

# Comparison of Nitrification Rates in Blueberry and Forest Soils

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**ABSTRACT.** Highbush blueberries (*Vaccinium corymbosum* L.) are long lived perennial plants that are grown on acidic soils. The goal of this study was to determine how blueberry cultivation might influence the nitrification capacity of acidic soils by comparing the nitrification potential of blueberry soils to adjacent noncultivated forest soils. The net nitrification potential of blueberry and forest soils was compared by treating soils with <sup>15</sup>N enriched (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, and monitoring nitrate (NO<sub>3</sub>-N) production during a 34-day incubation period in plastic bags at 18 °C. Net nitrification was also compared by an aerobic slurry method. Autotrophic nitrifiers were quantified by the most probable number method. Nitrate production from labeled ammonium (<sup>15</sup>NH<sub>4</sub><sup>+</sup>) indicated that nitrification was more rapid in blueberry soils than in forest soils from six of the seven study sites. Slurry nitrification assays provided similar results. Blueberry soils also contained higher numbers of nitrifying bacteria compared to forest soils. Nitrification in forest soils did not appear to be limited by availability of NH<sub>4</sub><sup>+</sup> substrate. Results suggest that blueberry production practices lead to greater numbers of autotrophic nitrifying bacteria and increased nitrification capacity, possibly resulting from annual application of ammonium containing fertilizers.

Nitrification, the oxidation of ammonium (NH<sub>4</sub><sup>+</sup>) to nitrate (NO<sub>3</sub>), is an important soil process that can affect fertilizer use efficiency, the potential for NO<sub>3</sub><sup>-</sup> movement into potable water sources, and loss of N<sub>2</sub>O to the atmosphere as a global warming gas (Robertson et al., 2000). Autotrophic bacteria are the primary nitrifiers in most soils. Although these bacteria have been thought to be inhibited by low pH (Watson, 1974), and acidic soils usually exhibit low nitrification rates (Dancer et al., 1973; Morrill and Dawson, 1967), some acidic soils have relatively high nitrification rates. High nitrification in acidic soils has been attributed variously to acid tolerant autotrophic bacteria (De Boer et al., 1990), nitrifying heterotrophic organisms (Stroo et al., 1986), or acid sensitive autotrophs that function within high pH microsites in the acidic soils (Hankinson and Schmidt, 1984) or by forming protective cell aggregates (DeBoer et al., 1991).

Highbush blueberries (*Vaccinium corymbosum*) require acid soils (optimum pH 4.5 to 5.0) and are usually grown on coarse textured, well drained soils where NO<sub>3</sub><sup>-</sup> may be prone to leaching. The fact that blueberries absorb NO<sub>3</sub><sup>-</sup> less readily than NH<sub>4</sub><sup>+</sup> (Peterson et al., 1988) may further increase the potential for NO<sub>3</sub><sup>-</sup> leaching. Nitrate may be toxic to blueberries under some conditions (Herath and Eaton, 1968). Although nitrification could impact fertilizer use efficiency and the potential for NO<sub>3</sub><sup>-</sup> contamination of water, information on NO<sub>3</sub><sup>-</sup> generation in blueberry soils is limited. Fertilizer NH<sub>4</sub><sup>+</sup> was nitrified at a moderate rate in one blueberry field study, but this soil had an atypically high pH that was reduced by S additions (Throop and Hanson, 1998).

Ammonium fertilizer applications appeared to increase the nitrification capacity in native stands of lowbush blueberry (Eaton and Patriquin, 1988) and in annual crop production on loam soils (Tabatabai et al., 1992). Annual applications of NH<sub>4</sub>-N up to 70

kg·ha<sup>-1</sup> are recommended for blueberries in Michigan (Hanson and Hancock, 1996), and many older plantings have received fertilizer for decades. A preliminary study comparing NO<sub>3</sub><sup>-</sup> production in Michigan blueberry soils to that of similar soils from adjacent noncultivated forest areas (Hanson and Mandujano, 1997) indicated that blueberry soils nearly always produced NO<sub>3</sub><sup>-</sup> at higher rates (mean 0.074 µg NO<sub>3</sub>-N/g soil per d) than adjacent forest soils (0.032 µg NO<sub>3</sub>-N/g soil per day). However, the blueberry soils also contained higher NH<sub>4</sub><sup>+</sup> levels, so it was not clear whether more NO<sub>3</sub><sup>-</sup> was produced because the pool of NH<sub>4</sub><sup>+</sup> substrate was larger or if there was greater nitrification capacity due to other factors. Therefore, the objective of the current study was to determine if previously reported differences in NO<sub>3</sub><sup>-</sup> production (Hanson and Mandujano, 1997) were due to differences in NH<sub>4</sub><sup>+</sup> substrate availability or nitrifier populations and inherent nitrification capacity.

## Materials and Methods

Soils chosen for this experiment exhibited either high or low nitrification potentials and a wide range in pH (3.5 to 5.7) as determined in a previous study (Hanson and Mandujano, 1997). Soils were sampled on 9 Sept. 1996 from seven blueberry plantings and adjoining noncultivated forest areas. Four plantings were in Van Buren County, Mich., and were classified as either as Pipestone-Kingsville complex (sandy mixed mesic Typic Endoaquad, mixed, mesic Mollic Psammaquents) (sites CH1, CH2, and MB) or Morrocco loamy sand (mixed mesic Aquic Udipsamments) (site KO). Three sites (GR1, GR2, and DE) were in Ottawa County, Mich., and mapped as Au-Gres Saugatuck complex loamy sands (sandy mixed frigid Typic Endoaquad, sandy, mixed, shallow, ortstein Typic Duraquad). Blueberry sites had been in production for 18 years or more. Although the forested areas may have been cultivated at some time, estimated tree age indicated that they had not been cultivated for at least 30 years (CH1, CH2, and GR2 sites), or 60 years (DE, GR1, KO, and MB sites). Forested sites contained mixed stands of hardwood species. The sampling areas were selected so that the blueberry fields and forest areas bordered one another and shared a similar soil type. Sampling areas varied from 50 to 200 m in length and 20 to 40 m in width.

**LABELED N NITRIFICATION.** On 9 Sept. 1996, twenty 20 cm deep soil cores were collected with a 2.5 cm diameter soil probe through-

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out each sampling area, usually by walking a staggered pattern across rows or through woods. The 20 soil cores were mixed in buckets, placed in 4-L plastic bags in an ice chest, transported to East Lansing, Mich., and placed in a cooler at 2 °C. Soil moisture content and water holding capacity (WHC) were determined for each soil so that the moisture content could be adjusted to 50% of WHC for the incubation study. Field moisture contents were determined by weighing subsamples of each soil before and after drying for 24 h at 60 °C in a forced air oven. WHC was determined by placing 30 g of dry soil in a funnel containing previously weighed filter paper. The soil was saturated with water, and allowed to drain for 3 h. The soil and filter paper were then weighed and the WHC was calculated as the difference between the dry and drained weight of soils minus that of the filter paper.

On 26 Sept. 1996, fresh soil, equivalent to 200 g dry weight, was placed in 4-L polyethylene bags. Each bag was treated with 30 mg (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> enriched to 99.8 atom % <sup>15</sup>N (Isotec, Inc., Miamisburg, Ohio) by spraying (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solutions with a hand sprayer over the bagged soil. The bags were shaken at least three times during the application to evenly distribute the N. The tracer was applied in enough water to bring each soil to 50% WHC. The application rate (N at 33.5 µg·g<sup>-1</sup> dry soil) was equivalent to an application of N at 73.4 kg·ha<sup>-1</sup> (assuming a 2,200,000 kg soil/ha furrow slice, 17 cm depth). Three bags of each soil served as replications. Bags were closed, and incubated at 18 °C in the dark. Bags were weighed weekly and water was added when needed to maintain the moisture content at 50% of WHC.

Subsamples were removed from each bag for N analyses 5, 8, 17, and 34 d after treatment, and extracted by shaking a suspension of 10 g soil in 75 mL of 1 M KCl at 150 rpm on a solution agitator (New Brunswick Scientific Co., Edison, N.J.) for 45 min. The extracts were passed through Whatman no. 5 filter paper, which had been rinsed previously with deionized water, and analyzed for total NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N with a Lachat Instruments autoanalyzer and Quikchem methods 12-107-06-1-A and 12-107-04-1-F (Lachat, Milwaukee, Wis).

A sequential diffusion procedure (Brooks et al., 1989) was used

to separate the NH<sub>4</sub><sup>+</sup>-N from NO<sub>3</sub><sup>-</sup>-N in the extracts. The extracts were first treated with MgO to raise pH and convert NH<sub>4</sub><sup>+</sup>-N to NH<sub>3</sub> gas. The gas was condensed on Whatman no. 3 filter paper disks treated with K<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>SO<sub>4</sub>. Devardas alloy with 50 µL of surfactant per sample cup was then used to convert NO<sub>3</sub><sup>-</sup>-N to NH<sub>3</sub> gas, which condensed on new treated disks. The <sup>15</sup>N enrichment of the NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N fractions was determined by mass spectrometry using a Europa Scientific (Crewe, England) Tracer Mass instrument (Harris and Paul, 1989). Sample discs were arranged according to concentrations to avoid errors due to carryover and drift as samples were analyzed with the mass spectrometer. Reference <sup>15</sup>N standards were included after every 12 samples.

Total soil N and atom % <sup>15</sup>N were measured after 34 d of incubation by analyzing 10 mg samples of dry soil with a commercial C–N analyzer–continuous flow isotope ratio mass spectrometer (Europa Scientific, Crewe, England) (Harris and Paul, 1989). The percentage of total N (inorganic and organic) originating from the fertilizer (NFF) was calculated as follows:  $NFF = 100(A - B)/(C - B)$ , where A = atom % <sup>15</sup>N of sample, B = atom % <sup>15</sup>N ambient (0.366%), and C = atom % <sup>15</sup>N in fertilizer (99.8%). Organic fertilizer N was calculated by subtracting the sum of inorganic fertilizer N (NO<sub>3</sub><sup>-</sup>-N and NH<sub>4</sub><sup>+</sup>-N determined by sequential diffusion as described previously) from the total fertilizer N. Organic matter content was measured by loss of weight on ignition at 360 °C (Combs and Nathan, 1998). Soil organic C was estimated by dividing the organic matter content by 1.724 (Nelson and Sommers, 1982). The C:N ratio was computed by dividing the organic C concentration by the total soil N concentration. Three replicate samples of each soil were analyzed for Bray-Kurtz-1 extractable P, ammonium acetate extractable Ca, Ma, and K and pH in a 1 soil/water slurry (by volume). Particle size analyses of single samples of each soil were conducted by the hydrometer method.

**SLURRY NITRIFICATION.** Additional forest and blueberry soil was collected from the CH1, CH2, GR1, MB, and KO sites on 7 Sept. 1999, using the same sampling procedure as in 1996. Soil nitrification potentials were determined by a shaken slurry method (Hart et al., 1994). After subsamples were taken from each soil for moisture

Table 1. Chemical and physical characteristics of soils collected in 1996 from blueberry fields and adjacent forest sites on seven southern Michigan farms. Organic matter, pH, and P, K, Ca, and Mg concentrations are means of three observations. Particle analysis data are single measurements.

Study		Organic matter		Mineral nutrient (mg·kg <sup>-1</sup> )				Particle analysis (%)		
site <sup>z</sup>	Cropping	pH	(%)	P	K	Ca	Mg	Sand	Silt	Clay
CH1	Blueberry	4.2	3.9	106	40	232	52	87	9	4
CH1	Forest	4.4	3.6	9	15	48	21	82	4	4
CH2	Blueberry	4.4	4.3	140	50	143	32	83	10	7
CH2	Forest	4.9	4.8	52	35	381	37	88	8	4
DE	Blueberry	4.6	7.8	70	35	334	200	87	7	6
DE	Forest	4.2	5.4	27	25	143	42	89	7	4
GR1	Blueberry	5.2	3.6	117	40	428	105	89	7	4
GR1	Forest	4.4	4.7	9	20	48	27	90	6	4
GR2	Blueberry	5.3	4.9	160	40	428	120	90	5	5
GR2	Forest	4.7	4.7	10	25	286	84	90	6	4
KO	Blueberry	4.2	3.4	22	85	192	69	77	13	10
KO	Forest	5.0	6.3	14	50	990	170	75	15	10
MB	Blueberry	4.5	6.1	96	40	191	53	86	9	5
MB	Forest	3.9	11.1	17	35	48	27	79	13	8
Means	Blueberry	4.6 a <sup>y</sup>	4.9 a	102 a	47 a	278 a	90 a	86 a	9 a	6 a
	Forest	4.5 a	5.8 a	20 b	29 b	278 a	58 a	85 a	8 a	5 a

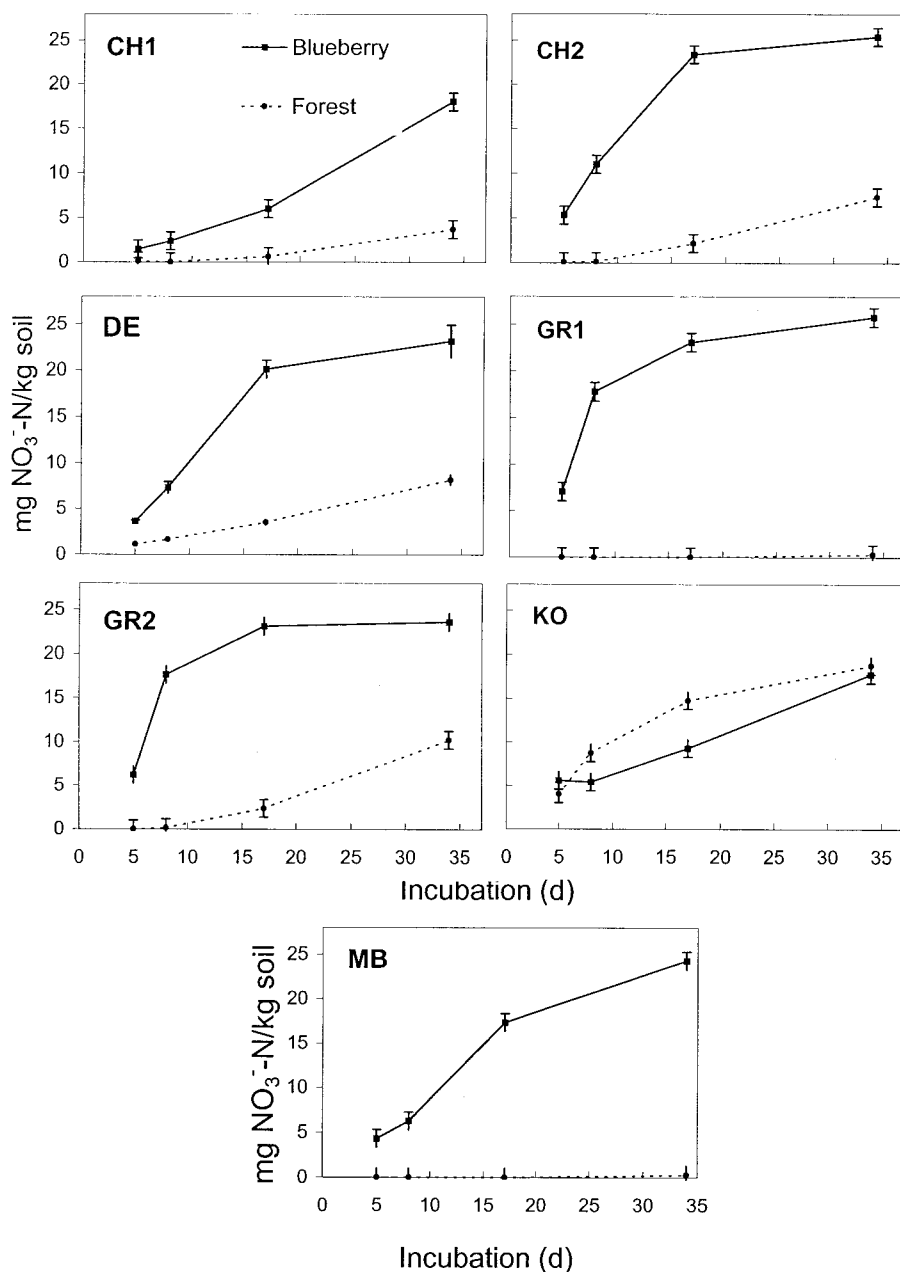
<sup>z</sup>See Materials and Methods for description.

<sup>y</sup>Mean separation by paired *t* test (*P* = 0.05). See materials and methods for details.

determinations, 10 g of sieved, field-moist soil was placed in a 250-mL Erlenmeyer flask containing 100 mL of a solution containing 1.5 mM of  $\text{NH}_4^+$  and 1 mM of  $\text{PO}_4^{3-}$ . Three flasks of each soil served as replications. Flasks were sponge-stoppered and agitated for 24 h on an orbital shaker at 180 rpm. Samples were removed from each flask after 2, 6, 10, 20, and 24 h of incubation and centrifuged at 8000  $g_n$  for 10 min. The supernatant was decanted into polypropylene scintillation vials, preserved with chloroform (2 mL of  $\text{CHCl}_3/\text{L}$  of sample solution) and frozen at  $-20^\circ\text{C}$  until  $\text{NO}_3^-$ -N analysis was conducted. The hourly rate of  $\text{NO}_3^-$ -N production (mg N/L) was calculated by linear regression of  $\text{NO}_3^-$ -N concentration versus time and converted to per unit dry soil ( $\text{mg}\cdot\text{kg}^{-1}$ ).

**NITRIFIER POPULATIONS.** Nitrifier populations in the 1999 sampled

Fig. 1. Changes in fertilizer derived  $\text{NO}_3^-$ -N levels following addition of  $^{15}\text{N}$ -enriched  $(\text{NH}_4)_2\text{SO}_4$  (N at  $35.5\text{ mg}\cdot\text{kg}^{-1}$  soil) to blueberry and forest soils sampled from seven Michigan farms in 1996. Vertical bars represent  $\pm$  SE ( $n = 3$ ).



soils were determined using the most probable number method (Rowe et al., 1977). Two replicate samples of each soil, consisting of 10 g moist soil, were blended with 190 mL 100 mM phosphate buffer (pH 7) in a Waring Blender (Waring Products Company, Winsted, Conn.). Coarse particles were allowed to settle for 1 min, then 10 mL of the slurry was transferred to a sterile plastic tray for further dilution in the microtiter plates. Before inoculation, all wells of the microtiter plates were filled with 100 mL of 1 $\times$  Schmidt and Belser (1994) medium. A multichannel pipettor was used to transfer eight 100 mL aliquots from the plastic tray to the first row of wells in the microtiter plates, mixed well with the medium, and then 100 mL of this mixture was transferred to the next row of wells, thus making a 1:2 dilution. A series of 12 twofold dilutions (equal to one microtiter plate) were made after the first 1:20 dilution in the blender.

The inoculated plates were double wrapped in plastic with a moist towel to help maintain moisture levels and then incubated in the dark at  $25^\circ\text{C}$  for 8 weeks. Uninoculated microtiter plates kept under the same conditions were used as controls. After 8 weeks, each plate was checked for the presence of  $\text{NO}_3^-$  and/or  $\text{NO}_2^-$  by adding an indicator (0.2 g of diphenylamine in 100 mL of concentrated  $\text{H}_2\text{SO}_4$ ) (Rowe et al., 1977). A blue color reaction was scored as positive. Two replicates of each soil were measured.

**BACTERIAL POPULATIONS.** Total bacterial populations, cell sizes, and biomass were determined in the 1999 sampled soils using the methods of Bloem et al. (1995). Three replicate 10 g samples of each soil were blended in 190 mL of filtered, distilled water for 1 min. The coarse particles were allowed to settle for 30 s and 20 mL bulk samples were placed in sterile tubes and treated with 0.1 mL formalin as a preservative. The samples were vortexed and 4 mL aliquots of each were placed in 6 mm wells on slides (Bellco Glass, Inc., Vineland, N.J.) and allowed to air dry completely. The dried smears were then flooded with a 8 mL DTAF [5-(4,6-dichlorotiazin-2-yl) amino fluoroscein; Sigma Chem. Co., St. Louis] stain solution (2 mg DTAF in 10 mL of a buffer solution consisting of 0.05 M  $\text{Na}_2\text{HPO}_4$  and 0.85% NaCl, adjusted to pH 9) for 1 h at  $20^\circ\text{C}$  in a covered container with wet tissue. The slides were rinsed three times for 20 min with the  $\text{Na}_2\text{PO}_4$ -NaCl buffer solution and finally briefly with water. After air drying, a cover slip was mounted with a small drop of immersion oil. The slides were stored in the dark at  $20^\circ\text{C}$  until observation.

Counts were performed with a 63 $\times$  oil immersion objective lens and 1.6 $\times$  zoom setting (total magnification  $\approx 1000\times$ ) using a Leitz Orthoplan 2 epifluorescence microscope, a Lep HBO 50 mercury lamp, and a Leitz I3 filter block (BP 450-490 excitation filter, RKP 510 beam splitter, and LP 515 suppression filter) (Leica Microsystems, Wetzlar, Germany). A charge-coupled device camera

Table 2. Effect of site and cropping history (blueberry vs. forest) on fertilizer derived and soil derived (native)  $\text{NO}_3^-$ -N,  $\text{NH}_4^+$ -N, and total N at the end of a 34 d incubation.

Study site <sup>z</sup>	Cropping	Fertilizer derived N (mg/kg soil)			Soil derived N (mg·kg <sup>-1</sup> soil)		
		$\text{NH}_4^+$ -N	$\text{NO}_3^-$ -N	Total N	$\text{NH}_4^+$ -N	$\text{NO}_3^-$ -N	Total N
CH1	Blueberry	0.2	18.0	38.7	2.0	29.0	1787
	Forest	11.6	3.7	28.5	14.0	4.3	821
CH2	Blueberry	0.1	25.4	28.9	2.1	30.7	1368
	Forest	6.4	7.4	31.8	12.5	13.0	1641
DE	Blueberry	0.1	23.1	36.3	1.3	40.0	2633
	Forest	9.5	8.2	46.7	13.6	11.0	2305
GR1	Blueberry	0.0	25.8	44.9	1.3	26.8	1620
	Forest	11.9	0.3	39.2	23.5	0.5	1758
GR2	Blueberry	0.0	23.6	43.1	1.0	25.7	1879
	Forest	4.1	10.2	35.5	11.4	17.5	1541
KO	Blueberry	6.7	17.8	45.2	11.4	40.0	1350
	Forest	1.6	18.7	39.0	10.3	33.6	2521
MB	Blueberry	0.1	24.3	34.0	1.6	26.1	2052
	Forest	8.7	0.3	28.8	29.2	0.4	3161
Significance (LSD <sub>0.05</sub> )							
Site (S)		**	**	*	*	*	***
Cropping (C)		***	***	NS	***	**	NS
S × C		**(3.8)	**(10.7)	NS	**(6.6)	**(12.5)	**(1890)

<sup>z</sup>See materials and methods for details.

NS, \*\*, \*\*\*, \*\*\*\* Nonsignificant or significant at  $P < 0.05$ , 0.01, or 0.001, respectively. Numbers in parentheses are LSD values (0.05) for S × C interaction.

(Princeton Instruments, Trenton, N.J.) was used to capture random images of each sample and transferred to a Power Macintosh 7100/66 via an ST135 detector/controller and GPIB interface card (Natl. Instruments, Austin, Texas). The images were then analyzed by IP Lab Spectrum image analysis software (Signal Analytics Corp., Vienna, Va.).

Data were subjected to analysis of variance using SAS protocols (SAS Inst., Inc., Cary, N.C.). A split-split-plot design was used for the labeled N incubation study, with the seven sites serving as main plots, cropping history (blueberry cultivated or forest) as subplots, and sampling time (5, 8, 17, and 30 d after treatment) as sub-subplots. Data from the slurry incubations and bacterial populations were analyzed as a split plot with the five sites serving as main plots and cropping history as subplots.

## Results

**Labeled N Nitrification.** Comparisons of selected chemical and physical characteristics of soils collected in 1996 (Table 1) indicated that blueberry soils contained higher levels of P and K than forest soils. The average C:N ratio of forest soils (17.3) was not statistically different than that of cultivated soils (15.5).

ANOVA indicated highly significant ( $P < 0.0001$ ) effects of sampling site, cropping (blueberry vs. forest), sampling time, and all interactions with respect to levels of fertilizer derived and total  $\text{NO}_3^-$ -N, and fertilizer derived and total  $\text{NH}_4^+$ -N. Fertilizer derived  $\text{NO}_3^-$ -N accumulated more rapidly in the blueberry soils than adjacent forest soils from all but the KO site, where the blueberry and forest soils accumulated fertilizer derived  $\text{NO}_3^-$ -N at similar rates (Fig. 1). Total  $\text{NO}_3^-$ -N (native plus labeled) accumulation followed similar patterns (data not presented). By the end of the 34 d incubation, all blueberry soils except that from the KO site contained higher fertilizer derived and total  $\text{NO}_3^-$ -N levels than the corresponding forest soils (Table 2).

Fertilizer derived  $\text{NH}_4^+$ -N levels declined at rates inversely proportion to  $\text{NO}_3^-$ -N accumulation (data not presented), and ap-

proached zero in most blueberry soils after 34 d of incubation (Table 2). By day 17, the CH1, CH2, DE, GR1, GR2, KO and MB blueberry soils contained 6.1, 0.5, 1.0, 0.1, 1.3, 4.0, and 4.0 mg fertilizer derived  $\text{NH}_4^+$ -N/kg soil, respectively, while levels in the forest soils from the same sites were 14.1, 9.8, 12.1, 13.0, 7.3, 14.6, and 9.0 mg. Since fertilizer derived  $\text{NH}_4^+$ -N levels in some soils were low enough after 17 d to limit subsequent  $\text{NO}_3^-$  production, net nitrification potentials were compared based on the slopes of linear regressions of fertilizer derived  $\text{NO}_3^-$ -N over time between days 5 and 17 (Fig. 2). All blueberry soils exhibited higher net nitrification potentials than adjacent forest soils, except for those from the KO site, where the forest soil had a higher nitrification potential than the blueberry soil (Fig. 2).

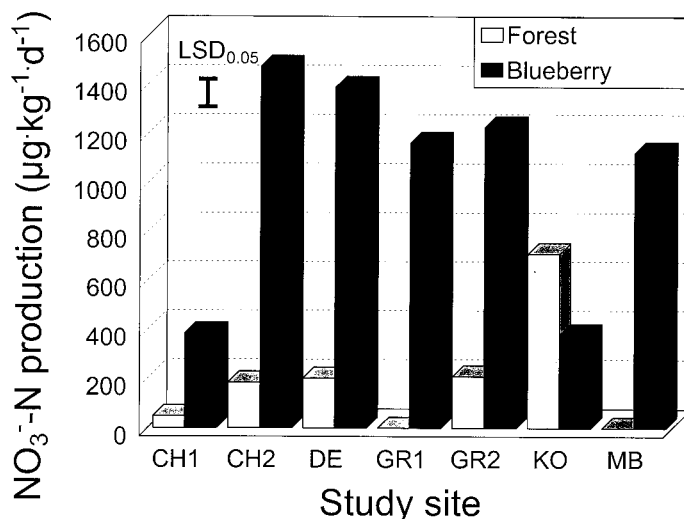


Fig. 2. Daily fertilizer derived  $\text{NO}_3^-$ -N production following addition of  $^{15}\text{N}$ -enriched  $(\text{NH}_4)_2\text{SO}_4$  to blueberry and forest soils sampled in 1996. Rates were derived by regressing fertilizer derived  $\text{NO}_3^-$ -N concentrations over time between day 5 and day 17 following  $(\text{NH}_4)_2\text{SO}_4$  addition.

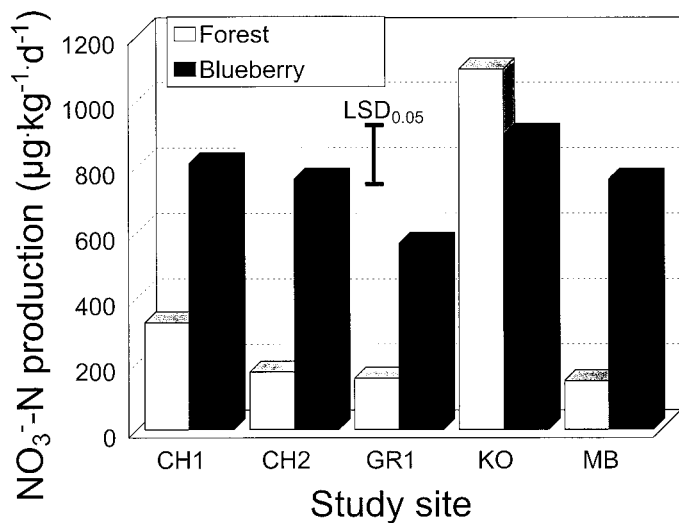


Fig. 3. Daily NO<sub>3</sub>-N production/kg soil during a 24-h slurry incubation of five blueberry and five forest soils sampled from Michigan farms in 1999. Rates were derived by regressing changes in NO<sub>3</sub>-N concentrations over time.

Blueberry and forest soils contained similar amounts of total fertilizer-derived N after 34 d (35.2 and 37.5 µg·g<sup>-1</sup> dry soil, respectively), but significantly more ( $P = 0.033$ ) of the fertilizer derived N was present in organic forms in the forest soils (56%) than the blueberry soils (32%) (Table 2).

**SLURRY NITRIFICATION.** ANOVA of nitrification rates indicated that the effects of the site, cropping (blueberry vs. forest), and the site × cropping interaction were highly significant ( $P < 0.001$ ). Blueberry soils from the CH1, CH2, GR1, and MB sites exhibited higher net nitrification rates than corresponding forest soils, whereas nitrification rates in the blueberry and forest soils from the KO site did not differ (Fig. 3).

**MICROBIOLOGICAL STUDIES.** Total bacteria numbers, average bacterial cell volume, and total bacterial biomass C varied significantly between sites (Table 3), but were not consistently affected by cropping history (blueberry vs. forest). The one exception was the CH1 site, where total bacteria numbers and biomass C were higher in the blueberry than the forest soil (Table 3). The population of

autotrophic nitrifying bacteria did not vary significantly between sites, but was significantly higher in blueberry soils ( $3.4 \times 10^3$ /g soil) than forest soils ( $6.1 \times 10^2$ ).

## Discussion

Two incubation procedures indicated that net nitrification potential of blueberry soils is higher than that of most similar forest soils. Most blueberry soils also contained greater numbers of autotrophic nitrifying bacteria compared to the forest soils. Higher nitrifier numbers were also observed in soils used for conventional crop rotations compared to similar nontilled grassland soils (Bruns et al., 1999). Other measures of bacterial activity or health (total bacteria numbers, average bacterial cell volumes, and bacterial biomass C) were similar in blueberry and forest soils, and were comparable to values reported for other soils (Bruns et al., 1999).

The low nitrification rates in most forest soils did not appear to result from limited availability of NH<sub>4</sub><sup>+</sup> substrate. Each forest soil in the labeled N incubation study, except that from the KO site, contained more total and fertilizer derived NH<sub>4</sub><sup>+</sup>-N than the corresponding blueberry soils after 17 and 34 d of incubation, indicating that NO<sub>3</sub><sup>-</sup>-N production in forest soils was not limited by NH<sub>4</sub><sup>+</sup> supply.

On average, forest soils immobilized fertilizer N more rapidly than blueberry soils. After 34 d of incubation, 56% of the fertilizer N in forest soils was in organic forms, compared to 32% in the blueberry soils (Table 2). Since the forest collection areas had not been fertilized with N, applied N was expected to be immobilized more rapidly in forest than the blueberry soils. Soils with high C:N ratios tend to immobilize added N more rapidly than those with lower ratios. The average C:N ratio of forest soils (17.3) was not statistically different than that of blueberry soils (15.5). However, soil C levels in this study were only estimated by multiplying soil organic matter content by a common conversion factor (1.724).

The incubation procedures used in this study measured net, rather than gross, nitrification rates because they did not account for NO<sub>3</sub><sup>-</sup> removal. Recent work indicates that microorganisms in some forest soils immobilize substantial amounts of NO<sub>3</sub><sup>-</sup> (Hart et al., 1994; Stark and Hart, 1997) so that net nitrification rates may underestimate gross nitrification rates. The low NO<sub>3</sub><sup>-</sup>-N levels in

Table 3. Total bacteria population, average cell volume, bacterial biomass, and autotrophic nitrifying bacteria populations in blueberry and forest soils sampled from Michigan sites in 1999.

Study site <sup>2</sup>	Cropping	Bacteria cells (10 <sup>9</sup> /g soil)	Cell volume (µm <sup>3</sup> )	Bacterial biomass carbon (mg·g <sup>-1</sup> soil)	Nitrifying bacteria (cells/g soil)
CH1	Blueberry	6.1	0.071	88	2,080
	Forest	2.0	0.072	28	960
CH2	Blueberry	5.7	0.074	85	1,230
	Forest	5.9	0.084	98	760
GR1	Blueberry	2.9	0.072	41	5,010
	Forest	2.3	0.077	35	50
KO	Blueberry	6.6	0.094	123	5,350
	Forest	7.6	0.094	143	1,200
MB	Blueberry	2.9	0.071	41	3,540
	Forest	2.8	0.084	46	60
Significance (LSD <sub>0.05</sub> )					
Site (S)		***	***(0.011)	***	NS
Cropping (C)		NS	NS	NS	**(620)
S × C		*(2.0)	NS	*(11)	NS

<sup>2</sup>See materials and methods for details.

NS, \*, \*\*, \*\*\* Nonsignificant or significant at  $P < 0.05$ , 0.01, or 0.001, respectively. Numbers in parentheses are LSD values (0.05).

most forest soils in our study did not appear to result from  $\text{NO}_3^-$  immobilization. If the low  $\text{NO}_3^-$ -N levels in our forest soils resulted from microbial immobilization, fertilizer derived  $\text{NH}_4^+$ -N levels would have been depleted at more rapid rates in forest soils. The fact that forest soils both accumulated  $\text{NO}_3^-$ -N and lost  $\text{NH}_4^+$ -N more slowly than blueberry soils indicates that the  $\text{NO}_3^-$ -N levels in forest soils were not low due to immobilization.

The amount of fertilizer-derived N present at the end of the incubation (35.2 and 37.5  $\mu\text{g N/g}$  dry soil in forest and blueberry soils, respectively) was 5% to 12% greater than the 33.5  $\mu\text{g N/g}$  dry soil added initially. This small difference may reflect sampling and measurement errors. The fact that the final fertilizer-derived N was higher than the application rate suggests that losses of fertilizer-derived N via denitrification were negligible.

Why net nitrification in the KO forest soil was so high compared to other forest soils and the KO blueberry soil is not clear. The KO forest soil had the highest net nitrification rate of any forest soil in both incubation tests, and a higher nitrification rate than the KO blueberry soil in the labeled N incubation test (Fig. 2). The KO forest soil also had high nitrifier numbers relative to other forest soils (Table 3), and both KO soils had relatively high total bacteria counts, average cell size, and bacterial biomass C (Table 3), indicating that these soils supported high overall bacterial populations. The KO soils also differed from most other soils in that the silt and clay contents were unusually high (Table 2). Nitrification rates of some soils are positively correlated with clay quantity (Neill et al., 1997) and type (Sarathchandra, 1978). The KO forest site was similar to other forest sites in terms of maximum estimated tree age (about 60 years) and species composition [mixed maple (*Acer* L.) and oak (*Quercus* L.) sp.].

Although autotrophic nitrifying bacteria are inhibited by acidic conditions in culture (Weber and Gainey, 1962), no relationship between pH and net nitrification rate was apparent in this study. Mean pH of blueberry soils (4.6) was not significantly different from that of forest soils (4.5), when compared with a paired *t* test. Also, soil pH was not significantly correlated with nitrification rates (Fig. 2) of blueberry soils ( $r = 0.738$ ,  $n = 7$ ), forest soils ( $r = 0.44$ ,  $n = 7$ ), or blueberry and forest soils combined ( $r = 0.52$ ,  $n = 14$ ).

Why nitrifier populations and nitrification rates were higher in most blueberry soils is not clear. We speculate that the annual use of  $\text{NH}_4^+$  fertilizers induced a larger nitrifier population in these blueberry soils, since enhanced nitrifier populations and/or nitrification capacity have been associated with  $\text{NH}_4^+$  fertilization in other cropping (Bruns et al., 1999; Eaton and Patriquin, 1988; Tabatabai et al., 1992) and forest systems (Martikainen, 1985). However, the soils in these blueberry sites were altered in other ways that could conceivably influence nitrifier populations and nitrification potentials. Soil P and K were higher in blueberry soils than the corresponding forest soils (Table 2), presumably due to past fertilization, and nitrification rates in some forest soils are positively correlated with soil P content (Martikainen, 1984; Pastor et al., 1984). Blueberry sites are also cultivated prior to planting, and the soil beneath the plants that was sampled in this study may or may not have been cultivated again after the initial disturbance. Cultivation stimulates organic matter oxidation and N mineralization (Reinhorn and Avnimelech, 1974), and nitrification rates in some ecosystems are increased initially, then decline with time after disturbance (Rice and Pancholy, 1972). Less organic debris was observed on the surface of these blueberry soils compared to the forest soils, and N mineralization and nitrification rates in forest soils are influenced by the composition and quantity of litter (Zak and Pregitzer, 1990). Nitrification may also have been influenced by drainage differ-

ences. Since these soils are either poorly drained or somewhat poorly drained, drainage is often improved by surface ditching or tiling when developing sites for blueberry culture.

Data herein indicate that an increase in nitrifying bacteria populations and nitrification capacity usually accompany blueberry culture. The practical significance is that optimum timing of fertilization may depend on the nitrification capacity. On soils that nitrify readily, multiple applications at lower N rates may reduce leaching losses and increase fertilizer use efficiency. A single application may be as effective as multiple applications in soils with low nitrification capacities since these soils may retain N in the less leachable  $\text{NH}_4^+$  form. Blueberry producers have observed that new plants grow more slowly when replanted on old blueberry sites compared to virgin soils. Perhaps one factor contributing to slow growth on replanted sites is higher nitrification rates which increase leaching losses and reduce efficiency of N fertilizer use. Since over 40% of the Michigan blueberry acreage is older than 25 years (Fedewa et al., 1998), and much of the younger acreage is likely to have been replanted to blueberries, increased nitrification rates may be common in the industry.

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