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Denitrification and Nitrous Oxide Production in Successional and Old-Growth Michigan Forests[1]

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ABSTRACT

Soils from 12 successional and old-growth forests in Michigan were examined for N_2O production under each of three incubation conditions: without amendment, in a 20% (vol/vol) acetylene atmosphere, and in an acetylene-amended atmosphere after a simulated rainfall. Intact cores were taken during the growing season and incubated in the laboratory for 90 to 180 min in a porespace-recirculation system that sampled for N_2O and CO_2 at 10-min intervals. Measurable rates of N_2O production occurred in cores from all sites and under all incubation conditions; in general but not always, rates were higher in the presence of C_2H_2 than in its absence, and increased upon simulated rainfall. Under acetylene- and water-amended conditions, mean production was highest in an old-growth and a late-successional hardwood site and in a recent clearcut (30 to 80 mg N_2O-N m^{-2} d^{-1}); intermediate rates (2 to 3 mg N_2O-N m^{-2} d^{-1}) were observed in soils from a midsuccessional hardwood stand and from two old-field communities, and low rates (<0.6 mg N_2O-N m^{-2} d^{-1}) occurred in soils from a young sand dune community, from a midsuccessional and a late-successional hardwood stand, and from a managed, a midsuccessional, and an old-growth conifer community. Nitrate production, CO_2 production and water content could explain 65% of the variation in rates of N_2O production among sites under acetylene-amended conditions. Nitrate pool sizes and pH differed substantially among sites but were not well correlated with N_2O production. The presence of a class of cores that produced N_2O only in the absence of acetylene suggests that sources of N_2O other than denitrifiers may be important in some sites.

Additional Index Words: ecological succession, nutrient cycling, nitrogen, clearcutting, soil respiration, nitrification.

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DENITRIFICATION has long been recognized as an important pathway for N loss in agricultural systems, but estimates of its importance in noncropped and other unfertilized systems have thus far been largely speculative because of methodological limitations. Mass balance calculations (e.g., Bormann and Likens, 1979; Salati et al., 1982) and estimates of denitrifier biomass or enzyme activity (e.g., Westerman and Tucker, 1978; Muller et al., 1980; Melillo et al., 1983; Jordan et al., 1982; Salati et al., 1982; George and Antoine, 1982) have suggested that significant rates of denitrification are likely to occur in soils of many nonfertilized communities, but until recently it has not been feasible to estimate these fluxes directly. The use of ^{15}N in these systems, for example, has been precluded by the fact that it usually boosts existing inorganic N pools severalfold. Consequently, our knowledge of rates of denitrification in unfertilized forests and our understanding of the factors that regulate these rates in situ have been significantly restricted.

The recent development of the acetylene-inhibition technique for examining denitrification in agricultural soils (Klemedtsson et al., 1977; Tiedje, 1982) offers promise for corresponding studies in nonagricultural areas. While results from different approaches to the application of this technique must be interpreted with care (Jury et al., 1982; Parkin et al., 1984), we believe that short-term incubations of intact soil cores in recirculating aerobic atmospheres can provide significant insight into both absolute and comparative rates of denitrification among different sites, as well as into the factors that may be affecting rates of N_2 and N_2O production in situ. In this paper we describe the results of a series of experiments designed to examine comparative rates of denitrification and N_2O production in 12 noncultivated communities in central Michigan that differ widely in vegetation cover, nutrient stores, and presumably nutrient cycling properties.

SITE DESCRIPTIONS

One old-growth or late-successional site and two earlier successional sites at each of four Michigan locations (41°50′ to 44°45′N, 84°35′ to 86°42′W) were sampled during the midgrowing season (June–July), 1982. Study areas included the Warren Dunes State Natural Area, Berrien County, near the southeastern shore of Lake Michigan; the W.K. Kellogg Biological Station, Kalamazoo County; the Rose Lake Wildlife Research Area, Shiawassee County, and the Hartwick Pines State Forest in Crawford County, ca. 300 km north of the other sites.

At Warren Dunes (WD), sample sites included a grass-shrub community near the base of the second foredune from the Lake, a midsuccessional mixed hardwood forest behind the first set of major foredunes, and an old-growth, undisturbed beech-maple forest several kilometers inland. The first two sites (sec. 26, T.6S, R.20W) were underlain respectively by well-drained sandy Entisols (Psamments) with no defined soil profile, and by soils in the Oakville fine sand series, also well drained. The old-growth site (sec. 27, T.7S, R.20W) was on somewhat poorly-drained Selfridge loamy sand soils. Vegetation dominants in the first site included *Ammophila breviligulata* and *Prunus pumila*; in the second site, *Quercus, Tilia, Fagus, Prunus* and *Acer* spp., and in the undisturbed site, *Fagus grandifolia* and *Acer saccharum*. The areas have been described at length by Cain (1935) and others (Tague, 1947; MNAC, 1979).

Sites at Kellogg Biological Station (KBS) (Section 8, T.1S, R.9W) included a late-successional oak-hickory forest (>100 yr since systematic disturbance) and two abandoned fields flanking either side of this ca. 2 ha stand. All three sites were on Oshtemo sandy loam. The younger of the fields had been planted to corn as recently as 12 yr previously, and at the time of the study was dominated by perennial grasses (*Agropyron repens* and *Bromus inermis*) and perennial and biennial dicots (including *Daucus carota, Potentilla recta,* and *Cirsium arvense*) (Gross and Werner, 1983). The older field had been abandoned since 1964, and at the time of sampling supported various forbs (mainly *Solidago* spp., *Aster* spp., and *Melilotus repens*) and staghorn sumac (*Rhus typhina*),

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which had been planted sometime prior to 1960 (Werner and Harbeck, 1982).

The Rose Lake (RL) sites (sec. 21, T.5N, R.1E) included a recent clearcut, a midsuccessional oak-hickory (*Quercus-Carya* spp.) forest, and a late-successional oak-hickory forest, all on well-drained Spinks loamy sand. Based on ring counts of felled trees, the clearcut forest was ca. 120 yr old at the time of cutting, 2 months previous to the mid-June sample date; at the time it was sampled, the site supported various annual and perennial herbs, grasses and root sprouts. Slash from the harvesting had been piled in windrows on the site. The adjacent late-successional forest appeared to have been about the same age as the cleared forest before cutting, and the midsuccessional forest, ca. 100 m from the others, about 60 yr old.

The Hartwick Pines sites (sec. 15, T.27N, R.3W) included a densely planted ≥30-year-old red pine (*Pinus resinosa*) plantation, a midsuccessional (ca. 60 yr) mixed conifer stand, and an adjacent, ca. 100 ha, old-growth white pine (*Pinus strobus*) community. All three sites were underlain by well-drained Rubicon Sand soils (Entic Haplorthods). The old-growth forest had not been cut since before settlement in the early 1800s, and included some red pine, hemlock (*Tsuga canadensis*), and scattered hardwoods. White pine, red pine, hemlock, red maple (*Acer rubrum*) and birch (*Betula papyrifera*) dominated the mixed conifer stand. Undergrowth was sparse or absent in all stands.

METHODS

Denitrification Assays

All sites were sampled at least 3 d after the most recent rainfall during the mid-1982 growing season. Cores for denitrification assays were taken at 10-m intervals along a 100-m transect located randomly within each site. Intact soil cores 4.7 cm in diameter by 20 cm deep were collected inside a 24-cm length of polyvinyl chloride (PVC) tubing using a steel punch-auger driven by a hand-operated slide hammer. The auger penetrated small stones and roots to 3-cm diam with minimal disturbance. Compaction of the soil cores kept for analyses was never greater than 5%. After collecting a core, both ends of the PVC liner were capped with butyl rubber stoppers and the core was placed on ice for transport to the laboratory.

In the laboratory, cores were brought up to temperature over 4 h in sets of eight, and incubated at 22°C (± 0.5°) on a soil atmosphere recirculation system (Parkin et al., 1984). The atmosphere inside each core liner was pumped continuously from the headspace of the liner through 5 m of 0.32 cm o.d. stainless steel tubing. At 10-min intervals, a valve along the sample stream shunted 0.5 mL of recirculating gas to the injection port of a Varian 3700 or Perkin Elmer 910 gas chromatograph (GC). Each GC was fitted with four Porapak Q columns in a 55°C oven; at alternate intervals each column was in line with either an electron capture detector operated at 300°C or a backflush stream of carrier gas (95% argon, 5% methane). Gas flows were optimized for separation of N_2O and CO_2. Sample peaks were integrated with Hewlett-Packard computing integrators. Detection limits were ca. 0.1 ng N_2O-N mL^{-1} and 3.0 μg CO_2 mL^{-1}. Total airspace volume inside each core was measured with a pressure transducer (Parkin et al., 1984); volumes generally ranged from 150 to 300 mL.

All cores were assayed for N_2O production under each of three conditions: (i) unamended, (ii) amended with 50 mL acetylene to ca. 20% (vol/vol) C_2H_2, and (iii) amended with acetylene as before but 4 h after a 1.4-cm (27 mL) simulated (distilled H_2O) rainfall. Preliminary experiments had shown N_2O production in wetted cores from two sites examined to peak at ca 3 h and remain constant for ca. 12 h following wetting.

Each core was assayed for N_2O production under each condition for up to 180 min after an initial 20-min equilibration period; in general, cores with moderate and higher activity exhibited linear production of N_2O within 80 min and analyses were stopped at that point. Cores that failed to exhibit significant production after 150 min (as indicated by regression slopes that were not significantly different from 0 at $p < .05$) were judged to produce N_2O at indetectable levels; the lowest increase detectable with the system was ca. 100 ng N m^{-2} h^{-1}. At no times during any incubations were N_2O concentrations below detection limits.

After incubation, the soil in each core was mixed, stones and roots judged unable to pass through a 4-mm sieve removed, and three 10-g subsamples taken out for KCl extractions and two 10-g subsamples removed for pH analysis as described below. The remaining soil was dried at 105°C for % H_2O and bulk density determinations, and then later ground with mortar and pestle for organic matter analyses by Walkley-Black procedures (Allison, 1965).

Site Characterizations

Respiration potentials and NH_4^+, NO_3^-, pH, organic matter and bulk density analyses were performed on soils from soil cores assayed for N_2O production. On dates different from sampling dates for N_2O assays, soil cores were taken for other soil analyses. At each of five subsites located at 20-m intervals along the 100-m transect, one soil core was taken and transported as described above and assayed for nitrification and mineralization activity. Each core was incubated intact at 25°C (± 0.5) after being first brought to ca. 60% water-holding capacity with distilled H_2O added to the top of each core. Core liners were loosely capped and soils were incubated for 15 d. After incubation, the soil in each core was mixed, five 10-g subsamples were removed for KCl extractions, and then the remaining soil was dried and weighed as described above. Extracts were later analyzed for NO_3^- and NH_4^+ as described below.

At the same time that sites were sampled for mineralization potentials, samples were taken for static nutrient pool analyses. Three 2.7-cm diameter by 13-cm long cores were taken with a modified polypropylene syringe within 50 cm of each mineralization subsite. These smaller cores were taken to the laboratory on ice, mixed and sieved as described above, and within 48 h subsets were extracted in 2*M* KCl, 2*M* NH_4OAc, and 0.1*N* HCl solutions. For each extraction, three replicate 10-g subsets of wet soil were mixed with 100 mL extractant, shaken on a rotary shaker for 1 h, and then filtered through glass fiber filter paper. Remaining soil was weighed and dried as above. Later, ground samples of each of these soils were digested in a Technicon block digestor and analyzed for total N (Technicon Instruments, 1977). Potassium chloride extracts were analyzed for mineral N colorimetrically with a Technicon autoanalyzer using the cadmium reduction technique for nitrate (Technicon Instruments, 1973). NH_4OAc and HCl extracts were analyzed for various cations and P with a Beckman 55-3a plasma emission spectrophotometer.

Statistical Analyses

All data but pH were subjected to a log-normal transformation before parametric analysis to accomodate homogeneity of variance assumptions. Analyses of variance, multiple regressions, and multivariate statistics (canonical correlation and discriminant function analysis) were performed using the SPSS statistical package (Hull and Nie, 1981). Regression analyses included factors in a two-step hierarchy; the first step included measured factors presumed to affect denitrification in most soils (% moisture, C availability, NO_3^- availability, pH) while the second step included all other factors measured. Within each of the two main

Table 1—Selected soil properties (to 20-cm depth) of sites described in text.

Site	Bulk density†	Organic C†	Total N‡	C/N	Respiration potentials§	Mineralization potentials‡	
						NO_3^--N	N_{min}¶
	g cm^{-3}	%				mg N m^{-2} d^{-1}	
Warren Dunes							
1. Grass-shrub (> 20 yr)	1.60 (0.04)	0.10 (0.01)	0.003 (0.0013)	55.8 (18.7)	9.1 (8.8)	−12.6 (6.6)	−16.9 (5.3)
2. Hardwoods (> 80 yr)	1.01 (0.06)	1.75 (0.14)	0.173 (0.034)	11.7 (0.3)	8.3 (2.7)	133. (27.0)	193. (13.)
3. Old growth (> 300 yr)	1.14 (0.05)	1.62 (0.36)	0.102 (0.002)	20.9 (6.4)	18.0 (8.6)	142. (28.0)	181. (32.)
Kellogg Biological Station							
1. Old field (ca. 12 yr)	1.29 (0.03)	1.27 (0.07)	0.131 (0.012)	10.1 (0.3)	56.6 (43.3)	6.7 (1.2)	−94.3 (11.7)
2. Old field (ca. 18 yr)	1.32 (0.01)	1.09 (0.05)	0.104 (0.005)	10.2 (0.7)	58.4 (28.4)	9.3 (2.8)	105. (13.6)
3. Hardwoods (> 100 yr)	1.06 (0.04)	2.03 (0.13)	0.160 (0.012)	13.2 (0.9)	21.0 (12.2)	25.0 (7.1)	10.6 (1.3)
Rose Lake							
1. Recent clear cut (< 0.2 yr)	0.88 (0.07)	2.49 (0.17)	0.190 (0.018)	12.3 (1.5)	54.1 (27.0)	60.3 (15.7)	211. (68.1)
2. Hardwoods (> 60 yr)	0.95 (0.04)	2.03 (0.13)	0.154 (0.008)	13.4 (0.4)	21.9 (12.8)	9.1 (5.6)	35.7 (6.1)
3. Hardwoods (> 120 yr)	0.98 (0.05)	1.82 (0.09)	0.126 (0.011)	15.1 (1.3)	14.7 (9.5)	28.1 (11.8)	85.1 (10.1)
Hartwick Pines							
1. Red pines (ca. 25 yr)	1.03 (0.02)	1.16 (0.06)	0.075 (0.010)	16.9 (1.2)	63.3 (27.7)	4.5 (0.8)	−3.8 (0.7)
2. White pine (> 80 yr)	1.04 (0.08)	1.02 (0.12)	0.070 (0.003)	15.2 (2.4)	8.9 (0.6)	−1.1 (0.4)	25.2 (10.5)
3. White pine (> 300 yr)	0.86 (0.05)	1.92 (0.17)	0.065 (0.005)	22.5 (2.2)	32.4 (20.0)	−8.2 (1.2)	76.0 (14.7)

† Mean (± SE) of 10 subsites per site; 3 analytical replicates per subsite.
‡ Mean (± SE) of 5 subsites per site; 3 analytical replicates per subsite.
§ g CO_2 m^{-2} d^{-1} determined during N_2O assays.
¶ NO_3^- + NH_4^+-N; can be < NO_3^--N where NH_4^+ was immobilized.

steps, factors were included in the order of greatest individual contribution to explained variance.

RESULTS

Site Characterizations

Results from organic C and total N analyses and respiration, mineralization and nitrification potentials (Table 1) suggest that site fertility varies widely among our sites. Organic C varied from 0.1 %C in the Warren Dunes grass + shrub site to 2.5 % C in the recent clearcut at Rose Lake, and total N, from < 0.01 % N to 0.19% N in these sites. Short-term soil respiration was extremely variable among cores within sites; rates of CO_2 production ranged from a low of 9.1 g CO_2 m^{-2} d^{-1} in the early Dunes sites to > 54 g CO_2 m^{-2} d^{-1} in others. In individual cores, CO_2 production was significantly but not strongly correlated with organic C concentrations ($r = 0.36$, $p < 0.01$, $n = 119$).

Highest rates of apparent N availability, as indicated by net mineralization potentials, were observed in the Rose Lake clearcut site. In this site > 210 mg N m^{-2} d^{-1} were mineralized over the 15-d laboratory incubation period. Net immobilization occurred in soils from three sites: the early succession Warren Dunes site, the youngest of the two old fields at Kellogg, and the youngest of the Hartwick Pine sites. Nitrification of the N mineralized during the 15-d intact-core incubations also varied among sites; nitrate was immobilized in the early Warren Dunes site and in two of the Hartwick Pines sites, while 60 to 142 mg NO_3^--N m^{-2} d^{-1} was produced in the two older Warren Dunes sites and the Rose Lake clearcut.

Extractable nutrients and minerals also varied sub-

Table 2—Selected chemical characteristics of soil from experimental sites.

Site	pH†	NO_3^--N‡¶	NH_4^+-N‡¶	P‡§	K‡#	Ca‡#	Al‡¶
		μg·g soil^{-1}					
Warren Dunes							
1	7.1 (0.04)	0.84 (0.26)	0.75 (0.31)	18.6 (0.93)	3.8 (0.2)	227 (8.4)	0.85 (0.75)
2	5.6 (0.3)	5.02 (1.02)	5.38 (0.39)	11.6 (1.3)	27.6 (2.7)	1644 (185)	2.74 (0.58)
3	3.6 (0.07)	5.46 (0.41)	3.32 (0.39)	19.3 (5.4)	86.5 (25.0)	153 (29)	2.20 (0.68)
Kellogg							
1	5.0 (0.1)	0.36 (0.25)	1.96 (0.70)	33.6 (7.1)	78.3 (7.4)	463 (54)	1.41 (0.17)
2	4.7 (0.1)	0.39 (0.01)	0.80 (0.15)	33.4 (5.3)	84.9 (7.4)	363 (24)	1.22 (0.16)
3	5.3 (0.2)	0.31 (0.14)	7.48 (4.6)	58.2 (19.4)	79.7 (10.3)	852 (210)	1.81 (0.29)
Rose Lake							
1	5.4 (0.4)	0.74 (0.20)	4.33 (0.60)	7.6 (3.9)	40.3 (4.4)	1385 (281)	1.50 (0.60)
2	4.3 (0.2)	0.11 (0.03)	4.72 (0.30)	5.1 (1.7)	36.7 (5.1)	600 (105)	2.37 (0.87)
3	4.0 (0.1)	0.07 (0.03)	6.92 (0.58)	10.2 (1.1)	46.1 (6.6)	552 (162)	2.60 (0.29)
Hartwick Pines							
1	3.8 (0.2)	0.07 (0.03)	6.09 (0.41)	4.37 (0.93)	33.5 (1.7)	253 (56)	14.2 (2.5)
2	3.7 (0.03)	0.14 (0.01)	10.7 (4.9)	4.38 (0.53)	34.4 (6.1)	241 (64)	8.26 (1.6)
3	3.4 (0.2)	0.36 (0.01)	8.43 (0.77)	6.63 (1.1)	39.9 (4.5)	231 (43)	25.6 (7.6)

† Mean (± SE) of 10 subsites per site (3 replicate extractions per subsite); 20-cm sample depth; determined for soils used in N_2O assays.
‡ Mean (± SE) of 5 subsites per site (3 replicate extractions per subsite); 13-cm sample depth.
§ HCl-extractable.
¶ KCl-extractable.
# NH_4OAc extractable.

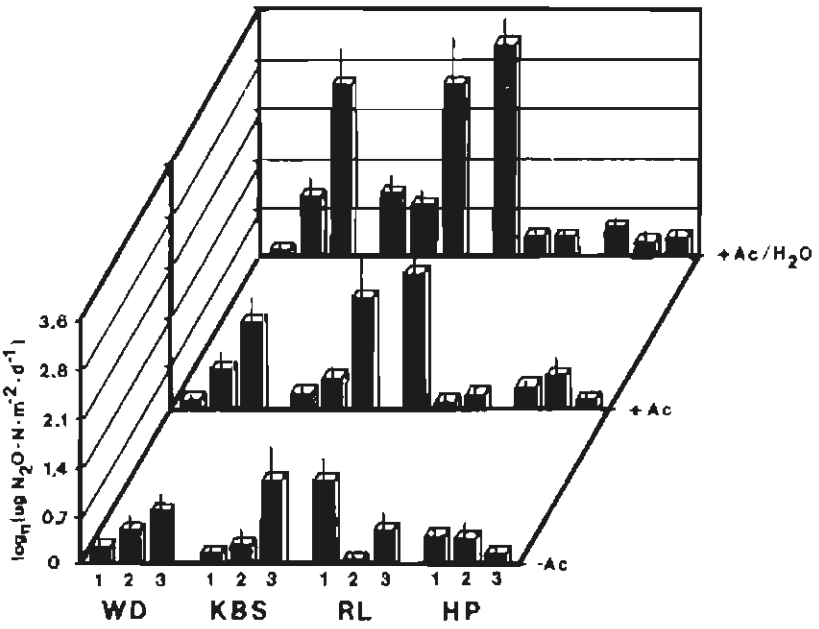

Fig. 1—Nitrous oxide production in soils from 12 successional and old-growth communities in Michigan during short-term aerobic incubations. Intact soil cores were incubated sequentially (i) without acetylene (− Ac), (ii) in the presence of 20% (vol/vol) acetylene (+ Ac), and (iii) in the presence of acetylene 4 h after a 1.4-cm simulated rainfall (+ Ac/H_2O). Vertical lines bisecting bars represent within-site variation (± standard error of site mean, n = 10 soil cores).

stantially among sites (Table 2). Bulk soil pH was as low as 3.1 in some cores from the old growth Hartwick Pines site, and ranged to 7.1 in the early Warren Dunes community. Acid-extractable P ranged from < 5 to > 30 μg P g soil^{-1}, and other species, particularly Al, varied by similar magnitudes.

N_2O Production

We observed significant N_2O production in cores from all sites under each of the three incubation conditions. Rates of production varied substantially both between and within sites and among incubation conditions (Fig. 1). In general, rates were higher in the presence than in the absence of C_2H_2, and higher after simulated rainfall than before. After wetting and in the presence of C_2H_2, rates ranged from > 76 mg N m^{-2} d^{-1} in cores from the late-successional Kellogg site to a mean rate of < 0.1 mg N m^{-2} d^{-1} in the early Warren Dunes site. These rates were < 1 to 3 × higher than the mean rates of N_2O production observed before wetting, which ranged from 0.15 to 28 mg N m^{-2} d^{-1} (Table 3). Rates of N_2O production in the acetylene atmosphere were highest in soils from the old-growth Warren Dunes site, the late-successional Kellogg site and the Rose Lake clearcut site; intermediate rates were observed in soils from the Warren Dunes mid-successional forest and the two old-field communities at Kellogg, and very low but measurable mean rates occurred in soils from the remaining sites.

N_2O production in the absence of C_2H_2 was observed in all sites; in many cases this production was equivalent to or greater than subsequent production in the presence of acetylene. In the three sites where N_2O production in the presence of acetylene was highest, N_2O production in incubations without acetylene was > 1/3 of corresponding rates in the presence of acetylene. A number of individual cores, however, produced no discernible N_2O until C_2H_2 was added (Fig. 2, Class II); likewise, about the same number of cores produced N_2O only in the absence of acetylene (Fig. 2, Class III), and still another distinct group (27% of the total number of cores) produced no discernible N_2O under either condition (Fig. 2, Class I). The positive correlation between N_2O production in the presence and in the absence of acetylene among Class IV soils (Fig. 2) may indicate that within this class N_2O production is mainly of denitrifier origin, or that conditions favorable to N_2O production by nondenitrifiers are also favorable for N_2O production by denitrifiers.

Discriminant function analysis using variables that had been measured for each core (CO_2 production, soil mass, water content, pH, NO_3^-, and % organic C) showed that response Classes II and III could be differentiated from one another on the basis of a single discriminant function comprised of values for pH, water content, and bulk density (canonical correlation = 0.39, p = 0.087). Those cores that produced N_2O only in the absence of acetylene (Class III) had a lower pH (4.6 vs. 5.2, p <0.05, n = 33 cores), less soil moisture (17.0 vs. 27.9 % H_2O at time of collection, p = 0.087, n = 33), and a slightly lower bulk density (0.97 vs. 1.12 g soil cm^{-3}, p = 0.141, n = 33).

Of the site characteristics examined, NO_3^- production (nitrification potential) and acid-extractable P could each explain the greatest proportion of the variation in rates of N_2O production under all incubation conditions (r > 0.56 for NO_3^- production and r > 0.69 for HCl extractable P, p < 0.05, n = 12). Under one or another incubation condition, N_2O production rates were also correlated with salt-extractable Al (r ≥ −0.54, p < 0.07, n = 12), organic C (r ≤ 0.46, p < 0.05, n = 118), CO_2 production (r ≤ 0.23, p <

Table 3—Nitrous oxide production in soil cores from various successional and old growth communities in Michigan.†

Site	N_2O production (mg N m^{-2} d^{-1})‡		
	− C_2H_2	+ C_2H_2	+ C_2H_2/H_2O
	Warren Dunes		
1	0.36 (0.18) abc	0.16 (0.06) a	0.06 (0.04) a
2	1.11 (0.80) abc	1.35 (0.62) c	2.72 (1.4) bcd
3	1.97 (1.06) c	5.73 (2.7) c	34.4 (16.9) de
	Kellogg		
1	0.31 (0.25) a	0.55 (0.46) a	2.16 (0.90) bcd
2	0.87 (0.87) d	0.85 (0.40) abc	1.94 (1.08) bc
3	10.8 (6.2) abc	27.7 (14.4) c	75.9 (37.6) cde
	Rose Lake		
1	4.48 (1.85) c	11.0 (3.7) c	36.2 (12.8) e
2	0.10 (0.07) a	0.15 (0.08) a	0.43 (0.21) ab
3	1.42 (1.01) abc	0.36 (0.28) ab	0.37 (0.18) ab
	Hartwick Pines		
1	0.59 (0.18) bc	0.44 (0.14) abc	0.61 (0.18) bcd
2	0.97 (0.63) ab	1.28 (0.68) abc	0.37 (0.27) a
3	0.19 (0.09) abc	0.18 (0.13) a	0.43 (0.25) ab

† Cores were incubated without added acetylene (− C_2H_2), in the presence of 20% (vol/vol) acetylene (+ C_2H_2), and in the presence of added acetylene 4 hours after simulated rainfall (+ C_2H_2/H_2O).

‡ Mean (± SE) of 10 cores per site; common superscripts within columns indicate no significant differences ($p > 0.05$) between means. Multiply values by 1.8 to approximate kg N ha^{-1} 6 $months^{-1}$ under incubation conditions shown. See Table 1 for explanation of site abbreviations.

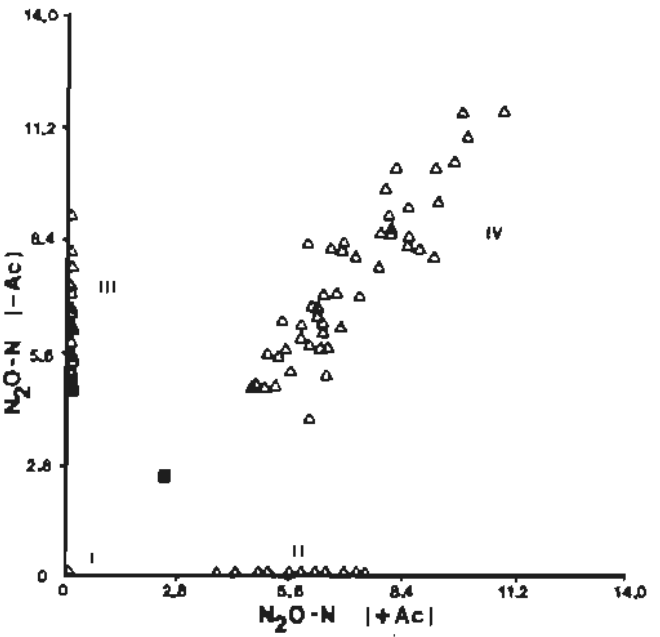

Fig. 2—Nitrous oxide production in soil cores from sites described in text during aerobic incubations; production in the absence of C_2H_2 (− Ac) is plotted against subsequent production in the presence of 20% (vol/vol) C_2H_2 (+ Ac). Each point represents a separate soil core from one of 12 sites; 32 points are clustered at the origin (Class I). The closed square represents detection limits.

0.05, $n = 118$), extractable K ($r \leq 0.63$, $p < 0.05$, $n = 12$), and % moisture ($r \leq 0.60$, $p < 0.05$, $n = 120$). Neither pH nor soil nitrate concentrations were significant predictors of N_2O production under any of the three incubation conditions ($r < 0.19$, $p > 0.05$, $n = 20$).

Nitrification potentials could account for as much as 31% of the variation in rates of N_2O production among sites under acetylene-free conditions ($p < 0.06$, $n = 12$), and for 35 to 36% of the variation in both the acetylene-amended incubations and incubations with acetylene and added H_2O ($p < 0.04$, $n = 12$) (Table 4). Including water content in the regression for the third incubation condition and respiration potentials in the regressions for all conditions brings these proportions to 45%, 49%, and 67%, respectively.

DISCUSSION

Measurable rates of N_2O production occurred in soils from all of the 12 sites examined in this study. Rates varied substantially both within and among sites, but in general, rates were highest in those sites with high potentials for nitrification and respiration, high concentrations of soil P and organic C, and low concentrations of extractable Al, and generally increased after additions of C_2H_2 and again after additions of 1.4 cm H_2O.

In our three most active sites, results suggest that from 6 to 30 mg N m^{-2} d^{-1} may be lost via denitrification (N_2O production in the presence of acetylene) under normal growing season conditions, with periodic increases to 2 to 3 times these rates during periods of precipitation. As much as an additional 1.5 to 10 mg N_2O-N m^{-2} d^{-1} may be lost from these sites during non-rainy periods via direct N_2O production by denitrifiers, nitrifiers, or other producers of N_2O. The potential importance of this last flux under water-amended conditions was not assessed.

These estimates for the three most active sites extrapolate to ca. 2 to 12 kg N ha^{-1} $month^{-1}$, a flux that can be several times greater than N inputs via precipitation in most temperate forests (Frissel and Kolenbrander, 1977; Likens et al., 1977), and perhaps of the same magnitude as precipitation and N_2 fixation combined. Consequently, in some forested and successional systems, denitrification and other pathways of N_2O loss may have a significant bearing on long-term N balances and hence on primary productivity and other ecosystem processes.

In a number of our sites, denitrification occurred at very low rates that extrapolate to < 0.1 kg N ha^{-1} $month^{-1}$. In most of these cases, low rates were the

Table 4—Best predictors of N_2O production in soils from all sites.

Incubation condition	Site factors	Additive r^2†	Significance (p)
Without C_2H_2	NO_3^- production	0.311	0.060
	CO_2 production	0.451	0.067
	NO_3^-, organic C	0.595	0.130
	pH, H_2O	0.666	0.298
With C_2H_2	NO_3^- production	0.356	0.041
	CO_2 production	0.488	0.049
	% H_2O	0.631	0.094
	NO_3^-, pH, organic C	0.563	0.479
With C_2H_2 + H_2O	% H_2O	0.364	0.038
	NO_3^- production‡	0.563	0.024
	CO_2 production	0.668	0.026
	Organic C	0.768	0.022
	pH, NO_3^-	0.795	0.109

† The proportion of the variation in rates of N_2O production under a given incubation condition that can be explained by a particular factor (or set of factors) plus all preceding factors taken together. See Methods. n = 12 sites.

‡ r^2 without % H_2O = 0.36.

net result of a large proportion of inactive soil cores (< apparent detection limits of 0.02 mg N_2O-N m^{-2} d^{-1}) that substantially diluted the positive effects of remaining cores that produced N_2O as actively as cores from any other site. Several Hartwick Pines cores, for example, produced the equivalent of 5 mg N m^{-2} d^{-1}, although mean within site N_2O production was < 1 mg N m^{-2} d^{-1} because of a high number of inactive cores. This high spatial variability (coefficients of variation > 100%) appears to be typical for even cultivated systems that are heavily fertilized. Parkin et al. (1984), for example, found coefficients of variation > 70% among intact cores collected within the same m^2 in a plowed and fertilized field previously planted to corn. The causes of this variation are not well understood, although the variability has important implications for both N losses and sampling strategies.

The dominant source of N_2O in these soils appears to be denitrification; in most sites at least as much and usually more N_2O was produced in the presence of a 20% (vol/vol) acetylene atmosphere as in an aerobic headspace. However, significant amounts of N_2O were produced in incubations without acetylene. Nitrous oxide produced under these conditions may have come from a variety of sources, including denitrifiers, nitrate respiring bacteria, fungi, yeasts, and nitrifiers (Blackmer et al., 1980; Smith and Zimmerman, 1981; Bleakley and Tiedje, 1982). Discrimination among these sources is not possible with the evidence available in this study, although the presence of a class of cores that produced N_2O under only acetylene-free conditions (Class III, Fig. 2) suggests that sources of N_2O other than denitrification are important in at least some cores of several sites.

Nitrous oxide response classes (Fig. 2) were not associated with specific sites. Discriminant analysis, however, showed that soil pH, % moisture, and bulk density could be used to discriminate successfully ($p < 0.09$) between the 33 cores in Classes II & III. Characteristic of those cores that produced N_2O only in the absence of acetylene (Class III), as opposed to those that produced N_2O only in acetylene's presence (Class II), was a lower pH, reduced soil moisture, and a slightly lower bulk density.

Results from this study plus preliminary evidence from studies in other nonfertilized forest sites (Myrold et al., 1982; Strauss and Firestone, 1982; Robertson and Tiedje, unpublished results) suggest that denitrification is a highly variable process both among and within temperate forests. Laboratory and field studies of agricultural soils have shown that favorable conditions for respiration, an adequate nitrate supply, a circumneutral soil pH, and reduced O_2 tensions tend to favor gaseous N losses in most soils. It is premature to generalize about patterns in rates of denitrification in successional and forested systems; however, analyses of regression trends in data from the present study suggest that nitrate production may be a principle determinant of N_2O loss in unfertilized temperate forests.

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