bioassay simulated a single leaching of the forest floor by a 2.54-cm precipitation event. Beneath unburned forest floor, inhibitors may be added during each precipitation event which reaches the soil. A significant accumulation of inhibitors in the soil could occur after many precipitation events or after spring showmelt.

Given the above scenario, either extremely wet conditions which would leach water-soluble inhibitors or extremely hot and dry conditions which would volatilize low-temperature volatile organics may significantly alter nitrogen mineralization and nitrification rates. In addition, the inhibitory organic could be consumed (decomposed) by soil microorganisms. This suggests that inhibitory organics may be extremely labile. The labile nature of such organics could result in drastically different rates of mineralization and nitrification dependent upon the environmental conditions prior to collection. The results of Vitousek and Matson (1985) from a pine ecosystem in Indiana and Gosz and White (in press) from a ponderosa pine ecosystem which displayed large annual and seasonal variation in rates of nitrogen mineralization and nitrification may represent the transitory effects of labile organic inhibitors.

In conclusion, the factor which primarily controls nitrogen mineralization and nitrification in this ponderosa pine ecosystem is allelochemic inhibition. It is unlikely that the inhibition was caused by tannins and polyphenols, which are the most commonly suggested inhibitors in the literature. The inhibition is more likely caused by low temperature volatiles, such as terpenoids. Also, the low mineralization rates demonstrated in ponderosa pine ecosystems are probably the result of fire restriction. In the absence of fire, the low temperature volatiles may build up to inhibitory levels. Periodic low-intensity fires would consume or volatilize the inhibitors and should result in greatly increased rates of nitogen mineralization. The mineralization of other nutrients (particularly phophate) also may be increased.

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