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Factors Controlling Nitrification in Soils of Early Successional and Oak/Hickory Forests in the Southern Appalachians

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ABSTRACT

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Factors regulating nitrification were examined in three forests of contrasting nitrifying activity in the southern Appalachians of North Carolina, U.S.A. NH_4-N availability was the main factor regulating nitrification in pine/mixed-hardwood and black locust *(Robinia pseudo-acacia* L.)-dominated early successional forests. Litter leachate solutions from black locust had high concentrations of N and other nutrients, but their influence upon nitrification as estimated in laboratory-amended soil incubations was relatively small.

In a mature oak/hickory forest, nitrification was not stimulated by NH_4-N amendments, nor by amendments of black-locust litter leachate solutions. Amendments with $CaCO_3$ and $CaCl_2$ stimulated ammonification but did not stimulate nitrification in the soils of this forest. Laboratory incubations of soils amended with oak/hickory live leaves, litter, and forest-floor extracts suggested a possible inhibitory action on nitrification from oak leaves. Low nitrification was also found in glucose-amended laboratory incubations of black-locust soils, suggesting that an increase of the C:N ratio of the soil following amendment with extracts could be responsible for low nitrification rates.

INTRODUCTION

Nitrogen availability often limits plant productivity in terrestrial ecosystems (Chapin, 1980). Factors controlling N mineralization and nitrification have been studied because these processes determine the availability of N for

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plant and microbial uptake. Nitrification results in the formation of nitrate ions, which can be leached, taken up by plants or reduced and lost in gaseous forms. Recent reviews on nitrification include those of Painter (1970), Focht and Verstraete (1977), and Schmidt (1982). The main factors that affect nitrification in soil are temperature, moisture, pH, and the substrates NH_4-N, O_2 and CO_2 (Stevenson, 1986). Evidence that nitrification is inhibited by decomposition products of organic residues in soils, or by metabolites excreted by plants or microorganisms, is still not conclusive (Schmidt, 1982).

Studies on nitrification started at the Coweeta Hydrologic Laboratory (North Carolina, U.S.A.) in the mid-1970s. Results of previous studies showed higher nitrifier populations in successional than in more mature forests (Todd et al., 1975; R. Rowe, unpublished Coweeta Lab. files, 1978). Nitrification rates were higher in an early successional watershed (WS6) dominated by black locust (*Robiniapseudo-acacia* L.), a nitrogen-fixing tree, than in a reference watershed (WS14) with more mature hardwoods (Montagnini, 1985; Montagnini et al., 1986). Within the early successional watershed, areas dominated by black locust had higher nitrification rates than areas with pine/mixed hardwoods. Ammonification rates were the main factor controlling nitrification in early successional stands, and there was no evidence of inhibition of nitrification in the hardwoods soils (Montagnini et al., 1986). The aim of the present study was to further examine factors controlling nitrification in these forests of contrasting nitrifying activity and treatment history. The following factors were examined: (1) general soil physical and chemical characteristics; (2) availability of ammonium; (3) availability of other soil mineral nutrients; (4) calcium content and soil pH; (5) populations of nitrifying bacteria; and (6) allelochemical inhibition of nitrification.

STUDY SITE

The 2185-ha Coweeta Hydrologic Laboratory (35°N, 83°W) is part of the Blue Ridge province of the southern Appalachian mountains of North Carolina, U.S.A. Precipitation is rather evenly distributed throughout the year, with an annual mean of 1800 mm at lower elevations and 2500 mm on the upper slopes; the mean annual temperature is 13°C (Swank, 1986). The forests of the reference watersheds have remained relatively undisturbed since 1924 (Johnson and Swank, 1973) and the vegetation consists of mature mixed hardwoods. Seventy percent of the basal area at low elevations consists of oak (*Quercus prinus, Q. coccinea, Q. rubra* and others), hickory (*Carya glabra* and other species) and red maple *(Acer rubrum* L.) (Day and Monk, 1974).

The reference WS14 and the successional WS6 are adjacent and northwest-facing. The mixed-hardwood forest of WS6 was cut in 1958 and sawlogs were removed. The watershed was limed, fertilized, seeded to fescue grass in 1959, and refertilized in 1965. In 1966 and 1967 the grass was killed by herbicide

applications and the watershed was left for revegetation to occur (Johnson and Swank, 1973). In 1969 it was dominated by herbaceous species, in 1970 there were abundant woody shrubs, and by 1980 it was dominated by black locust, blackberries (*Rubus* spp.), and other woody species. The soils of both watersheds are Evard-Cowee gravelly loams (Typic Hapludults) on the slopes and Saunookee gravelly loams (Humic Hapludults) at lower elevations and coves (Swank, personal observations, 1987).

METHODS

Soil sampling

Soil was sampled at nine points along three transects running from the stream to the ridge in a pine/mixed hardwood and in a dense black-locust stand in the successional watershed (WS6) and in an oak/hickory forest stand in the adjacent reference watershed (WS14). Since the three stands were on the same soil series, major differences in soil characteristics among them may primarily reflect differences in treatment and vegetation type.

Samples of the 0-5 and 5–15-cm soil depths were collected with a 2.5-cm-diameter soil-corer. These are the zones of highest nitrifier activity (Montagnini, 1985), and correspond to the upper and lower portions of the A horizon. At each sampling point a composite of at least five samples was collected from each depth. Sampling was carried out in May, July, October (just before leaf-fall), and November (after leaf-fall) in 1983, and monthly from April to October in 1984.

Soil physical and chemical characteristics

Soil texture was measured with the hydrometer method (Day, 1983). Soil bulk density was estimated from soil cores 4.8 cm diameter and 5 cm long. Soil pH was measured in a 1:1 mixture of soil: deionized water (McLean, 1982) using a glass electrode. Total Kjeldahl nitrogen (TKN) and organic matter were determined on air-dried sub-samples of the <2-mm fraction. Kjeldahl-N was measured by acid digestion on a heater block, followed by colorimetric determination of NH_4-N in a Technicon Auto-Analyzer (Anonymous, 1977). Organic matter was measured by the Walkley-Black technique (Allison, 1975). Moisture content was determined by drying sub-samples at 70°C for 48 h.

Soil incubations

Nitrification rates were measured in aerobic laboratory incubations (Keeney, 1982). Samples were refrigerated at 4°C until analysis and were processed within 72 h after collection. The <4-mm fraction of field-moist soils was in-

cubated in glass vials, and aerated every 3-4 days. Incubations were run in a dark cabinet, at $22 \pm 2°C$, for 4 weeks. In 1983, time-course incubations were run using replicate sub-sets. Seven to nine sub-sets were extracted with *2N* KC1 before incubation. The extracts were analyzed colorimetrically with a Technicon Auto-Analyzer (Anonymous, 1970) to obtain the initial concentrations of NH_4-N and NO_3-N. At the end of the incubation time, other sub-sets were also extracted and analyzed as described above. Final-minus-initial values of NH_4-N + NO_3-N gave net N-mineralization rates, and final-minus-initial concentrations of NO_3-N gave a measurement of net nitrification rate. Final-minus-initial NH_4-N concentrations indicated NH_4-N consumption or production (ammonification). Net ammonification rates can be negative when all the NH_4-N present in the soil before incubation is consumed. Net nitrification rates can be lower than net N-mineralization rates when all the initial NH_4-N is consumed and part of the NH_4-N is immobilized by heterotrophic microorganisms. Net nitrification rates can be negative when NO_3-N is immobilized or denitrified, giving a lower value at the end of the incubation.

Forest-floor incubations

The forest floor (O_1 layer) in black locust, pine/mixed hardwoods and oak/hickory was collected in October 1984 with a 15-cm diameter plastic ring. The samples were pooled by stand, put in plastic bags and refrigerated until analysis. Following the procedures described for soils, 2 g of fresh material was incubated, and pH, TKN and percent moisture were determined.

Buried-bag incubations

For testing the effect of site temperature on nitrification, buried-bag incubations (Westermann and Crothers, 1980) were done in April and June of 1984. Soils of the 0-5-cm depth from the same points as already described were put in polyethylene bags and buried at the depth of sampling. Sub-sets were taken to the laboratory to measure the initial mineral N concentrations. Bags were collected after 4 weeks and soils were extracted. Incubations were also run in the laboratory for comparison.

Amended soil incubations

Soils of the nine sampling points of each stand were pooled, and sieved through 2-mm sieves. Soils were allowed to dry for 1/2 day until they reached 17-20% (*w/w*) moisture. Amendments were done by adding solutions at rates which would restore water content of 30-33%. Deionized water amendments served as controls. Soils were homogenized with a spatula after additions of solutions or deionized water.

(1) Ammonium additions

Ammonium was added to soils collected in April 1984. NH_4-Cl was added at a rate of 100 mg NH_4-N/kg of moist soil. This was calculated as the maximum amount of NH_4-N which could be nitrified in the soils of higher nitrifier activity (the black-locust soils), in a 4-week period (Montagnini et al., 1986).

(2) Calcium additions

Calcium amendments were performed in September 1984. Calcium was added at a rate of 20 g/kg of moist soil as either a 13.33% solution of $CaCO_3$ or a 12% solution of $CaCl_2$. $CaCO_3$ was added to increase the pH of the soil by 1-2 units, while $CaCl_2$ was added to decrease the pH by 1-2 units, to investigate the effects of the interaction between Ca availability and H^+-ion concentration on nitrification. Amendments were done to soils from all three sites and to a 1:1 mixture of oak/hickory:black-locust soils. Black-locust soils were used as an inoculum of high nitrifying activity to the oak/hickory soils.

(3) Forest-floor leachate additions

Amendments to soil incubations with forest-floor leachates were done in October 1984. It was expected that immediately after the initiation of leaf-fall, the forest-floor leachates would have the greatest effect on nitrification, with temperatures still high and a high rate of leaching of nutrients from the fresh leaf-litter. Black-locust forest-floor leachates were added to pine/mixed hardwoods and oak/hickory soils to examine the influence of a nutrient-rich solution on nitrification. Oak/hickory forest-floor leachates were added to all three soils to test a possible inhibitory action. Forest-floor leachates were collected in plastic containers which were set on top of the mineral soil. The bottom of the containers had fiberglass screens to hold the forest floor. Several holes were punched in the bottom to allow leachates to pass through and be collected in a plastic holder. The collectors were set at the sampling points of each forest stand, and collections were made as soon as possible following rains. Leachates were analyzed for pH, NH_4-N, NO_3-N and total N. Cations were analyzed by Atomic Absorption Spectrophotometry, and PO_4-P was analyzed by the colorimetric method of Strickland and Parsons (1972) using a spectrophotometer.

(4) Amendments with extracts of live leaves, leaf-litter, forest floor, and with glucose

Extracts of live leaves, leaf-litter and forest floor from oak/hickory were added to black-locust and to oak/hickory soils in October 1984, to test for the possible influence of substances inhibitory to nitrification. Leaves of oak, rhododendron and sassafrass were collected from tree seedlings in the oak/hickory site. Samples of fresh-fallen litter and the forest floor were collected from inside a 15-cm-diameter plastic ring, which was set at the top of the mineral soil. At least 10 pooled samples were taken at each sampling point. All materials

were refrigerated for transportation to the laboratory, and kept at 4°C until processing. Leaves were pooled by species, and 6 g were diced and macerated in 30 ml of deionized water with a mortar and pestle. This viscous material was centrifuged at 3000 rpm for 5 min and the supernatant used. Litter samples were pooled and 20-g portions were blended with 200 ml deionized water. Forest-floor samples were processed similarly to the litter. All amendments were at a rate of 0.75 ml/g dry soil.

Low nitrification rates may result from competition for NH_4-N by heterotrophic microorganisms, whose activity may be stimulated by the addition of a carbon source with the extracts. To evaluate this effect, C was added in the form of glucose, at a rate of 1.0 g/100 g moist soil. This rate was calculated to correspond to the C addition in annual litter-fall, and it was also calculated to increase the soil C:N ratio to 40:1.

Statistical analysis

A correlation analysis between nitrification rates and soil parameters was done with the Correlation Procedure of the Statistical Analysis System (Helwig and Council, 1979). Analysis of variance on the results of nitrification potentials was done using the General Linear Models (GLM) Procedure and Duncan's Multiple Range Test of SAS (Helwig and Council, 1979).

RESULTS

Soil physical and chemical characteristics

Soil texture and bulk density were similar in the three forest stands (Montagnini, 1985). Soil NO_3-N concentrations (Table 1) were higher in black-locust than in pine/mixed hardwoods, and they were very low or undetectable in oak/hickory. There were no significant differences in NH_4-N concentrations, TKN or C:N among the three sites (Table 1). Although differences were not statistically significant, soil C concentrations were lower in pine/mixed hardwoods than in oak/hickory or black locust. Soil pH (Table 1) was similar in pine/mixed hardwoods and black locust, and was significantly lower in oak/hickory. Moisture percentage did not differ among the three sites. At all sites, NO_3-N, NH_4-N, TKN and C concentrations were lower at 5–15-cm than at 0-5-cm soil depth (Table 1). There were no differences in pH or soil moisture between the two depths. Patterns of differences between the three forests were similar to the 0-5-cm depth.

TABLE 1

Soil general characteristics in the forest floor (O_1) and at 0-5 and 5-15-cm soil depth, for the 1984 sampling season

	Depth (cm)	Pine/mixed hardwoods	Black locust	Oak/hickory
NO_3-N (mg/kg)	O_1	7.2[b]	49.5[a]	1.5[c]
	0-5	1.14[b]	11.7[a]	0.03[c]
	5-15	0.14[b]	3.3[a]	0.0[c]
NH_4-N (mg/kg)	O_1	79.1[a]	44.8[b]	74.4[a]
	0-5	3.07[a]	4.86[a]	2.0[a]
	5-15	1.79[a]	1.68[a]	3.7[a]
TKN (%)	O_1	1.28[a]	1.18[b]	1.49[c]
	0-5	0.28[a]	0.334[a]	0.319[a]
	5-15	0.145[a]	0.151[a]	0.171[a]
C (%)	0-5	3.88[a]	4.33[a]	4.56[a]
	5-15	2.43[a]	2.23[a]	2.63[a]
C/N	0-5	14.1[a]	13.6[a]	14.8[a]
	5-15	17.8[a]	15.2[a]	10.8[a]
pH	O_1	5.84[ab]	6.26[ab]	5.57[a]
	0-5	5.79[a]	5.60[a]	4.88[b]
	5-15	5.53[a]	5.65[a]	4.89[b]
Moisture (%)	O_1	29.9[c]	31.6[b]	68.1[a]
	0-5	27.3[a]	29.7[a]	33.1[a]
	5-15	27.4[a]	28.5[a]	30.2[a]

Means with non-matching superscript letters indicate significant differences among forest types for a given depth and analysis ($n=54$, $P<0.05$). (The forest floor was only sampled in October 1984.)

Nitrogen mineralization and nitrification rates by forest type

As the record was more complete in 1984, only those results are presented; results of 1983 were similar. In the 0–5-cm layer, net N-mineralization and nitrification rates were substantially higher in black locust and pine/mixed hardwoods than in oak/hickory (Table 2). Net nitrification rates followed closely total net N-mineralization rates for black locust and pine/mixed hardwoods. In contrast, in the oak/hickory site net nitrification rates were almost negligible. All the NH_4-N was consumed in pine/mixed hardwoods and black locust, but NH_4-N accumulated in oak/hickory. At 5-15 cm, N mineralization and nitrification were lower than at 0-5 cm (Table 2).

TABLE 2

Net N mineralization, nitrification and NH_4-N production in the forest floor (O_1) and at 0-5 and 5-15 cm soil depth, for the 1984 sampling season

Depth (cm)	Pine/mixed hardwoods	Black locust	Oak-hickory
N mineralization (mg NH_4-N+NO_3-N kg^{-1} 30 days)			
O_1	117.5[a]	221.9[bc]	719.9[c]
0-15	26.3[a]	33.0[a]	12.2[b]
0-15	5.4[b]	26.9[a]	8.8[b]
Nitrification (mg NO_3-N kg^{-1} 30 days)			
O_1	7.9[a]	59.1[a]	1.7[b]
0-5	27.0[a]	36.0[a]	2.0[b]
5-15	6.5[b]	25.8[a]	1.6[c]
NH_4-N production (mg kg^{-1} 30 days)			
O_1	109.5[a]	9.6[b]	718.2[c]
0-5	−1.2[b]	−3.6[b]	10.3[a]
5-15	−1.0[b]	0.75[b]	7.1[a]

Means with non-matching superscript letters indicate significant differences among forest types for a given depth and analysis ($n=54$, $P<0.05$).

Net N-mineralization was higher in the oak/hickory forest floor than in pine/mixed hardwoods or black locust. Nitrification rates and nitrate concentrations were higher in black locust than in pine/mixed hardwoods and oak/hickory forest floor (Tables 1 and 2). NH_4-N concentrations were higher in pine/mixed hardwoods and in oak/hickory than in black-locust forest floor. The TKN concentration was higher in oak/hickory than in pine/mixed hardwoods or black-locust forest floor. The pH was lower and the percent moisture was higher in the oak/hickory than in the pine/mixed hardwoods and black-locust forest floor.

Correlation of nitrification rates with soil parameters

The highest correlations of nitrification rates were: net N-mineralization rates ($r^2=0.85$, $P<0.0001$); total mineral N ($r^2=0.38$, $P<0.001$); and initial NO_3-N concentrations ($r^2=0.37$, $P<0.0001$; Montagnini, 1985). There were also significant, but low, positive correlations between nitrification and initial soil NH_4-N concentration ($r^2=0.09$, $P<0.001$), TKN content ($r^2=0.07$, $P<0.0001$) and negative correlations with NH_4-N production ($r^2=-0.18$, $P<0.0001$) and with soil H^+ ion concentration ($r^2=-0.13$, $P<0.0001$). Correlation analysis was also done with data from black locust and pine/mixed

hardwoods only, to obtain indication of factors controlling nitrification in the two successional forests. When the oak/hickory forest site was deleted from the correlation analysis, the correlation coefficient of nitrification and H^+ ion concentration decreased from $r^2 = -0.13$ ($P<0.0001$) to -0.03 ($P<0.008$). The correlation coefficient between nitrification and net N-mineralization increased from $r^2 = 0.85$ to $r^2 = 0.97$. Correlation coefficients between nitrification and TKN and C:N ratio also increased from $r^2 = 0.13$ to 0.28 and from $r^2 = -0.07$ to -0.12, respectively. Correlations between nitrification and percent soil moisture and carbon concentration became significant with $r^2 = 0.15$ and 0.14, respectively. Values of other parameters remained similar to those obtained when the oak/hickory site was included in the analysis (Montagnini, 1985).

Comparison between field and laboratory incubations

The comparison of nitrification rates as measured in the laboratory and in the field for April is shown in Table 3. Similar results were obtained in June (Montagnini, 1985). Net N-mineralization and nitrification rates in pine/mixed hardwoods were about 2 x higher in the laboratory than in the field. Net N-mineralization and nitrification rates of black-locust soils were 1.7 X higher in laboratory than in field incubations. Net N mineralization was 5 X higher, and nitrification 10X higher in oak/hickory laboratory incubations than in the field. NH_4-N was consumed during incubation of pine/mixed hardwoods and black-locust soils, whereas NH_4-N accumulated in oak/hickory, with ac-

TABLE 3

Comparison of net N mineralization, nitrification and NH_4-N production rates in soils from pine/mixed hardwoods, black locust and oak/hickory forest stands incubated in the laboratory and in the field, for April, 1984

Pine/mixed hardwoods		Black locust		Oak/hickory	
Lab.	Field	Lab.	Field	Lab.	Field
N mineralization ($mg\ NH_4+NO_3\text{-}N\ kg^{-1}$ 30 days)					
28.8^a	14.9^a	33.4^a	19.2^a	22.0^a	4.1^a
Nitrification (mg NO_3-N kg^{-1} 30 days)					
28.7^a	14.4^{ab}	32.8^a	19.1^a	8.3^b	0.9^b
NH_4-N production (mg NH_4-N kg^{-1} 30 days)					
0.12^a	0.58^b	0.6^a	0.1^b	13.7^a	3.2^a

Means with non-matching superscript letters indicate significant differences among forest types for a given analysis (but not between laboratory vs. field; $n=9, P<0.05$).

cumulation about 4 X higher in the laboratory. Differences in rates among sites were the same for both incubation methods: net N mineralization was higher in black locust than in pine/mixed hardwoods or oak/hickory (although differences were not statistically significant in either field or laboratory) and nitrification was higher in black locust and pine/mixed hardwoods than in oak/hickory (difference was statistically significant in both field and laboratory).

Amended soil incubations

(1) Ammonium additions

All the added NH_4-N was nitrified in black locust and pine/mixed-hardwood soils (Table 4). Nitrification rates exceeded total net N-mineralization rates in both soils. The addition of NH_4-N did not increase nitrate production in oak/hickory. No NH_4-N was found at the end of the incubation in oak/hickory, suggesting that it had been immobilized by heterotrophic soil microorganisms.

TABLE 4

Net N mineralization, nitrification and NH_4-N production rates, initial NO_3-N and NH_4-N concentrations in control (unamended) and NH_4-N-amended pine/mixed hardwoods, black locust and oak/hickory soils

Pine/mixed hardwoods		Black locust		Oak/hickory	
Control	Amended	Control	Amended	Control	Amended
N mineralization (mg NH_4-N+NO_3-N kg^{-1} 30 days)					
27.4^a	-1.6^b	49.5^a	-0.78^b	18.1^a	-107.8^b
Nitrification (mg NO_3-N kg^{-1} 30 days)					
31.3^b	126.4^a	52.1^b	152.6^a	1.2^a	0.47^a
NH_4-N production (mg NH_4-N kg^{-1} 30 days)					
-3.9^a	-128.0^b	-2.6^a	-153.3^b	16.9^a	-108.3^b
Initial NO_3-N concentration (mg/kg)					
2.2^a	2.11^a	13.5^a	12.8^a	0.0^a	0.24^a
Initial NH_4-N concentration (mg/kg)					
4.7^b	33.6^a	3.9^b	153.4^a	7.3^b	144.4^a

Means with non-matching superscript letters indicate statistically significant differences between control and amended for a given soil ($n=9$, $P < 0.05$).

TABLE 5

pH, N mineralization, nitrification, NH_4-N consumption rates, NO_3-N and NH_4-N concentrations for control and Ca-amended soils

Pine/mixed hardwoods			Black locust			Oak/hickory			Black locust/Oak-hickory 1:1 mixture		
Control	+$CaCO_3$	+$CaCl_2$	Control	+$CaCO_3$	+$CaCl_2$	Control	+$CaCO_3$	+$CaCl_2$	Control	+$CaCO_3$	+$CaCl_2$
pH											
6.16[a]	6.88[a]	5.04[b]	5.48[b]	6.63[a]	4.44[c]	5.21[b]	6.48[a]	4.05[c]	5.33[b]	6.67[a]	4.23[c]
N mineralization (mg NH_4-N+NO_3-N kg^{-1} 30 days)											
21.7[b]	18.7[b]	66.3[a]	48.9[b]	51.5[b]	100.5[a]	21.4[b]	26.3[b]	138.8[a]	46.1[b]	39.6[b]	119.6[a]
Nitrification (mg NO_3-N kg^{-1} 30 days)											
21.3[a]	23.9[a]	0.50[b]	56.5[b]	63.1[a]	3.7[c]	0.93[b]	1.4[a]	0.78[b]	46.4[a]	44.6[a]	2.3[b]
NH_4-N production (mg NH_4-N kg^{-1} 30 days)											
0.40[b]	−5.2[b]	66.3[a]	−7.6[b]	−11.6[b]	96.8[a]	20.5[b]	26.3[b]	138.8[a]	−0.25[b]	−4.9[b]	117.2[a]

Means with non-matching superscript letters indicate significant differences between treatments for a given soil ($n=7, P<0.05$).

(2) Calcium additions

The addition of $CaCO_3$ increased soil pH by 0.72-1.34 units, and the addition of $CaCl_2$ decreased soil pH by 1.04-1.16 units (Table 5). $CaCO_3$ amendment increased nitrification in black locust, but resulted in only a small increase in oak/hickory, and did not increase nitrification in oak/hickory:black-locust soils mixture (Table 5). The rate of nitrification in the oak/hickory:black-locust unamended mixture was much higher than in the oak/hickory $CaCO_3$ amended and unamended soils, with values close (82%) to those of the unamended black-locust soils (Table 5).

$CaCl_2$ amendment resulted in very low nitrification rates in all cases (Table 5). NH_4-N accumulated in all the $CaCl_2$-amended incubations: the highest values of NH_4-N accumulation were found in oak/hickory followed by the oak/hickory:black-locust mixture, black locust, and pine/mixed hardwoods.

(3) Forest-floor leachate additions

The pH of the black-locust forest-floor leachate was higher (7.17) than that of oak/hickory (5.96). Cation concentrations in black-locust leachates were: Ca, 50.9; K, 88.8; and Mg, 17.7 mg/l, 2–3 X higher than in oak/hickory. PO_4-P was 3.2, NO_3-N was 9.5 and NH_4-N was 8.8 mg/1, with respective values 14.0, 23.0 and 31.0 X higher than in oak/hickory (Montagnini, 1985).

(a) Black-locust forest-floor leachate amendments. Amendments did not result in pH changes in either pine/mixed hardwoods or oak/hickory soils (data not presented). Net nitrification was higher in pine/mixed-hardwoods amended (37.2 mg NO_3-N kg^{-1} 30-days) than in unamended (33.2 mg kg^{-1} 30-days) soils, but differences were not statistically significant. Net nitrification rates were slightly lower in the amended (1.2 mg kg^{-1} 30-days) than in the unamended (2.1 mg kg^{-1} 30-days) oak/hickory soils, and the difference was statistically significant.

(b) Oak/hickory forest-floor leachate amendments. In pine/mixed hardwoods, there were no significant differences in pH between the amended and the unamended soils (data not presented). Net nitrification was 9.4% lower in the amended soils, although differences were not statistically significant. Net N mineralization rates were 10.9% lower in the unamended soils, but differences were not statistically significant ($P < 0.05$). All the NH_4-N was consumed during the incubation of amended and unamended pine/mixed-hardwood soils.

In black locust, the pH was significantly lower in the amended than in the unamended soils, but the difference was only 0.04 units. In contrast to the response of pine/mixed-hardwoods soils, nitrification rates were significantly higher in black-locust amended (59.6 mg kg^{-1} 30-days) than in the unamended (51.3 mg kg^{-1} 30-days) soils. As in the pine/mixed-hardwood soils,

all the NH_4-N was consumed during the incubations of amended and unamended black-locust soils.

In oak/hickory, there were no differences in pH between amended and unamended soils. Net N mineralization and nitrification rates were slightly lower in the amended than in the unamended soils, but differences were not statistically significant.

(4) Amendment with leaf, litter and forest-floor extracts and glucose

In oak/hickory, nitrification was undetectable following amendments of leaf, litter, and forest-floor extracts, and in the unamended soils (Table 6). NO_3-N production was higher and NH_4-N accumulated in the glucose-amended soils. There were no differences in pH (data not presented) between amended and unamended soils.

In black locust, the nitrification rate was negative following amendment with oak-leaf extracts, indicating either denitrification or immobilization of NO_3-N (Table 6). In soils amended with sassafrass and rhododendron-leaf extracts, and in soils amended with litter and forest-floor extracts, nitrification rates were lower than or similar to the unamended soils. The NH_4-N was consumed during the incubation of black-locust soils amended with extracts and in the controls. Nitrification rates were negative and NH_4-N accumulated in the glucose-amended black-locust soil. There were no differences in pH between amended and control soils (data not presented).

TABLE 6

Nitrate and ammonium production for oak/hickory and black-locust soils amended with extracts of *Quercus, Sassafrass* and *Rhododendron* leaves, litter, forest floor and glucose

Oak	Sassafrass	Rhododendron	Litter	Forest floor	Glucose	Control
Oak/hickory						
NO_3-N production (mg kg^{-1} 30 days)						
n.d.	n.d.	n.d.	n.d.	n.d.	0.67^a	n.d.
NH_4 production (mg kg^{-1} 30 days)						
-1.0^b	0.04^b	-4.4^b	-0.5^b	9.2^a	1.2^a	14.5^a
Black locust						
NO_3-N production (mg kg^{-1} 30 days)						
-15.1^b	10.2^a	25.4^a	12.6^a	25.1^a	-0.8^a	27.1^a
NH_4-N production (mg kg^{-1} 30 days)						
-2.1^a	-2.8^a	-6.32^{ab}	-5.6^b	-8.1^b	1.2^a	-9.5^b

Means with non-matching superscript letters indicate significant differences between treatments for a given site ($n=l$, $P<0.05$, n.d. = not detectable).

DISCUSSION

Factors controlling nitrification rates: influence of general soil chemical and physical characteristics

The influence of soil physical characteristics on differences in nitrification between sites is likely to be minimal. Results of buried-bag incubations, in which soil structure is preserved, indicated the same differences between sites as in the laboratory incubations. The soil manipulations (sieving, mixing) in the laboratory may create more-aerated conditions as compared with the field. The interaction of constant temperature and soil manipulation increased nitrification in laboratory incubations as compared to buried bags, with similar effects on all soils. Site temperature did not appear to influence differences in nitrification between sites, because the same magnitudes of differences were found in the field as in the laboratory (Table 3).

Results of the laboratory and field incubations, the correlation analysis, and the NH_4-N amendments suggest that nitrification is controlled by ammonification rates in pine/mixed hardwoods and black locust but not in oak/hickory. Apparently, pine/mixed-hardwoods and black-locust soils have a high capacity to nitrify above the average levels shown in Table 2, with no other factors preventing oxidation of the added NH_4-N. The nitrification rates in pine/mixed hardwoods and black-locust NH_4-N-amended soils (Table 4) are equivalent to the highest rates reported for temperate forests (Robertson, 1982a).

In the black-locust-dominated areas, with higher nitrification, the availability of NH_4-N must be higher, possibly as a result of increased N inputs from N fixation. The leaching of mineral and organic N, cations and P from black-locust forest-floor may stimulate nitrification in the mineral soil. Black-locust forest-floor leachate amendments non-significantly increased nitrification in pine/mixed-hardwoods soils, although the effect was small. The amount of NH_4-N added with the leachate cannot account for the observed increase in nitrification. More likely, the increase resulted from the addition of mineral and organic N and other nutrients with the leachate. Concentrations of Ca, Mg, K and P were higher in the black-locust litter leachates, than in pine/mixed-hardwoods and oak/hickory. PO_4-P has been demonstrated to influence nitrification (Purchase, 1974; Hue and Adams, 1984). Inputs of dissolved organic N from forest-floor leaching may also stimulate ammonification and nitrification. If black-locust litter leachates contain dissolved organic N in a labile form, they may increase NH_4-N availability for nitrification.

Influence of soil Ca content and pH on N mineralization and nitrification

The increase of pH and Ca content through $CaCO_3$ amendment did not enhance nitrification in the oak/hickory soils (Table 5). Hence, low pH or low

Ca content do not appear to cause low nitrification in oak/hickory. Soil pH does not appear to influence differences in nitrification between black locust and pine/mixed hardwoods; pH differences between these two sites were small, and the amendment with $CaCO_3$ resulted in only a 10% increase in nitrification in both sites. Amendments with $CaCl_2$ resulted in decreased nitrification in all soils. Net ammonification was higher in the soils amended with $CaCl_2$, than in those amended with $CaCO_3$. Suppression of NH_4-N oxidation could only partially explain NH_4-N accumulation: 30% in pine/mixed-hardwoods, 55% in black locust and 15% in oak/hickory. Therefore, in spite of lowered pH, the addition of $CaCl_2$ increased net ammonification in all soils, with greatest effects in oak/hickory. The increase in the production of NH_4-N was apparently the result of lower immobilization of NH_4-N and higher ammonification rates.

Effects of decreased soil pH on soil N transformations vary with soil type, length of the experiments and soil chemistry (Strayer et al., 1981; Novick et al., 1984). Diminished soil nitrification at low pH appears to be due to inhibitory effects of free nitrous acid on *Nitrobacter* spp. (Prosser and Cox, 1982). Effects of salt amendments on ammonification and nitrification vary depending on the amounts and salts and soil type (McClung and Frankenberger, 1985). These authors reported that, in three different soils, nitrification was inhibited more by $CaCl_2$ than by NaCl and Na_2SO_4, whereas ammonification was either not affected or was stimulated, with greatest effects following addition of $CaCl_2$ and NaCl, in that order. Similar effects of chloride salts on nitrification have been reported by Laura (1974), Westerman and Tucker (1974), and Heilman (1975). Mechanisms of decreased nitrification following salt amendments to soils consist of both osmotic and indirect ion effects (Runge, 1983).

Factors controlling nitrification in the older-growth oak/hickory forest

Nitrification occurred occasionally and at low rates in oak/hickory. The lack of response to NH_4-N and $CaCO_3$ amendments and accumulation of NH_4-N suggested allelochemical inhibition of nitrification was occurring in oak/hickory. However, in black-locust: oak/hickory 1:1 mixture, nitrification rates were greater than expected from the mixture, suggesting tht part of the NH_4-N of oak/hickory had been nitrified. These results agree with those of an earlier experiment (Montagnini, 1985).

Christensen and MacAller (1985) reported that nitrification did not increase following NH_4-N additions, or addition of nutrients other than N to four hardwood soils in the piedmont of North Carolina, and suggested that factors other than nutrient availability may limit N transformations in those soils. Vitousek et al. (1982) observed lags in nitrification in eight of 17 forest sites in the U.S.A., and attributed this response to low nitrifier bacterial pop-

ulations due to competition by roots, mycorrhyzae and heterotrophs, or to inhibition of nitrification by plant secondary compounds. Populations of nitrifiers in the oak/hickory forest of WS18, adjacent to WS14, were very low (Todd et al., 1975); R. Rowe (unpublished data, Coweeta Lab. files, 1978) also reported low nitrifier populations at the oak/hickory forest of WS2, another reference watershed at Coweeta. Vitousek (cited in Montagnini, 1985) measured nitrification in oak/hickory soil and in mixtures with soils of high nitrifying activity, and identified low bacterial populations as one of the factors controlling nitrification in the oak/hickory forest of WS2.

The oak/hickory forest-floor leachate amendment depressed nitrification in pine/mixed hardwoods and oak/hickory, suggesting an inhibitory effect. However, these amendments stimulated nitrification in black locust; possibly, in black locust with high nitrification, the inhibitory action was not enough to depress nitrifier activity.

Amendments with extracts of leaves, litter, and forest floor did not stimulate nitrification in oak/hickory, but NH_4-N was consumed during the incubation, suggesting that the extracts might have provided a C source which stimulated heterotrophic growth and immobilization of NH_4-N.

This evidence suggests that allelochemical inhibition does not have an influence on nitrification in oak/hickory. Rates of nitrate production were not affected by amendments of ground-litter and whole-leaf extracts to incubated soils in a North Carolina piedmont secondary sere (Montes and Christensen, 1979). Robertson (1982b) investigated the presence of labile inhibitors of nitrification in a secondary sere in the New Jersey piedmont by applying soil, litter and whole-leaf washings and extracts to incubated soils. Allelochemical inhibition of nitrification did not appear to regulate nitrification in these secondary successional sites.

Competition for NH_4-N by heterotrophic microorganisms may influence low nitrification in oak/hickory forest soils at Coweeta. This is supported by the results of glucose amendments, which resulted in very low nitrification rates in black-locust soils. Competition for NH_4-N may be enhanced through inputs of organic matter of high C:N ratio to the soils, such as oak leaves and litter. Competition for NH_4-N heterotrophic soil microbes decreases numbers of nitrifiers, as they are weaker competitors (Jones and Richards, 1977), but competition for other nutrients such as Ca and P may later result in even lower nitrifier populations in the mature forests.

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