Soil Biol. Biochem. Vol. 23, No. 3, pp. 299–302, 1991
Printed in Great Britain.

0038-0717/91 \$3.00 + 0.00

SHORT COMMUNICATION

RELATIONSHIPS BETWEEN DENITRIFICATION, CO_2 PRODUCTION AND AIR-FILLED POROSITY IN SOILS OF DIFFERENT TEXTURE AND DRAINAGE

PETER M. GROFFMAN* and JAMES M. TIEDJE
Departments of Crop and Soil Science and Microbiology and Public Health, Michigan State University, East Lansing, MI 48824, U.S.A.

(Accepted 30 September 1990)

There is great interest in developing predictive models of denitrification and other microbial processes in soil. These models are needed for further understanding the importance of microbial processes in nutrient cycling in natural and managed ecosystems. Results from such models could have implications for fertilizer-use efficiency, water quality and atmospheric chemistry questions. Models must be based on quantitative relationships between specific processes and environmental variables derived from field experiments. Moreover, these models must be capable of depicting microbial activity over temporal and spatial scales relevant to ecosystem nutrient cycling, water quality and fertilizer-use efficiency.

Microbial process models are frequently based on relationships between microbial activity and soil moisture and temperature. Temperature is an important regulator of all biochemical processes, and moisture directly affects both microbial respiration and oxygen availability. Most models link temperature and moisture relationships to other models and measurements of soil N and C dynamics to account for multifactor controls under field conditions (Rolston *et al.*, 1984; Schlentner and van Cleve, 1985; Schimel *et al.*, 1988; Parton *et al.*, 1988).

Using relationships between moisture and temperature and microbial processes as a basis for modeling is complicated by the high spatial and temporal variability of microbial processes in general, and denitrification in particular (Schimel *et al.*, 1988). The inability to establish strong predictive relationships between denitrification and environmental variables raises two distinct questions: (1) Do we need more sophisticated sampling and statistical techniques to deal with high variability? or (2) Is our understanding of how the process is regulated in nature incomplete?

We have presented data from a landscape scale study of denitrification that found distinct patterns of annual denitrification activity in forest soils of different texture and drainage (Groffman and Tiedje, 1989a, b). These patterns should be useful for several types of denitrification modeling efforts. At the field scale, we should be able to establish relationships between hourly or daily denitrification rate and measurable factors such as soil moisture, temperature, soil nitrate and CO_2 production. These factors can then be used to drive field scale models of denitrification activity. We can also determine if relationships between denitrification and environmental factors vary with texture and drainage. If so, this variability can be incorporated into landscape scale models. Finally, we can analyze weaknesses in the relationships between denitrification and environmental factors to determine how we can improve our models either through more sophisticated sampling and statistical techniques, or through better understanding of denitrification in the environment.

The methods used in this study were detailed in Groffman and Tiedje (1989a). Three catenas of different soil texture (loam, clay loam, sand) consisting of well-, somewhat-poorly and poorly-drained soils were located within 80 km of East Lansing, Mich., U.S.A. (42.5° lat. N, 84.3° long. W). Soils were sampled 12 times between April and November 1985. There were seven sample dates (once a week) from mid-April through May and monthly samplings from June through November. Soils were sampled to 15 cm depth using a 2 cm dia punch auger containing plexiglass inserts. At each sampling date, five cores were taken at four locations (located at the corners of a randomly-selected 5 × 5 m square) within each site (20 cores total per soil series per sampling date). At each of the four locations, bulk samples for soil moisture were taken within the area where soil cores were taken. Soil moisture data was combined with bulk density values from each soil core to compute air-filled porosity. For statistical calculations, data from each sampling location were aggregated so that there were four replicate values for air-filled porosity, denitrification and CO_2 production for each soil for each sampling date.

Denitrification and CO_2 production were measured using the soil core technique described by Groffman and Tiedje (1989a). In this method, soil cores were exposed for 6 h, to the presence of acetylene (C_2H_2), at room temperature, within 24 h of sampling. The headspace of the cores was sampled at 2 and 6 h. Gas samples were stored in evacuated glass vials and were later analyzed for N_2O and CO_2 by gas chromatography. Acetylene was added to all cores to inhibit N_2O reduction to N_2. Our measurements of N_2O and CO_2 production thus represent the rate of total N gas production by denitrification and CO_2 production from all forms of soil respiration respectively. Acetylene does not affect respiration during short incubations of soil that has not been treated with C_2H_2 (Terry and Duxbury, 1985; Topp and Germon, 1986). Rates of denitrification and CO_2 production were corrected for field temperatures using a Q_{10} factor of 2 (Rolston *et al.*, 1984).

Relationships between denitrification, CO_2 production and air-filled porosity were computed by linear regression. Logarithmic (base 10) transformations were done to normalize data where appropriate. Except where noted, only regressions with a slope that was significant at $P < 0.10$ or less are reported.

*Current address: Department of Natural Resources Science, University of Rhode Island, Kingston, RI 02881, U.S.A.

Table 1. Regression parameters of $\log_{10}$ denitrification rate vs air-filled porosity analysis

Soil	Slope	Vertical intercept	r^2
Somewhat poorly-drained loam	−0.03	1.08	0.47
Poorly-drained loam	−0.04	2.03	0.44
Well-drained clay loam	−0.02	1.21	0.14
Somewhat poorly-drained clay loam	−0.02	0.61	0.16
Poorly-drained clay loam	−0.01	1.27	0.11

Table 2. Horizontal axis intercepts of plots of denitrification rate vs air-filled porosity

Soil	Horizontal intercept	Transformed[1] intercept
Somewhat poorly-drained loam	63	70
Poorly-drained loam	54	63
Well-drained clay loam	72	89
Poorly-drained clay loam	71	113

[1]The value on the horizontal axis when the vertical axis equals −1 in a plot of $\log_{10}$ denitrification rate vs air-filled porosity.

Although there was considerable scatter in the relationships studied, several interesting patterns were evident. Relationships between denitrification and air-filled porosity differed by texture but not by drainage (Table 1, Fig. 1). The loam soil regressions had steeper slopes and higher coefficients of determination (r^2) than the more fine-textured clay loam soils. Horizontal axis intercepts also differed by texture (Table 2). In the clay loam soils, denitrification activity was zero at higher air-filled porosity values than in loam soils. Intercepts in plots of log-transformed data were calculated for a value of −1 on the vertical axis, due to the greater detail at low values produced by the log transformation. A regression line calculated using all of the loam soils was significantly different ($P < 0.01$) from a regression line calculated using all of the clay loam soils. Sand soils showed no significant relationships between denitrification and either air-filled porosity or soil water.

Differences in relationships between denitrification and air-filled porosity among the soils of different texture were likely caused by the effects of soil texture on oxygen availability. Fine-textured soils have smaller pores that more easily become anaerobic than the larger pores present in loam and sand soils (Papendick and Campbell, 1980). Even when clay loam soils were well-aerated, anaerobic microsites were likely to be present and denitrification occurred at relatively high air-filled porosity values. In coarse-textured soils, anaerobiosis is more directly controlled by the absolute amount of water present than in fine-textured soils. This control is illustrated by the fact that the loam soils had more strongly negative slopes and higher r^2-values than the clay loam soils in regressions of denitrification rate vs air-filled porosity.

The lack of any significant relationships between denitrification and air-filled porosity in the sand soils was due to very low denitrification activity. The well- and somewhat poorly-drained sand soils always had high air-filled porosity, and levels of soil nitrate were low (Groffman and Tiedje, 19891a). Denitrifier populations in these soils were small (Groffman and Tiedje, 1989b) and denitrification events were insignificant and infrequent.

Relationships between CO_2 production and air-filled porosity differed by drainage but not by texture (Fig. 2, Table 3). In both the clay loam and loam catenas, the regression for the well-drained soil had a positive slope, while the poorly-drained soil regression had a negative slope. The somewhat poorly-drained soils and the sand soils had no significant relationships between CO_2 production and air-filled porosity.

Previous investigations of CO_2 production–moisture relationships have produced a range of contradictory results. While some studies have found a parabolic relationship between moisture and CO_2 production (Douglas and Tedrow, 1959; Linn and Doran, 1984), other investigators have found either positive (Froment, 1972; Pal and Broadbent, 1975; Orchard and Cook, 1983; Schlentner and van Cleve, 1985; Coxson and Parkinson, 1987) or negative (Kucera and Kirkham, 1971; DeSanto *et al.*, 1976; Kowalenko *et al.*, 1978) relationships between these factors. Other investigators have found variable or non-significant relationships between soil moisture and CO_2 production (Miller and Johnson, 1964; Reiners, 1968; de Jong *et al.*, 1974; Buyanovsky *et al.*, 1986). In general, wet forest soils tend to exhibit high CO_2 production at moisture contents near or above saturation (Schlentner and van Cleve, 1985; Coxson and Parkinson, 1987), possibly due to the development of microbial populations tolerant of low aeration status (Griffin, 1980). Miller and Johnson (1964) showed that the development of these populations can be fairly rapid (weeks).

Microbial adaptation to *in situ* moisture conditions was probably a factor in this study. It is logical that populations in wet soils should be more active under wet conditions and vice-versa. The fact that the soils of intermediate moisture (somewhat poorly drained) showed no significant moisture–CO_2 production relationships strengthens this argument since these soils should have a mixed population that shows no strong overall adaptation to either wet or dry conditions.

CO_2 production and denitrification in our soils were probably affected by the pattern of available carbon production. Freezing and thawing events, drying and rewetting cycles, and litterfall are processes that increase carbon availability and are known to stimulate both CO_2 production and denitrification (Birch, 1958; de Jong *et al.*, 1974; Orchard and Cook, 1983; Edwards and Kilham, 1986;

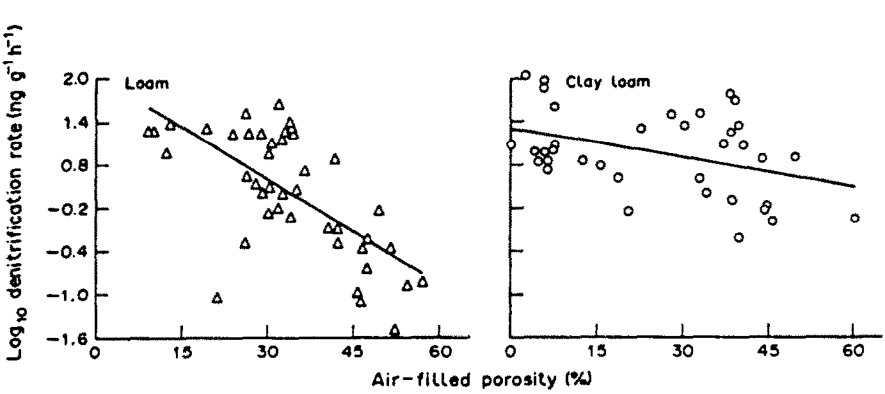

Fig. 1. $\log_{10}$ denitrification rate vs air-filled porosity for poorly-drained loam and clay loam soils with regression lines drawn through the points.

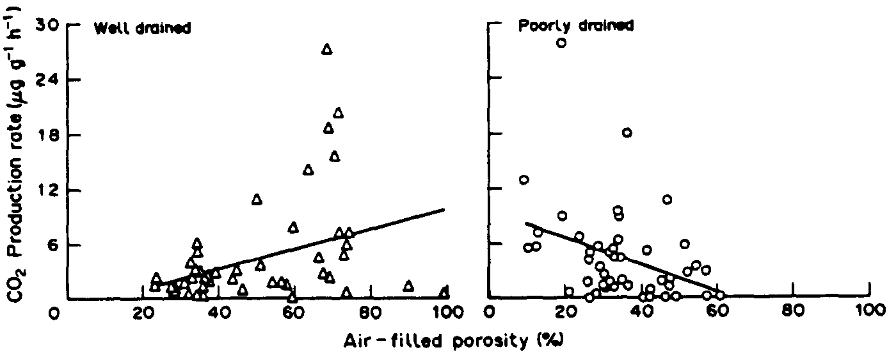

Fig. 2. Respiration rate in poorly- and well-drained loam soils with regression lines drawn through the points.

Tiwari *et al.*, 1987; Skoglund *et al.*, 1988; Groffman and Tiedje, 1988; Christensen and Tiedje, 1990). Freeze–thaw cycles were especially common in the poorly-drained soils in early spring and fall when the soils were very wet and both denitrification and CO_2 production were high (Groffman and Tiedje, 1989a). Increased C availability from freeze–thaw cycles and litterfall during spring and fall may exert selective pressure on microorganisms to be active in very wet soils.

Drying and rewetting cycles likely affected carbon availability, denitrification and CO_2 production during late spring and summer. Approximately 2 cm of rain fell before the June sampling date, causing a pulse of CO_2 production at several sites. Eliminating this date greatly improved the significance and r^2-value of the regression for CO_2 production versus air-filled porosity for the poorly-drained clay loam soil. In general, many of the outlying values in plots of both CO_2 production and denitrification with air-filled porosity could be attributed to wetting and drying events. de Jong *et al.* (1974) concluded that annual variation in soil respiration was mainly caused by the number of wetting and drying cycles.

Denitrification–CO_2 production relationships were closely tied to CO_2 production–air-filled porosity relationships (Table 4). In the poorly-drained soils that respired vigorously under wet conditions, there was a significant relationship between denitrification and CO_2 production. Aerobic respiration may have played an important role in stimulating denitrification through consumption of oxygen. Conversely, denitrification may have produced a significant percentage of the CO_2 in these soils. In the drier soils, there were weak negative relationships between denitrification and CO_2 production, suggesting that aerobic respiration and denitrification were not strongly linked. In general, CO_2 production and denitrification should be significantly correlated when respiration significantly reduces oxygen supply in soil. This often occurs when respiration rates are very high (Rice *et al.*, 1988; Parkin and Robinson, 1989) or when soils are very wet and oxygen diffusion is slow (given adequate NO_3^- supply).

Table 3. Regression parameters of CO_2 production rate vs air-filled porosity analysis

Soil	Slope	Vertical intercept	r^2
Well-drained loam	0.11	−1.09	0.14
Poorly-drained loam	−0.15	9.60	0.14
Well-drained clay loam	0.16	−3.18	0.32
Poorly-drained clay loam[1]	−0.06	5.26	0.11

[1]Data from one sampling date that was affected by drying and rewetting effects are not included for this soil.

Table 4. Regression parameters of $\log_{10}$ denitrification rate vs respiration rate analysis

Soil	Slope	Vertical intercept	r^2
Well-drained loam	−0.27	−0.45	0.02[1]
Poorly-drained loam	1.04	−0.05	0.19
Well-drained clay loam	−0.31	0.18	0.03[2]
Poorly-drained clay loam	0.35	0.82	0.10

[1]Regression not significant at $P < 0.10$.
[2]Regression not significant at $P < 0.10$. Regression with unlogged data was significant at $P < 0.10$.

Despite the considerable scatter in the relationships observed, there are several useful conclusions that can be drawn from our results. First, the data show that relationships between denitrification and air-filled porosity vary with soil texture, and that relationships between respiration and air-filled porosity vary with soil drainage class. Denitrification and CO_2 production models centered around moisture–activity relationships should incorporate texture and drainage information and should account for the variable response with these factors.

More importantly, the data suggest that our understanding of regulation of denitrification and CO_2 production under field conditions is incomplete. Moisture did not account for the majority of variability in activity in any of the soils studied, and although our results do not permit quantitative analysis of temperature–activity relationships, temperature was not a strong controller of activity. Rates of both denitrification and CO_2 production were highest in the early spring and declined into the summer, suggesting negative relationships between temperature and activity. Soil nitrate was also not a strong predictor of activity (Groffman and Tiedje, 1989a). These results suggest that factors other than temperature and moisture, likely related to C and N dynamics in soil, need to be considered when modeling denitrification. Wheatley and Williams (1989) and Thompson (1989) found similar seasonal patterns and a similar lack of relationships between temperature, moisture and denitrification activity. They also concluded that C availability may be a more important regulator of dentrification activity under field conditions than has previously been thought.

Previous investigators (Parton *et al.*, 1988) have effectively accounted for available N dynamics in denitrification models, and our data suggest some approaches for addressing available C dynamics. Adaptation of microbial populations to *in situ* conditions (e.g. cold and wet soils), and factors controlling carbon supply to those populations, appear to strongly influence microbial activity in the field. Better understanding of microbial community dynamics, and explicitly addressing events such as freeze–thaw and wet–dry cycles, should improve our ability to effectively model denitrification in soil.

Acknowledgements—We thank Delbert Mokma for help with site selection, John Golphin for help with laboratory analysis, and Don Zak, Søren Christensen and Stephen Simkins for valuable reviews of this manuscript. This research was supported by a U.S. National Science Foundation research grant.

REFERENCES

Birch H. F. (1958) The effect of soil drying on humus decomposition and nitrogen availability. *Plant and Soil* **10**, 9–31.

Buyanovsky G. A., Wagner G. H. and Gantzer C. J. (1986) Soil respiration in a winter wheat ecosystem. *Soil Science Society of America Journal* **50**, 338–344.

Christensen S. and Tiedje J. M. (1990) Brief and vigorous N_2O production by soil at spring thaw. *Journal of Soil Science* **41**, 1–4.

Coxson D. S. and Parkinson D. (1987) Winter respiratory activity in aspen woodland forest floor litter and soils. *Soil Biology & Biochemistry* **19**, 49–59.

DeSanto A. V., Alfani A. and Sapio S. (1976) Soil metabolism in beech forests of Monte Taburno (Campania Appenines). *Oikos* **27**, 144–152.

Douglas L. A. and Tedrow J. F. C. (1959) Organic matter decomposition rates in arctic soils. *Soil Science* **88**, 305–312.

Edwards A. C. and Killham K. (1986) The effect of freeze/thaw on gaseous nitrogen loss from upland soils. *Soil Use and Management* **2**, 86–91.

Froment A. (1972) Soil respiration in mixed oak forest. *Oikos* **23**, 273–277.

Griffin D. M. (1980) Water potential as a selective factor in the microbial ecology of soils. In *Water Potential Relations in Soil Microbiology*, pp. 141–151. *Special Publication Number 9*, Soil Science Society of America, Madison.

Groffman P. M. and Tiedje J. M. (1988) Denitrification hysteresis during wetting and drying cycles in soil. *Soil Science Society of America Journal* **52**, 1626–1629.

Groffman P. M. and Tiedje J. M. (1989a) Denitrification in north temperate forest soils: spatial and temporal patterns at the landscape and seasonal scales. *Soil Biology & Biochemistry* **21**, 613–620.

Groffman P. M. and Tiedje J. M. (1989b) Denitrification in north temperate forest soils: relationships between denitrification and environmental parameters at the landscape scale. *Soil Biology & Biochemistry* **21**, 621–626.

de Jong E., Schappert H. J. V. and MacDonald K. B. (1974) Carbon dioxide evolution from virgin and cultivated soil as affected by management practices and climate. *Canadian Journal of Soil Science* **54**, 299–307.

Kowalenko C. G., Ivarson K. C. and Cameron D. R. (1978) Effect of moisture content, temperature, and nitrogen fertilization on carbon dioxide evolution from field soils. *Soil Biology & Biochemistry* **10**, 417–423.

Kucera C. L. and Kirkham D. R. (1971) Soil respiration studies in tallgrass prairie in Missouri. *Ecology* **52**, 912–915.

Linn D. M. and Doran J. W. (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. *Soil Science Society of America Journal* **48**, 1267–1272.

Miller R. D. and Johnson D. D. (1964) The effect of soil moisture tension on carbon dioxide evolution, nitrification, and nitrogen mineralization. *Soil Science Society of America Proceedings* **28**, 644–647.

Orchard V. A. and Cook F. J. (1983) Relationship between soil respiration and soil moisture. *Soil Biology & Biochemistry* **15**, 447–453.

Pal D. and Broadbent F. E. (1975) Influence of moisture on rice straw decomposition in soils. *Soil Science Society of America Proceedings* **39**, 59–62.

Papendick R. I. and Campbell G. S. (1980) Theory and measurement of water potential. In *Water Potential Relations in Soil Microbiology*, pp. 1–22. *Special Publication Number 9*, Soil Science Society of America, Madison.

Parkin T. B. and Robinson J. A. (1989) Stochastic models of soil denitrification. *Applied and Environmental Microbiology* **55**, 72–77.

Parton W. J., Mosier A. R. and Schimel D. S. (1988) Rates and pathways of nitrous oxide production in a shortgrass steppe. *Biogeochemistry* **6**, 45–58.

Reiners W. A. (1968) Carbon dioxide evolution from the floor of three Minnesota forests. *Journal of Ecology* **58**, 497–519.

Rice C. W., Sierzega P. E., Tiedje J. M. and Jacobs L. W. (1988) Stimulated denitrification in the microenvironment of a biodegradable organic waste injected into soil. *Soil Science Society of America Journal* **52**, 102–108.

Rolston D. E., Rao P. S. C., Davidson J. M. and Jessup R. E. (1984) Simulation of denitrification losses of nitrate fertilizer applied to uncropped, cropped, and manure-amended field plots. *Soil Science* **137**, 270–279.

Schimel D. S., Simkins S., Rosswall T., Mosier A. R. and Parton W. J. (1988) Scale and the measurement of nitrogen-gas fluxes from terrestrial ecosystems. In *Scales and Global Change* (T. Rosswall, R. G. Woodmansee and P. G. Risser, Eds), pp. 179–194. Wiley, Chichester.

Schlentner R. E. and van Cleve K. (1985) Relationships between CO_2 evolution from soil, substrate temperature, and substrate moisture in four mature forest types in interior Alaska. *Canadian Journal of Forest Research* **15**, 97–106.

Skoglund T., Lomeland S. and Goksøyr J. (1988) Respiratory burst after freezing and thawing of soil: experiments with soil bacteria. *Soil Biology & Biochemistry* **20**, 851–856.

Thompson R. B. (1989) Denitrification in slurry treated soil: occurrence at low temperatures, relationships with soil nitrate and reduction by nitrification inhibitors. *Soil Biology & Biochemistry* **21**, 875–882.

Terry R. E. and Duxbury J. M. (1985) Acetylene decomposition in soils. *Soil Science Society of America Journal* **49**, 90–93.

Tiwari S. C., Tiwari B. K. and Mishra R. R. (1987) The influence of moisture regimes on the population and activity of soil microorganisms. *Plant and Soil* **101**, 133–136.

Topp E. and Germon J. C. (1986) Acetylene metabolism and stimulation of denitrification in an argicultural soil. *Applied and Environmental Microbiology* **52**, 802–806.

Wheatley R. E. and Williams B. L. (1989) Seasonal changes in rates of potential denitrification in poorly-drained reseeded blanket peat. *Soil Biology & Biochemistry* **21**, 355–360.