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FACTORS REGULATING NITRIFICATION IN PRIMARY AND SECONDARY SUCCESSION[1]

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Abstract. A six-point primary sere developed on sand dunes and a four-point secondary sere developed on old fields were studied to examine the regulation of nitrification in succession. Soils from sites along the seres were incubated in funnel-microlysimeters leached weekly with NH_4Cl and other nutrient solutions. Nitrate output in soils from the five youngest sites along the primary sere was rapidly stimulated by NH_4^+-N additions. Nitrification in soils from all four sites of the secondary sere was also stimulated by added ammonium. Added NH_4^+-N had no effect on the last primary sere site; in this site $CaCO_3$ was the only treatment that stimulated potential nitrification.

The possibility that labile inhibitors of nitrification were present in these sites was assessed by applying soil, litter, and whole-leaf washings, and whole-leaf and litter extracts to incubated soils. Soils amended with oxidizable carbon and pH-buffer solutions served as controls. Clear evidence for ecologically meaningful allelochemical inhibition of nitrification was found only in some subsites in the next-to-last site of the primary sere, which in previous incubations had had higher rates of nitrate production than any other site along this sere. No evidence for inhibition was found in the secondary sere.

The effects of moisture and temperature on the rates of nitrification in these sites were also investigated. Results suggested that laboratory incubations may overestimate relative field rates of nitrification for early primary sere sites.

Nitrification appears to be controlled by ammonium availability in at least the first four sites of the primary sere and perhaps the fifth. Allelochemical inhibition may also be important in this fifth site. A lag in nitrification that is counteracted by $CaCO_3$ is an important regulator of nitrification in the last site. In the secondary sere NH_4^+-N availability appears to control nitrification in all sites.

Key words: allelopathy; dunes; Indiana; inhibition; microlysimeters; mineralization; New Jersey; nitrate; nitrification; nitrogen; nutrient cycling; piedmont; succession.

INTRODUCTION

The control of nitrification in terrestrial ecosystems has attracted considerable attention in recent years. Much of this interest has stemmed from a heightened awareness of the importance of nitrification for controlling nitrogen and cation losses from terrestrial ecosystems (Nye and Greenland 1960, Likens et al. 1969, Vitousek et al. 1979, Kurtz 1980). Both directly (Blackmer et al. 1980) and by regulating the rate at which nitrate becomes available to denitrifiers, nitrification can also affect the production of N_2O, believed to play a significant role in the destruction of atmospheric ozone (Crutzen 1983).

Ecological succession has provided a useful context for examining nitrification. Since Warren's (1965) observation that apparent nitrification changed monotonically during the successional development of a South African grassland, a number of studies have examined changes in nitrification with succession (e.g., Neal 1969, Rice and Pancholy 1972, 1973, 1974, Todd et al. 1975, Reeder and Berg 1977, Lodhi 1979, Montes and Christensen 1979, Lamb 1980, Robertson and Vitousek 1981, D. J. Vogt and R. L. Edmonds, *personal communication*). Results from many studies appear to support Rice and Pancholy's (1972) hypothesis that nitrification decreases in the course of succession due to increasingly effective inhibition of nitrifying bacteria by later successional vegetation. Other studies, however, in particular those that have used nitrification potentials to indicate relative rates of nitrification (Coile 1940, Reeder and Berg 1977, Montes and Christensen 1979, Lamb 1980, Robertson and Vitousek 1981), have either failed to find a successional trend or found the reverse from that predicted by the hypothesis.

Nitrification potentials (measurements of nitrate production in incubated soils) provide an index of the activity of the nitrifying population in a soil at the time of sampling. Studies that use this measure rather than only in situ mineral-nitrogen and nitrifier-population pool sizes to indicate relative nitrification along seres thus avoid many of the assumptions that have clouded the interpretation of earlier studies. Robertson and Vitousek (1981) have discussed the importance of these assumptions, and in addition have argued that within any given sere, nitrogen availability is more likely to limit nitrification than is allelochemical inhibition. In 9 of the 10 sites they studied, nitrification potentials directly reflected nitrogen mineralization potentials. Further, Montes and Christensen (1979) had found that added NH_4^+-N stimulated nitrate production in all incubated soils from different stages of a three-point North Carolina Piedmont sere, and Lamb (1980) found

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similar results for soils from a two-point subtropical rainforest sere in Australia.

Implicit in these soil incubation studies are two important assumptions. The first is that differences in in situ moisture and temperature conditions among sites in a sere are not great enough to affect significantly the relative rates of nitrification predicted by incubations under identical laboratory conditions. Otherwise, laboratory incubations will overestimate relative field rates of nitrification in some sites. For example, a site with lower relative nitrification in the field because of consistently drier soils may nitrify at rates equivalent to those from the wetter sites when all soils are incubated under the same high-moisture laboratory conditions. This assumption could be important in primary and perhaps secondary successions where early-site soils with little canopy or litter cover are subjected to greater insolation, greater evaporation, and less transpiration than are soils in older sites.

The second and less easily tested assumption is that labile inhibitors are not differentially present among sites in the field. These are inhibitors of nitrification that could degrade quickly in soil, but under natural conditions could be continuously replenished by throughfall or root exudates (Moleski 1976). Since most laboratory incubations run for several weeks or longer, the presence of exudates that degrade within the first few days of an incubation could easily be overlooked.

A number of attempts to assess the importance of this inhibition have been made. The most common approach for testing for the presence of inhibitors has been to add suspected sources of inhibition such as extracts and washings of vegetation, litter, and soil to incubated soil microcosms (e.g., Boquel and Suavin 1972, Rice and Pancholy 1973, 1974, Rychert et al. 1974, Melillo 1977) or to pure cultures of nitrifers (Rice 1964, Munro 1966*a*, *b*, Neal 1969). Subsequently depressed rates of nitrate accumulation or reduced nitrifier populations relative to controls treated with distilled water are then usually interpreted to indicate the presence of allelochemical inhibitors in the extract or washing.

Rarely, however, are the results on which such interpretations are based free from ambiguity. First, readily oxidizable carbon is unavoidably added to soil microcosms with the suspected inhibitors, and this carbon may itself suppress nitrate production in soils (Purchase 1974, Melillo 1977). Nitrifiers are poor competitors for inorganic nitrogen (Jones and Richards 1977), so that when soil heterotrophs and subsequent nitrogen immobilization is stimulated by the addition of a substrate with a high C:N ratio, the nitrifiers may be suppressed by the lack of available NH_4^+-N rather than by allelopathic inhibitors of nitrification. Adding potential inhibitors to NH_4^+-N saturated soils (Moore and Waide 1971) does not avoid this problem because competition between nitrifiers and heterotrophs for other limiting resources (such as O_2 and space) may inhibit nitrifiers equally effectively (Purchase 1974). Furthermore, changes in soil pH brought about by suspected sources of inhibition, e.g., by highly buffered whole-tissue extracts, could further suppress nitrification in incubated soils (Weber and Gainey 1962, Focht and Verstraete 1978) independently of allelochemicals.

Second, although inhibition experiments with pure cultures of nitrifiers avoid immobilization interactions, ecologically meaningful interpretations of resulting inhibition are difficult to make. In these experiments potential interactions of an inhibitor with the biotic and physical components of natural systems are assumed unimportant, though such interactions clearly could mediate inhibitory effects. In the field, for example, naturally occurring heterotrophs could degrade a potential inhibitor before it reaches most nitrifiers. In addition, reactions of laboratory stock-culture nitrifiers to a potential inhibitor may be quite different from the reactions of nitrifiers that occur naturally at a site. Nitrifiers and site-specific conditions such as inhibitors could be closely coevolved (see, e.g., Ulyanova 1961, 1962, Mahendrappa et al. 1966, Monib et al. 1979). Molina and Rovira (1964), Odu and Adeoye (1973), and Purchase (1974) have all documented inhibitory effects of natural compounds in pure cultures of nitrifiers, but stimulation or no effect for the same inhibitors in soil microcosms.

A further problem with many existing inhibition studies is the poor match between the solution applied to incubations and what would normally occur under field conditions. While distilled-water leachings of whole leaves, litter, or soil may closely represent naturally occurring solutions, extracts prepared from ground plant parts may be less meaningful. Chemical compounds in nondesiccated, actively metabolizing tissue (e.g., whole leaves) may be quite different from compounds in the same tissue after senescence.

One way to assess the importance of allelochemical inhibitors in an ecosystem and avoid many of these problems may be to add distilled-water leachings of various ecosystem components to soil microcosms, and then to compare subsequent nitrate production in these soils with nitrate production in control microcosms to which have been added equivalent amounts of oxidizable -C and H^+. This approach could allow allelopathic effects of suspected inhibitors to be assessed independently of carbon and pH effects, and, if applied to short-term incubations, could document the presence of highly labile inhibitors.

The present study was designed to test the hypothesis that the availability of nitrogen regulates nitrification in ecological succession. The experimental approach involved monitoring nitrate production in incubated NH_4^+-amended soils from various sites along two well-defined seres, one a primary sere developed on sand dunes, and the other, a secondary sere de-

veloped on old fields. Washings from whole leaves, litter, and soil from these sites were also tested for their potential to inhibit nitrification, and potential nitrification of these soils at different moisture and temperature levels was examined to evaluate some of the bias introduced by laboratory incubation conditions.

Study Sites

A six-point primary sere at the Indiana Dunes on the southern shore of Lake Michigan and a four-point secondary sere on the New Jersey Piedmont were examined in this study. Sites along the primary sere included sand, grass, grass + shrub, pine, oak(1) and old-growth oak(2) stages of succession. Along the secondary sere, sites corresponded to annual, perennial, shrub, and old-growth forest stages.

Physical, chemical, and biotic features of sites along both seres have been described at length elsewhere (Robertson and Vitousek 1981), although sites in the present study sampled in 1979 were 1 yr older than described earlier. Significant changes in the primary sere over this interval were not discernible; changes in the secondary sere appeared mainly limited to the earliest, now 1-yr-old site. In this site primrose (*Oenothera* sp. [nomenclature follows Gleason and Cronquist 1963]), orchard grass (*Dactylis glomerata*), and Queen Anne's lace (*Daucus carota*) had invaded the near monoculture of ragweed (*Ambrosia artemisiifolia*). In other secondary sere sites minor changes followed patterns described by Frye (1978).

Methods

Nutrient amendment incubations

The hypothesis that the availability of NH_4^+-N limits nitrification in these seres was tested by monitoring nitrate production in laboratory-incubated soils treated with different combinations of nutrients. Soils were collected in the 1979 growing season from three subsites in each site along the primary sere and in the 1978 growing season from four subsites in each site along the secondary sere. Subsites were randomly located along a 100-m transect that crossed each site. Several 12 cm deep by 6 cm diameter soil cores were taken from within a 1-m^2 area at each subsite. These were combined (by subsite) in polyethylene bags and then refrigerated at 0–3°C for transport to the laboratory. Processing of the collected samples took place as soon as possible after collection; this was within 10 h of collection for the Indiana Dunes sites and within 30 h for the New Jersey sites.

In the laboratory, soils were passed through a 4-mm sieve and three 50-g (fresh mass) subsite replicates per nutrient treatment were placed in funnel microlysimeters (Fig. 1). Microlysimeters were mounted on plywood shelving (110 microlysimeters per shelf) served by a central vacuum system. Shelves were mounted on racks in a darkened controlled-environment cabinet at 21° (±0.5°C).

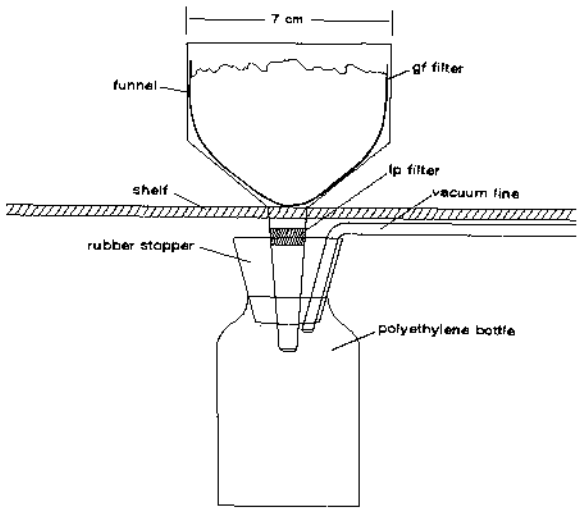

Fig. 1. Funnel-microlysimeter (110 funnels/shelf) used in nutrient-amendment experiment. lp refers to linear polyethylene, gf to glass fiber.

At the beginning of the incubation and at weekly intervals thereafter, soils were leached with nutrient solutions. One exception to this procedure was soils from the secondary sere perennial site, which were collected in late May rather than July 1978, and leached with distilled water after the initial nutrient-treatment leachings. All solutions were applied at a 1:1 (50 mL solution:50 g fresh soil) ratio and allowed to equilibrate with the soils for at least 15 min before being slowly drawn into polyethylene bottles suspended below each funnel. Aliquots from these bottles were preserved with phenylmercuric acetate (PMA) at 0.5 mg/L and stored at 0–3° until nitrate analysis. Nitrate was analyzed colorimetrically with a Technicon Autoanalyzer II system.

Nutrient solutions applied to Indiana Dunes soils included ammonium as NH_4Cl (10 mmol/L) and a modified Hoagland's solution (Epstein 1972) that contained HPO_4, Ca, K, Mg, Bo, Mn, Zn, Cu, Mo, Na, Fe, Cl, and SO_4 ions, but no nitrogen. A distilled water treatment served as control.

Secondary sere treatments included ammonium as described above, phosphorus as Na_2HPO_4 (1.6 mmol/L), and calcium as $CaCl_2$ (5 mmol/L). These three treatments were applied in a full factorial design, and modified Hoagland's solution as above but containing no N, P, or Ca was an eighth treatment. Distilled water served as control.

Inhibition experiments

Potential inhibitors were prepared from whole-leaf washings and extracts, forest-floor washings and extracts, and soil washings. Where possible, these substrates were sampled at each site along both seres, but not all of these sources were available at all sites (e.g.,

only soil washings were prepared for the primary sere sand site).

For soil-derived solutions, three soil cores (described earlier) and three 0.25 × 0.25 m forest floor samples were taken from each of three subsites at each site. Where there was no forest floor (e.g., at the early sites along both seres), standing dead litter was substituted. Whole leaves were taken from at least three individual plants of each dominant species (Robertson and Vitousek 1981) at each site. Each individual plant was sampled in at least three places, and at each site samples were combined by species. All samples were placed in opaque polyethylene bags and immediately refrigerated for transport back to the laboratory. Indiana Dunes sites were sampled in early July 1979, although the oldest Dunes site was resampled in late August because of equipment failure in July. New Jersey Piedmont sites were sampled in mid-August.

Washings were prepared by extracting 10 g of whole leaves, 20 g of forest floor or 10 g of soil subsite composites with deionized water at a 1:10 (fresh sample mass : solution volume) extraction ratio. Extraction jars were hand-shaken for several minutes and then allowed to equilibrate for up to 1 h before centrifuging at 2000 rpm on a bench-top (Sorvall GLC-2) centrifuge for 5 min. Leaf extracts were prepared by macerating 6.0 g (fresh mass) of diced leaf tissue (blade only) with 20–30 mL of deionized water in a mortar and pestle. Amounts of added water varied with the viscosity of the ground tissue. Forest-floor and litter extracts were prepared by blending 20 g of tissue in 200 mL of deionized water in a Waring blender for 10 min. Whole-leaf and forest-floor extracts were also centrifuged as above. All washings and extract supernatants were amended with NH_4^+-N (to 10 mmol/L of added NH_4Cl) before they were applied to soils in order to equalize available NH_4^+-N among treatments.

Each supernatant (0.5 mL) was applied to each of seven 2.5-g soil microcosms in large (9 × 160 mm) test tubes; soil + solution mixtures were then mixed vigorously with a laboratory spatula to ensure even distribution of supernatant. With one exception, soils were from the same site as the supernatant solution and had been preincubated for several days prior to treatment in order to increase the sensitivity of the test by building up nitrifier populations. Solutions prepared from samples of the oak(2) primary sere site, however, were applied to oak(1) soils preincubated for 1 d, because oak(2) soils nitrified very little even when preincubated for long periods (Robertson and Vitousek 1981). Treated soils were brought to ≈70% water-holding capacity (WHC) with deionized water before mixing, and five tubes of each set of seven were then plugged with glass wool and incubated in a darkened controlled-environment cabinet at 30° (±0.5°) for 3.5 d. WHC was determined gravimetrically (Peters 1965, Robertson and Vitousek 1981). The remaining two tubes per treatment were immediately analyzed for pH in a 1:1 (3 g fresh soil: 3 mL H_2O) distilled water soil slurry after 30–60 min of equilibration. Five tubes from each site were also set aside for initial nitrate analysis.

Remaining washings and extracts were stored at −20° for later organic carbon analysis. This analysis followed the Walkley-Black procedure for oxidizable matter (Allison 1965) using known-concentration soluble starch solutions as standards. This procedure yielded readily oxidizable carbon values in soluble-starch-equivalent units.

At the same time that soils were amended with potential inhibitors, 2.5-g microcosms from each site were also treated with 0.5 mL of one of the carbon/pH control solutions. These solutions were designed to control for the oxidizable carbon and H^+ added by the suspected inhibitors. Control solutions were applied in a 5 × 5 factorial design with 5 replicates per treatment. Levels of carbon were 0, 12, 24, 36, and 48 g/L reagent-grade soluble starch; pH solutions were site-specific concentrations of HCl and NaOH designed to alter soil pH by −1.0, −0.3, 0, 0.3, and 1.0 pH units. These ranges of carbon and H^+ were designed to include concentrations expected from tissue extracts. For distilled water washings a further 12 levels of carbon between 0 and 12 g/L soluble starch were each applied to five soil microcosms per site for four primary sere sites where further resolution of C effects was needed. These and the wide-range carbon/pH control solutions were amended with NH_4^+-N as for the extracts and washings above, and were incubated and analyzed in the same manner as soils treated with suspected inhibitors.

Moisture/temperature incubations

The effects of moisture and temperature on potential nitrification in soils from along these seres were investigated by incubating soils from these sites in a factorial experiment. The design incorporated four levels of soil moisture (10, 30, 50, and 70% WHC) and two levels of temperature (20° and 30°).

Soils were collected in June 1979 from both seres at five subsites per site, as described earlier. In the laboratory, soils were composited by site, sieved, and percent water determined as above. Soils were allowed to air-dry overnight where necessary, and then 45 15.0-g replicates from each site composite were put into 150-mL polyethylene cups. These were then brought to either 10, 30, 50, or 70% WHC, in sets of 10, with distilled water and stirred as described earlier. The remaining 5 were set aside for initial mineral-N analysis. Each cup was capped with a snap-on lid with a 5-mm hole punched near its center. Five of the 10 cups per site at each moisture level were then incubated in a darkened controlled-environment cabinet kept at 20° (±0.5°). Water loss from a subset of the cups was monitored gravimetrically; original water content in all cups was restored every other day with distilled water + stirring. Incubations lasted 30 d, after which soils were extracted for mineral-N analysis.

These ~15-g and earlier 3-g soil mineral-N ex-

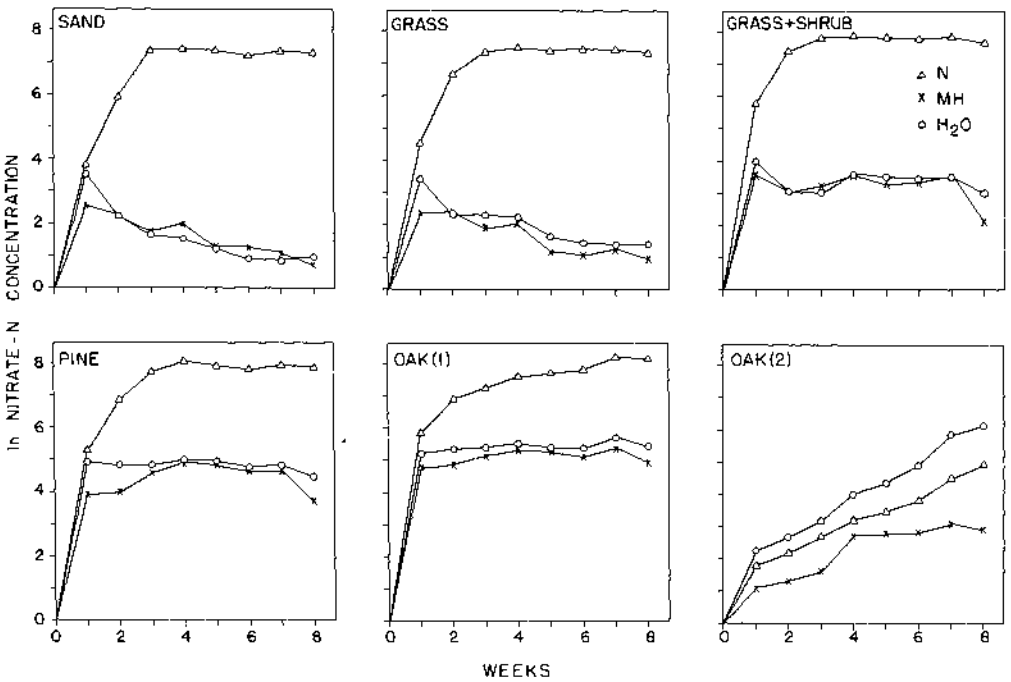

FIG. 2. Nitrate-nitrogen (NO_3^--N; milligrams per kilogram of soil) in weekly leachates from microlysimeters containing primary sere soils treated with distilled water (H_2O), NH_4Cl (N), or modified Hoagland's (MH) solutions. Values are unweighted means of three subsites with three replicates per subsite. See text for analysis of variance results.

tractions were performed at a 1:6 (fresh soil mass : extract solution) extraction ratio in 2 mol/L KCl (Jackson 1958) that also contained PMA at 0.5 mg/L to retard microbial growth. Extractions were shaken briefly and then allowed to equilibrate for 18–30 h before centrifuging. Supernatants were then analyzed for NO_3^-, NO_2^-, and NH_4^+ colorimetrically with a Technicon Autoanalyzer II system. Mineral N production was determined by subtracting mean initial nitrogen levels from final nitrogen levels; negative values can result and indicate net nitrogen immobilization.

Statistical analysis

Statistical analyses were performed on ln-transformed data in order to homogenize variance inherent in measuring chemical parameters. Analysis of variance was performed with the MANOVA subprogram of SPSS (Statistical Package for the Social Sciences) version 7.0 (Cohen and Burns 1977), except for contrasts which were calculated separately (Lindman 1973). Where transformed data failed to meet homogeneity of variance assumptions, nonparametric tests were substituted. Complete results of statistical analyses for all experiments are available in Robertson (1980).

RESULTS

Nutrient amendments

Primary sere.—NH_4^+-N stimulated nitrate production in soils from the first five sites of the primary sere (Fig. 2). Analyses of variance for each of the 1st 3 wk showed that nitrate output from NH_4^+-treated soils was significantly greater ($P < .05$) than control soils for the sand site (weeks 2 and 3), the grass site (weeks 1, 2, and 3), the grass + shrub site (weeks 1, 2, and 3), the pine site (weeks 2 and 3), and the oak(1) site (week 3). Soils from the oak(2) site were not significantly stimulated by added NH_4^+-N for any week.

Nitrogen-free Hoagland's solution had no more effect than did the distilled-water control, although in several cases a significantly decreased level of nitrate production was noted for this treatment for the 1st wk of incubation. In the oak(2) site this effect extended through 8 wk.

In no site but oak(2) was there evidence for a lag preceding the maximum rate of nitrate production, and the rate of production for NH_4^+-treated soils usually leveled off around week 3.

Secondary sere.—Soils from all sites along the secondary sere showed an overall increased nitrate output in response to NH_4^+-N treatment (Fig. 3). Analysis of variance for each of the 1st 3 wk showed that nitrate output from NH_4^+-treated soils (including those soils treated with ammonium-N + phosphorus [N + P]) was significantly greater ($P < .05$) than control soils for the annual site (weeks 1, 2, and 3), the perennial site (weeks 1, 2, and 3), the shrub site (weeks 1, 2, and 3), and the old-growth site (weeks 2 and 3).

The response to added NH_4^+-N was immediate for the annual, perennial, and shrub sites, and persisted

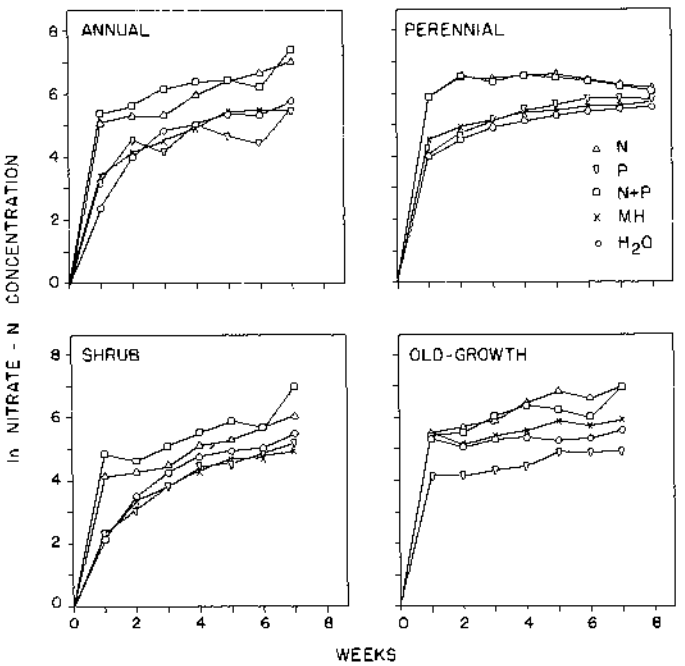

FIG. 3. Weekly nitrate-nitrogen (NO_3^--N; milligrams per kilogram of soil) output from microlysimeters containing secondary sere soils treated with distilled water (H_2O), NH_4Cl (N), Na_2HPO_4 (P), N + P, and modified Hoagland's (MH) solutions. For clarity, calcium treatments are not shown (see text). Values are unweighted means of four subsites with three replicates per subsite, except the perennial site with five subsites. After week 0 perennial site soils were leached with distilled water rather than nutrient solutions. See text for analysis of variance results.

throughout the incubation period except in the shrub. In this site the NH_4^+-N response persisted for only 2 wk, though the N + P treatment maintained this difference throughout and stimulated nitrate production significantly more than nitrogen alone during 2 of the 1st 3 wk.

Phosphorus applied in the absence of nitrogen appeared to inhibit nitrification in soils of the old-growth site; in no other cases along the sere did non-N treatments consistently affect nitrate production. Calcium and modified Hoagland's treatments showed no stimulatory effect other than that which could be ascribed to accompanying N or N + P.

Inhibition experiments

Primary sere.—Carbon additions to inhibition-control soils, applied in the range delivered by tissue extracts, quickly and severely depressed nitrate production in all soils where substantial nitrate production occurred. This was true regardless of pH effects (Fig. 4). Changes in pH within one unit of initial pH also affected nitrate production in all sites, but effects were less striking and trends varied somewhat among sites. In the sand and oak(2) sites, nitrification occurred too slowly to assess adequately carbon and pH effects, though some inhibition by carbon did occur at most pH levels.

Of the 49 potential sources of inhibition tested from the primary sere, 6 appeared to inhibit nitrification significantly over and above the effects of added C and altered pH (Table 1). These included *Ammophila breviligulata* leaf extracts from the grass site, *Prunus pumila* leaf washings from the grass + shrub site, and from the oak(1) site *Quercus rubra* leaf extract, two of three forest floor washings, and one of three soil washings. No inhibition was found for suspected inhibitors from the oak(2) site.

All of these inhibitors suppressed nitrate production in incubated soils more than was expected, based on nitrate production in the set of control soils with the closest but lower equivalent amount of H^+ and oxidizable C. Further resolution of the carbon effects was provided where needed by extrapolating expected nitrate production from low-level carbon-response curves (Fig. 5).

Secondary sere.—The effects on nitrate production of carbon applied to secondary sere soils are as striking as the effects on nitrate production noted for primary sere soils (Fig. 6), although soils from the secondary sere old-growth site appeared relatively resistant to added C. Reductions in pH at 0 g C depressed nitrate production in all sites, while increased pH in these soils stimulated apparent nitrification.

Of the 52 sources of potential inhibition tested in

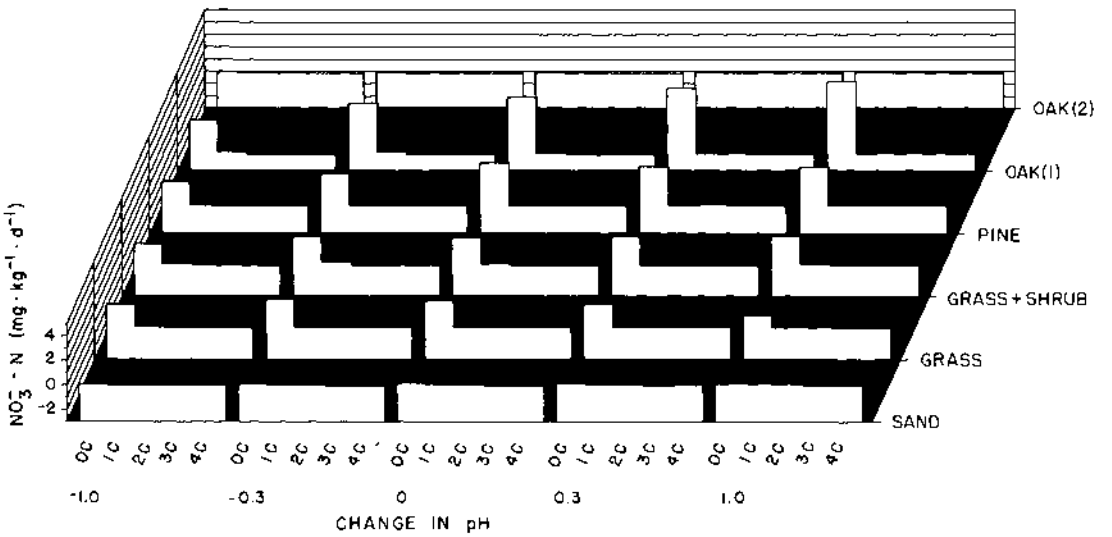

FIG. 4. Net nitrate production in soils from six stages of a primary succession treated with carbon and pH-buffer solutions and incubated for 3.5 d. 1C is equivalent to 0.5 mL of 12 gL soluble starch applied to 3.0 g wet soil, and corresponds to carbon additions of 800, 840, 800, 920, 1000, and 1200 mg C/kg dry soil for the sand through oak(2) sites, respectively. Note position of origin on the *Y*-axis; negative nitrate values indicate net nitrate immobilization. Each bar is the mean of five replicates; standard errors were usually <5% of the mean except where nitrate production was low (Robertson 1980).

this sere, none inhibited nitrate production more than would be expected based solely on the C and H^+ contents of the sources of suspected inhibitors.

Moisture/temperature incubations

Primary sere.—Both net nitrate-nitrogen and total mineral nitrogen (NH_4^+-N + NO_3^--N) production in this sere were stimulated by increased temperature and increased moisture, though the magnitude of these effects differed among sites (Fig. 7). Soils from older sites were affected more strongly than those from earlier sites except for oak(2) soils, in which nitrate production was very low. Except at lower moisture levels in the pine and oak(1) sites, a nearly constant proportion of the nitrogen made available by mineralization was subsequently nitrified, regardless of temperature.

Secondary sere.—Effects of temperature and moisture for secondary sere soils were similar to effects in the primary sere except that at 20°, moisture had a less predictable effect on nitrate and mineral nitrogen production (Fig. 8). At 30°, increased moisture (to 70% WHC) generally resulted in increased nitrogen pro-

TABLE 1. Sources of inhibition from sites along a primary sere and their effect on nitrate production (measured as N in dry soil mass) in preincubated soils from the same sites incubated for 3.5 d. No inhibition sources from sites along the secondary sere were detected. Added C (as a fraction of dry soil mass) refers to the readily oxidizable carbon that accompanied the inhibitor when it was added to incubated soil, and pH change indicates the corresponding change in soil pH. Expected values are based on extrapolations from low-level carbon response curves (Fig. 5) and most closely matching pH control microcosm. Values in parentheses are standard deviations. Negative values indicate net nitrate immobilization.

Site	Source of inhibitor	Added C (mg/kg)	pH change	Nitrate production (mg · kg^{-1} · d^{-1})	
				Actual	Expected
Grass	*Ammophila breviligulata* leaf extract	480	−0.75 (0.05)	−0.43 (0.006)	−0.35
Grass + shrub	*Prunus pumila* leaf washing	8	0.13 (0.18)	1.5 (0.08)	1.7
Oak(1)	*Quercus rubra* leaf extract	280	0.30 (0.05)	0.02 (0.21)	1.3
	Litter washing subsite 1	<8	0.40 (0.05)	2.9 (0.5)	3.9
	subsite 2	<8	0.30 (0.04)	2.9 (0.2)	3.8
	Soil washing subsite 1	<8	0.40 (0.04)	2.9 (0.3)	3.9

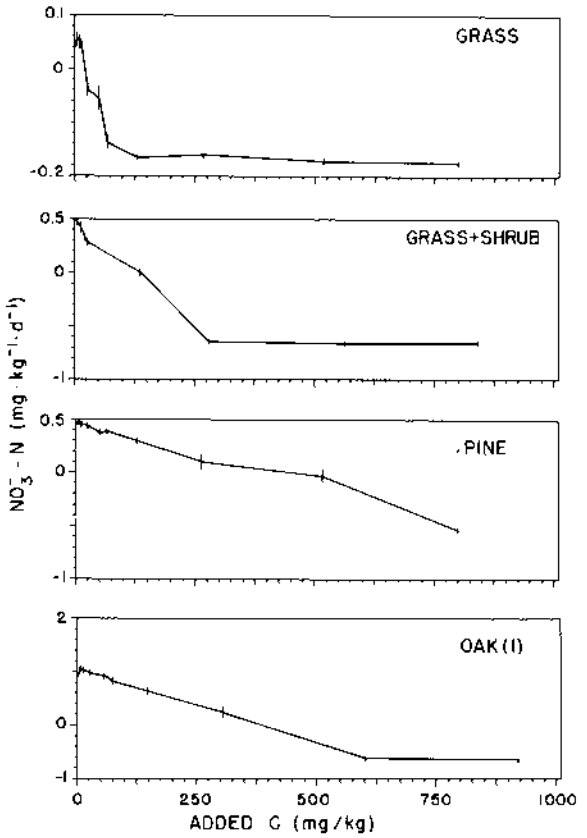

FIG. 5. Net nitrate production in soils from four stages of a primary sere treated with 12 different low-level carbon solutions. The highest concentration of carbon for each curve corresponds to 1C in Fig. 4 legend. Values are the means (±standard errors) of five replicates per carbon solution. Note position of origin on y-axis; negative values indicate net nitrate immobilization.

duction, though this nitrogen increase was minor in the perennial and the old-growth sites above moisture levels of 10% WHC. Within all sites but the shrub site, moisture levels above 10% WHC resulted in a nearly constant proportion of the mineral nitrogen's subsequent nitrification regardless of temperature.

DISCUSSION

If the rate of nitrification in the two seres studied is controlled by NH_4^+-N availability, then these experiments would be expected to yield two results. First, NH_4^+-treated soils incubated in microlysimeters should rapidly produce nitrate and at greater rates than soils from the same site treated with distilled water. Second, soils treated with sources of suspected inhibitors that are likely to be present under natural conditions (soil, litter, and whole-leaf washings but not extracts) should not inhibit short-term nitrate production in incubated soils when inhibitory effects attributable to added C and altered pH are removed.

Results show that ammonium stimulated nitrification in all but the final site along the primary sere and in all sites of the secondary sere. Only soils from the primary sere oak(2) site showed no response to added ammonium (Fig. 2).

Allelochemical inhibition was unambiguously (but not consistently) present only in the primary sere oak(1) site. Although leaf washings of *Prunus pumila* in the primary sere grass + shrub site also appeared to inhibit nitrate production in 3-d incubations, the effect was small and its importance as a factor regulating nitrification in this site is probably minor. Soil washings from this site caused no such effect and individual *P. pumila* are widely spaced on the dunes. Although leaf extracts from several sites also appeared to inhibit

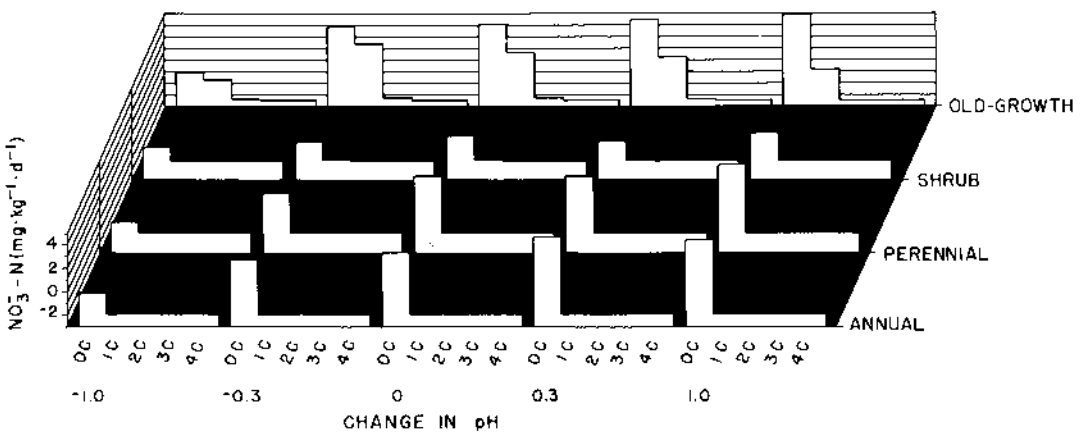

FIG. 6. Net nitrate production in soils from four stages of a secondary succession treated with carbon and pH-buffer solutions and incubated for 3 d. 1C (equivalent to 0.5 mL of 12 g/L soluble starch added to 3.0 g fresh soil) corresponds to a carbon addition of 1300, 1300, 1400, and 1300 mg/kg dry soil for the annual through old-growth sites, respectively. Note position of origin on the y-axis; negative values indicate net nitrate immobilization. Each bar represents the mean of five replicates; standard errors were usually <5% of the means (Robertson 1980).

nitrate production in soils from this sere, for reasons discussed earlier this inhibition is not likely to be ecologically meaningful.

Both litter and soil washings inhibited nitrification in incubated soils in the oak(1) site. The importance of this inhibition under natural conditions, however, is difficult to assess. Inhibitors were not apparent in litter washings from one of the three subsites nor in soil washings from two of the three subsites sampled, and in longer incubations, soils from this site consistently produced more nitrate than soils from any other site along the primary sere. These include a number of 15- and 30-d incubations (Robertson and Vitousek 1981 and Fig. 7) as well as 1st-wk and later nitrate production in control microlysimeters (Fig. 2). Further, the rate of nitrate production in these microlysimeter incubations was strikingly increased by added ammonium, although not significantly so until the 3rd wk of incubation.

In the one site along both seres where previous incubations strongly suggested the presence of inhibitors, no allelochemical inhibition could be documented. Primary sere oak(2) soils consistently produced very little nitrate despite high rates of nitrogen mineralization (Robertson and Vitousek 1981 and Fig. 7) and ammonium amendment (Fig. 2). Soil, litter, and leaf washings from this site failed to depress nitrate production (adjusted for added C and H^+) in preincubated oak(1) soils. In a subsequent experiment (Table 2), $CaCO_3$ was the only treatment that stimulated nitrate production in oak(2) soils. This treatment raised bulk soil pH from 4.3 to 6.3, while calcium as $CaCl_2$ failed to stimulate nitrification. Whether altered pH affected nitrifiers directly or neutralized a pH-sensitive inhibitor is unknown.

Suspected inhibitors appeared to stimulate nitrification in several cases. This occurred with both washings and extracts in both seres, and seemed particularly pronounced in the earlier sites. The effect was not likely related to differential ammonium availability in the treatment solutions since all had received ammonium amendments to 10 mmol/L.

Results from the moisture/temperature incubations suggest that uniform-moisture laboratory incubations of primary sere soils may systematically misrepresent relative rates of nitrification under field conditions. In the range examined (10–70% WHC and 20–30°), mois-

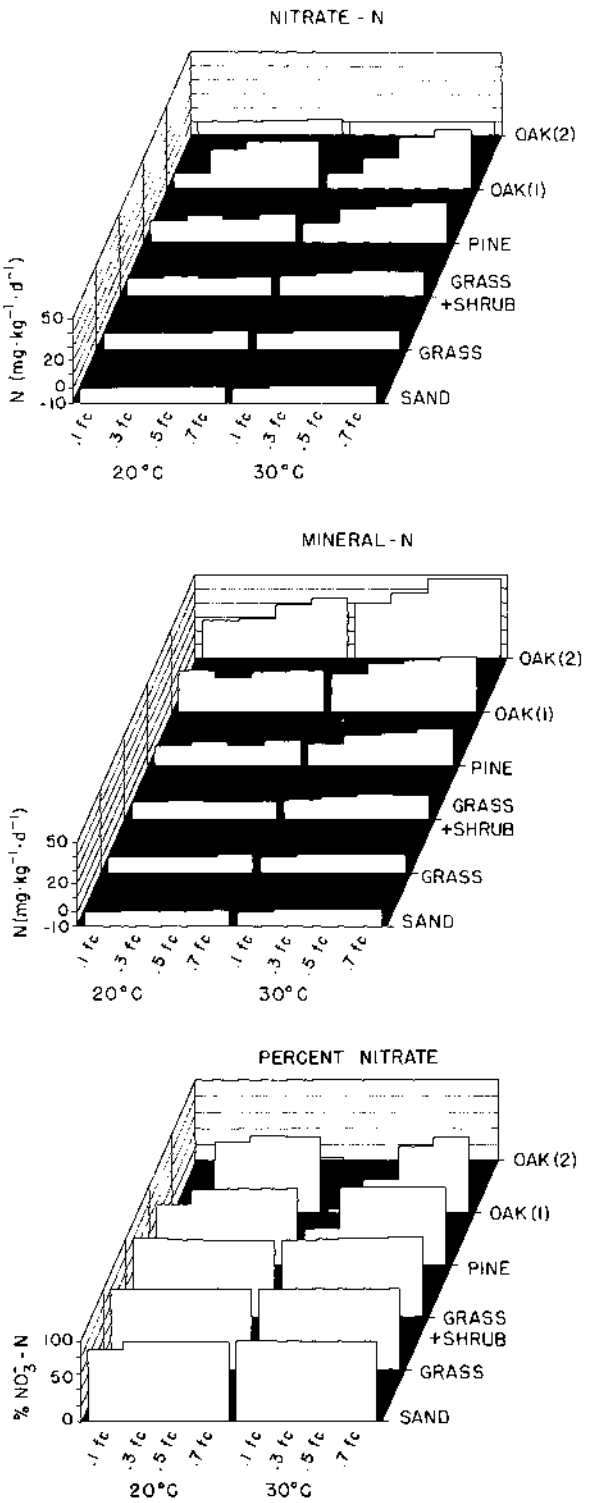

FIG. 7. Net nitrate-N production, net mineral-N (NO_3^--N + NH_4^+-N) production, and percent nitrate of total mineral-N accumulation (not net production) for soils from a primary sere incubated at different moistures and temperatures. Soils were either .1, .3, .5, or .7 of field capacity (fc, defined as gravitational water-holding-capacity, WHC) and were incubated at either 20° or 30°C. Negative values for nitrate production indicate net nitrate immobilization and nitrate values can be higher than total mineral-N values where immobilization of NH_4^+-N occurred. Each bar represents the mean of five replicates; standard errors were usually <5% of the means except where nitrate production was low (Robertson 1980).

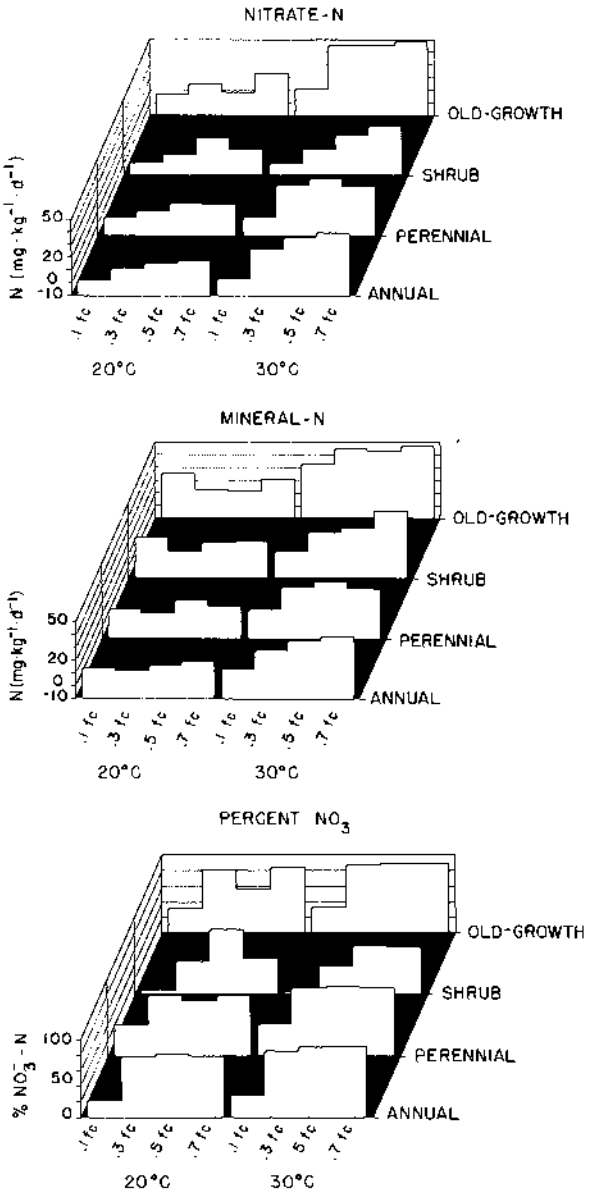

FIG. 8. Net nitrate production, net mineral-N (NO_3^--N + NH_4^+-N) production, and percent nitrate of total mineral-N accumulation (not net production) for soils from a secondary sere incubated at different moistures and temperatures. See Fig. 7 legend for further explanation.

ture levels had a greater effect on nitrification in soils from both seres than did temperature, with nitrification lower at lower water contents. For the primary sere, this may reinforce laboratory incubation predictions of higher relative nitrification later in the sere. Soils in early sites of this sere have relatively little vegetation cover and so are subjected to greater insolation and evaporation than are soils in older sites; thus early soils may be more moisture-stressed than later soils. These differences may be moderated somewhat by reduced transpiration early in succession, but on several sample dates, early soils were substantially drier than others (Table 3). Secondary sere laboratory-incubation estimates of relative nitrification, on the other hand, are probably not systematically different from relative rates in the field. Maximum moisture differences among sites in this sere do not appear great enough to affect relative nitrification significantly (Table 3 and Fig. 8).

TABLE 2. Net nitrate-N and total mineral-N (NO_3^--N + NH_4^+-N) production in 3 d as a proportion of dry soil mass in soils from the primary sere oak(2) site treated as indicated. Nitrogen as NH_4Cl, phosphorus as NaH_2PO_4, calcium as $CaCO_3$, and calcium as $CaCl_2$ were added at rates of 210 mg/kg, 150 mg/kg, 36 g/kg, and 36 g/kg dry soil, respectively. Modified Hoagland's solution (MH) was a complete nutrient solution less N and P as described in text for microlysimeters. Distilled water served as control. Soils were collected and combined at five subsites in August 1979 and processed as described for soils collected on other dates. Values are means of five replicates (±standard errors). nd = not determined because of added NH_4^+-N.

Treatment	Nitrogen production	
	NO_3^--N (mg/kg)	Total mineral-N (mg/kg)
Control	0.8 (0.1)	11.2 (0.5)
N	0.5 (0.04)	nd
P	0.6 (0.2)	9.3 (1.3)
MH	0.2 (0.02)	17.9 (1.5)
N + P	0.3 (0.02)	nd
N + MH	0.1 (0.02)	nd
P + MH	0.2 (0.02)	17.3 (1.0)
N + P + MH	0.1 (0.02)	nd
$CaCO_3$	61.5 (3.4)	61.3 (3.5)
$CaCl_2$	0.7 (0.1)	4.6 (0.7)

As Robertson and Vitousek (1981) reported for earlier incubations, in neither the microlysimeter incubations (Figs. 2 and 3) nor the moisture/temperature incubations (Figs. 7 and 8) were there progressive successional decreases in net nitrate production for either sere. In the primary sere, nitrification again increased through the first five sites and was low in the sixth site, though the increased nitrate production in oak(1) soils may be misleading due to the apparent presence of labile inhibitors in some locations in this site. This successional trend could be especially pronounced in the field, where moisture conditions may depress soil nitrate production in the earlier sites. Soils from the secondary sere again showed little trend except for slightly higher rates of nitrate production in old-growth soils. In neither sere is there consistent evidence that with succession a progressively smaller proportion of mineralized nitrogen is converted to nitrate. If the inhibitor found in oak(1) soils is an important regulator of nitrification in this site, however, nitrification may be inhibited late in succession in the primary sere.

Evidence that ammonium availability controls nitrification in numerous systems at a variety of succes-

TABLE 3. Percent H_2O and percent water-holding capacity (%WHC) of soils from the Indiana Dunes primary sere and the New Jersey Piedmont secondary sere sampled on four dates in 1978–79. Soils were collected without regard to previous precipitation, but were at least 3 d after heavy rain on all dates but May 1978 for both seres. Primary sere soils were snow-covered in January and the two earliest sites were frozen. WHC values (as a percentage of dry soil mass) are from Robertson and Vitousek (1981). All values are unweighted means of five subsites with three replicates per subsite except for June collections. June values are means of three subsites with two replicates per subsite (secondary sere) or of three-subsite site composites with three replicates per site composite (primary sere). Common superscripts within a column within a sere indicate no significant difference between values for those sites ($P < .05$) as determined by analysis of variance. nd = not determined.

Site	WHC (g/100 g)	May 1978		August 1978		December–January 1978–79		June 1979	
		%H_2O	%WHC	%H_2O	%WHC	%H_2O	%WHC	%H_2O	%WHC
Primary sere									
Sand	20.3 (0.2)	3.7 (0.3)	18.2 (1.4)^{ab}	2.3 (0.4)	11.1 (2.0)^{a}	nd	nd	1.3 (0.9)	6.4 (4.6)^{a}
Grass	21.3 (0.7)	4.9 (0.5)	23.0 (2.5)^{a}	0.9 (0.4)	4.1 (1.7)^{b}	nd	nd	1.5 (0.4)	7.0 (1.8)^{a}
Grass + shrub	23.1 (1.7)	3.3 (0.2)	14.1 (1.0)^{b}	1.1 (0.6)	4.6 (2.7)^{b}	4.7 (0.2)	20.4 (1.0)^{a}	0.3 (0.2)	1.3 (0.9)^{b}
Pine	27.6 (0.6)	8.6 (0.5)	31.3 (1.6)^{c}	3.6 (0.9)	13.0 (3.3)^{a}	12.0 (1.7)	43.5 (6.3)^{b}	3.8 (0.1)	13.8 (0.4)^{c}
Oak(1)	28.6 (0.5)	18.1 (3.8)	63.2 (13.2)^{d}	6.7 (0.4)	23.6 (1.4)^{c}	13.7 (1.7)	48.0 (5.9)^{b}	10.0 (0.2)	35.0 (0.8)^{d}
Oak(2)	33.7 (1.0)	nd	nd	11.7 (1.1)	34.7 (3.2)^{c}	15.5 (1.1)	46.1 (3.2)^{b}	11.4 (0.2)	33.8 (0.6)^{d}
Secondary sere									
Annual	36.1 (0.9)	20.6 (0.9)	57.2 (2.5)^{a}	21.4 (0.5)	59.3 (1.5)^{a}	20.6 (0.8)	57.0 (2.2)^{a}	19.2 (0.4)	53.1 (1.0)^{a}
Perennial	35.0 (0.8)	22.3 (0.6)	63.8 (1.7)^{a}	24.0 (0.6)	68.7 (1.4)^{b}	21.0 (0.6)	60.2 (1.7)^{a}	22.6 (0.2)	64.6 (0.6)^{b}
Shrub	28.4 (2.6)	22.6 (1.2)	79.6 (4.4)^{b}	21.0 (0.6)	73.8 (2.0)^{b}	21.8 (0.5)	76.9 (1.6)^{b}	21.8 (0.6)	76.9 (2.0)^{c}
Old-growth	39.9 (1.1)	28.0 (1.3)	70.1 (3.3)^{b}	21.9 (0.7)	54.9 (1.7)^{a}	26.3 (0.8)	66.0 (2.0)^{c}	28.1 (1.1)	70.4 (2.8)^{c}

sional stages is readily available. Though interpreations are usually complicated by the assumptions described earlier, increased nitrate production in incubated soils following in vitro ammonium fertilization is common (e.g., Stojanovic and Alexander 1958, Overrein 1967, Stevens and Reuss 1975, Melillo 1977, Ohta and Kumada 1979). In situ fertilization has also stimulated nitrate accumulation in soils from many systems (e.g., Krause and Batsch 1968, Johnson et al. 1980), though increased soil nitrate following ammonium treatments could be due to factors other than increased nitrification.

Delays in nitrification similar to that found in the primary sere oak(2) site have also been reported elsewhere. A number of early studies (noted by Romell 1935) described sites in which little apparent nitrification occurred before liming. More recent descriptions of such sites include Corke (1958), Brar and Giddens (1968), Williams (1972), and Jones and Richards (1977). The stimulatory effect of elevated pH in these soils could be due to degradation of an allelochemical inhibitor, but it could also be related to ionic competition between ammonium and hydrogen ions at the interface between the cell and the soil solution (Epstein 1972), to pH-effected chemical degradation of bacterial cell wall proteins, to aluminum toxicity at low pH (Brar and Giddens 1968), or to pH-sensitive microsite surface charges (Marshall 1980). Nitrifiers from most sites along these seres fit the general observation that nitrifiers are relatively pH sensitive, though low pH per se does not imply lower potential nitrification. Nitrifiers are extremely active in the secondary sere old-growth site, for example, where bulk soil pH is 4.4 (Robertson and Vitousek 1981). In all sites, however, altering soil pH by as little as 0.3 or 1.0 pH units significantly affected nitrate production (Figs. 4 and 6). Differential pH-sensitivity among sites could represent adaptations to site-specific pH, or it could result from differences in microsite heterogeneity.

CONCLUSIONS

1) Nitrification was stimulated by added ammonium in the first five of six sites along a primary sere at the Indiana Dunes. Apparent allelochemical inhibition was present in places and may be important in controlling nitrification in the fifth site. A lag in nitrification that is persistent and removed by $CaCO_3$ is an important regulator of nitrification in the sixth site.

2) Added ammonium stimulated nitrification in all four sites of a secondary sere on the New Jersey Piedmont. No evidence was found for the presence of inhibitors in these sites.

3) Uniform-moisture laboratory incubations of primary sere soils may overestimate in situ rates of nitrogen transformations in soils from early sites relative to rates in later sites. Relative rates of transformations in incubations of secondary sere soils probably approximate those in the field.

4) In general, ammonium availability appears to be more important than allelochemical inhibition as a regulator of nitrification in both seres. There is no consistent evidence for the progressive inhibition of nitrification with successional development in the secondary sere or in the earliest five sites of the primary sere.

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