

## DENITRIFICATION AT DIFFERENT TEMPORAL AND GEOGRAPHICAL SCALES : PROXIMAL AND DISTAL CONTROLS

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### ABSTRACT

*Study of denitrification in soil is hindered by the fact that each of the primary, or proximal, factors controlling denitrification is affected by a wide range of physical and biological factors (distal factors). As the spatial and temporal scale of investigation increases, one must focus on distal rather than proximal factors to gain an understanding of denitrification at the particular scale of study. Further, understanding proximal and distal controls of denitrification at small scales of investigation is essential for understanding denitrification at higher scales. Mechanistic relationships between the proximal factors controlling denitrification and a wide range of distal factors (including soil water, organic matter distribution and decomposition, soil texture, plant community composition and organismal competition) are important as foci of study at organismal, microsite, field, landscape, regional and global scales of investigation.*

### INTRODUCTION

Through denitrification, nitrogenous oxides, principally nitrate and nitrite, are reduced to dinitrogen gases (Tiedje 1987) and this activity constitutes a potential source of loss of N from the ecosystem. Most denitrification in soil is carried out by bacterial respiratory processes where nitrogen oxide reduction is coupled to electron transport phosphorylation (Payne 1981). Research over the last 100 years has addressed the dynamics of denitrification at scales of resolution ranging from the gene to the globe. This paper aims to discern linkages between the factors controlling denitrification at different scales of investigation and to show how this understanding can aid the design and interpretation of denitrification research.

Denitrification in soil is primarily controlled by three main factors: oxygen, nitrate and carbon. Any of these primary factors can exert fundamental, limiting control of denitrification at the cellular level. This study of denitrification in soil is hindered because the primary, or proximal, factors controlling it are affected by many physical and biological factors (distal factors). As the scale of investigation increases above the cellular level, it usually becomes necessary to focus on distal rather than proximal factors as controllers of denitrification. Understanding proximal and distal controls at different scales of investigation is important in the design, execution and interpretation of denitrification experiments, and can be crucial to evaluating the significance of denitrification at the scale of study.

In this paper, we examine the relationships between distal and proximal factors controlling denitrification. Our thesis is that as the temporal and spatial scale of investigation increases, distal factors become increasingly more significant as foci of study. Even though the activity of denitrifying organisms is always directly controlled by proximal factors, at different scales of investigation, different factors control the dynamics of the proximal factors. The investigator must focus on the proper factors if the study is to be successful in characterizing patterns of activity in time and space, and in quantifying the significance of denitrification at the scale required.

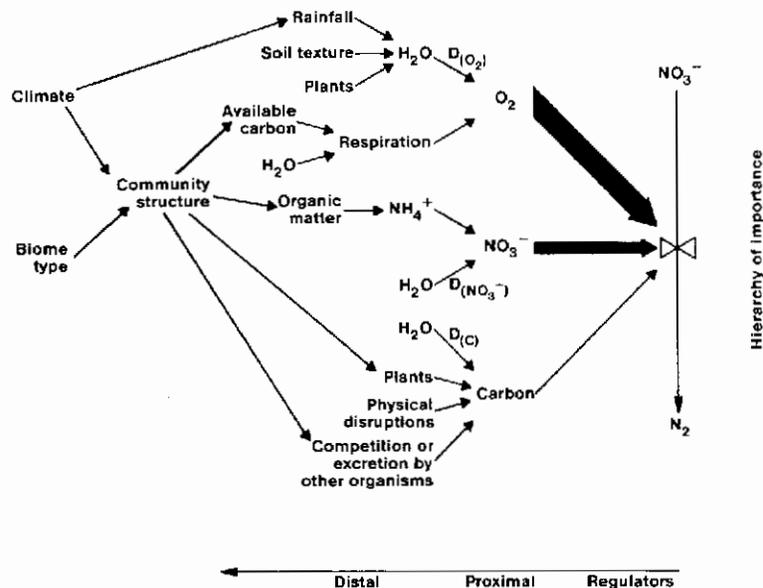
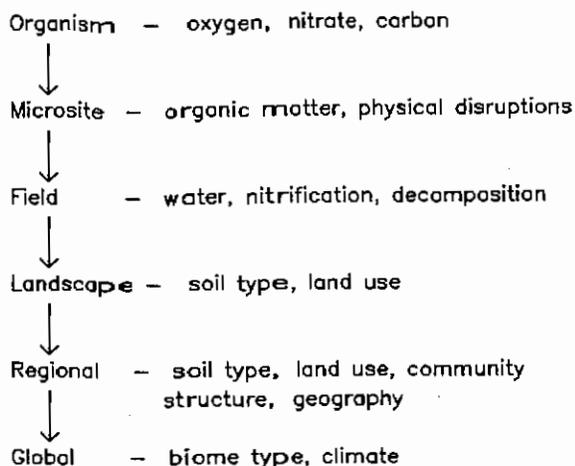


Fig. 1: Relationships between proximal and distal controlling factors of denitrification (Adapted from Tiedje 1987).

Figure 1 presents a simplified illustration of the mechanistic relationships between proximal and distal factors controlling denitrification. A more exhaustive set of linkages, including indirect and interactive effects, would be too complex to diagram. Figure 2 shows how the controlling factors change as the scale of investigation increases. Below, we discuss the mechanistic relationships presented in figure 1 and give examples of how the scale of investigation affects the significance of the different controlling factors.



**Fig. 2:** Factors controlling denitrification at different scales of investigation.

## RELATIONSHIPS BETWEEN PROXIMAL AND DISTAL FACTORS

### Oxygen

The level of  $O_2$  available to microorganisms in any habitat is a product of supply and demand. Oxygen flow in soil is hindered primarily by slow diffusion through water in porous media. Oxygen flow through air is c.  $10^{-3}/cm^2/s$  compared to flow through particle free water of c.  $10^{-5}/cm^2/s$  (Revsbech *et al.* 1980). The next distal factors controlling  $O_2$  in soil are the factors that control soil water. Rainfall is an obvious controller of soil moisture, as are plants, which are a major sink for water. Soil texture is also important because soil particle size affects the adhesion of water molecules to soil particles and the flow of water through the soil matrix (Papendick and Campbell 1980).

The main sink for  $O_2$  in soil is respiration by plant roots and by aerobic microorganisms. Consumption rates as high as  $70 \text{ mM } O_2/cm^2/h$  occur in soils (Singh and Gupta 1977) and can often exceed  $O_2$  supply rates. The distal factors affecting soil respiration are complex. Available C is the substrate

for microbial respiration and is controlled by the decomposition of plant materials, exudation and/or sloughing of material from plant roots, and decomposition of microbial cells (Helal and Sauerbeck 1986). Soil water affects respiration by controlling the activities of decomposer organisms (Griffin 1980) and plant roots (Smucker 1984). Sharp changes in soil water (drying and rewetting events) and other physical disturbances increase C availability by physical rearrangement of soil particles (Adu and Oades 1978) and by killing microbial cells (van Veen et al. 1985; Kieft et al. 1987).

### Nitrate

Nitrate is similar to  $O_2$  in that the level available to denitrifiers in soil is a function of supply, demand and diffusion. Nitrate is produced by nitrification or added in fertilizer; nitrate sinks include plant uptake, microbial uptake, dissimilatory reduction to ammonium, groundwater, and denitrification. Due to its diverse fates, distal control of  $NO_3$  can be quite complex. In N-fertilized soils,  $NO_3$  is not likely to be limiting to denitrification since denitrifiers generally have a high affinity for  $NO_3$  (Myrold and Tiedje 1985). In unfertilized soils, the supply of ammonium from mineralization is often a dominant control of nitrification and therefore of  $NO_3$  availability (Robertson 1982). Nitrogen mineralization in unfertilized soils is controlled by interactive effects of soil type, climate and plant community composition (Pastor et al. 1984; Pastor and Post 1986).

Soil water affects  $NO_3$  supply by controlling the activities of mineralizing and nitrifying organisms (Fisher and Gosz 1986). Water also facilitates diffusion and leaching of  $NO_3$  through the soil matrix. In many water saturated environments,  $NO_3$  levels are extremely low due to inhibition of nitrification by anaerobiosis.

### Carbon

As heterotrophs, the electron accepting denitrifying process is controlled by the supply of electron donor, usually organic C. The factors controlling C availability to microorganisms are discussed above in relation to soil respiration and  $O_2$  status. The effect of C on denitrifiers is more commonly expressed through indirect effects on  $O_2$  status than on direct effects of substrate availability. By stimulating heterotrophic activity carbon availability reduces  $O_2$  availability and thus favors denitrifiers over strictly aerobic organisms that cannot assimilate C under anaerobic conditions. Because competition for C is low when  $O_2$  is limiting, C is probably the least common factor directly regulating denitrification in soils.

## PROXIMAL AND DISTAL CONTROL OF DENITRIFICATION AT DIFFERENT SCALES OF INVESTIGATION

### Organism

When studying denitrification at the organismal level we need to focus only on the three proximal controllers. Regulation at this level is best studied using pure culture techniques under highly controlled laboratory conditions. It is now possible to extend these mechanistic studies to the molecular level, a likely area of progress in the near future. It is necessary to quantitatively understand the effects of proximal factors on denitrification at the organismal level to be able to confidently use distal factors as predictors of activity at higher spatial or temporal scales; recent data from our laboratory illustrate this point.

Table 1 shows rates of  $\text{NO}_3^-$  consumption by a denitrifying organism (*Pseudomonas fluorescens*) and a barley plant (*Hordeum vulgare*) at different  $\text{O}_2$  levels. Activity of both organisms was measured in a temperature controlled continuous culture vessel equipped with a polarographic  $\text{O}_2$  sensing electrode. These cultivation conditions allowed for vigorous mixing and rigid control of solution  $\text{O}_2$  concentrations. Only very small amounts of  $\text{O}_2$  (0.05%) completely inhibited denitrification by *P. fluorescens*.

**Table 1:** Nitrate reduction by a plant (*Hordeum vulgare*) and a denitrifier (*Pseudomonas fluorescens*) at different oxygen concentrations (data from Christensen and Tiedje 1987).

Oxygen level	Denitrification	Plant uptake
$I, v/v$	$\mu\text{g NO}_3^- \text{-N/mg biomass/h}$	
20	0	1.85
10	0	1.15
5	0	0.52
1	0	0
0.05	0	0
0.01	0.15	0
0	3.5	0

In some previous studies, denitrification activity could be measured at considerably higher (0.35 to 2.0%)  $\text{O}_2$  concentrations (Nelson and Knowles 1978; Dunn et al. 1979). Preliminary work in our study showed that unless the culture was vigorously mixed (greater than 1000 rpm) anaerobic microsites developed in the culture vessel even though the  $\text{O}_2$  electrode indicated significant concentrations of  $\text{O}_2$  in the medium. Development of anaerobic sites in culture vessels may account for the high  $\text{O}_2$  tolerance of denitrifiers

reported in other studies. Our data also show that plants and denitrifiers do not directly compete for  $\text{NO}_3$  at the organismal level. There was a wide range of intermediate  $\text{O}_2$  concentrations at which neither the plant nor the denitrifier could take up  $\text{NO}_3$ .

These results at this level have important implications for study of denitrification at higher scales of investigation. The low  $\text{O}_2$  tolerance of the denitrifier shows that for denitrification to occur in soil there must be virtually a complete absence of  $\text{O}_2$ . Also, at the organismal level plants and denitrifiers did not directly compete for  $\text{NO}_3$ , whereas at higher spatial and temporal scales of investigation, their competition for  $\text{NO}_3$  can be a major factor controlling denitrification. Organismal level studies show that a range of habitats must be present in soils where plants and denitrifiers are both active. These studies thus serve as guides for investigation of the spatial heterogeneity of the soil environment as it affects denitrification at the microsite and field scale.

### Microsites

The concept of microsites within a spatially heterogeneous environment has important application to study of denitrification in soil. The persistence and activity of denitrifying enzymes in apparently well aerated soil has been attributed to microsites of anaerobiosis within the soil matrix. Research and modelling efforts have addressed the presence of microsites of denitrification activity by focusing on the occurrence of anaerobic zones in soil aggregates (Smith 1980; Sexstone *et al.* 1985b; Lefelaar 1986). These studies have been only moderately successful at explaining the occurrence of microsite 'hotspots' of denitrification. This lack of success is due partly to the difficulty of studying soil aggregates *in situ*, but perhaps more fundamentally, it is because analysis of  $\text{O}_2$  dynamics within the soil matrix does not account for the C and  $\text{NO}_3$  factors that also control denitrification.

In addition to studying aggregates, another approach to understanding the occurrence of hotspots of denitrification in soil is to focus on the distribution and decomposition of organic C. Parkin (1987) showed that nearly 100% of the denitrification in a soil core could be attributed to a decomposing plant leaf that represented 0.08% of the mass of the core. Research in our laboratory (S. Christensen and T.M. Tiedje, unpublished data) confirmed that denitrification activity could be traced to microsites of decaying plant material and also showed that hotspots could be created by injecting concentrated portions of a non-diffusible C source (such as dead microbial cells) into soil. These results suggest that, at the microsite scale, it is useful to focus on the distribution and decomposition of organic C to understand the distribution of activity centers of denitrification in soil.

It is not surprising that organic C is a strong distal controller of denitrification at the microsite scale. Fresh plant residues or other readily

decomposable compounds can provide the C necessary for a strong respiratory sink for  $O_2$ , can be a source of  $NO_3$  through mineralization of organic N compounds present in the residue and, finally, provide reductant for the denitrifier.

The fact that organic C can provide all the necessary proximal factors for denitrification makes it a more important distal controller of denitrification activity at the microsite level than soil aggregate structure. While aggregate structure can produce anaerobic volumes of soil, C and  $NO_3$  may not be available to denitrifiers in the aggregate centers. A promising area of research is in understanding the relationships between aggregate formation and organic C dynamics. Macro-aggregate formation and stability are tied to the dynamics of readily decomposable C compounds (Chaney and Swift 1986; Elliott 1986). Biological activity (including denitrification) associated with aggregates should thus be studied in the context of C availability and aggregate formation and turnover.

### Field scale

Most investigations of denitrification have been at the field or plot scale, comparing different agricultural treatments, soil types or forest communities. Several reviews or summaries of large data sets have been published (Colbourn and Dowdell 1984; Rolston et al. 1984; Aulakh and Rennie 1986) and we can now draw some conclusions about the key factors controlling denitrification at the field scale. In agricultural soils, soil water appears to be the dominant factor controlling denitrification, since soil  $NO_3$  levels are usually high in these soils due to fertilization. In non-agricultural soils where competition for available N is high, soil  $NO_3$  production is frequently the key limiting factor. The role of soil C in controlling denitrification at the field scale appears to be important in some cases, but this factor has been less well studied than either  $NO_3$  or water.

Rolston et al. (1984) modelled an extensive denitrification data set using three main factors: soil water,  $NO_3$  concentration and available C. Soil water had the strongest effects on denitrification and  $NO_3$  concentration the least. The dynamics of available C was the most difficult factor to model, since there is no simple direct measurement of this parameter. Other studies have also found soil water to be the dominant factor controlling denitrification in field soils (Mosier et al. 1983, 1986; Burton and Beauchamp 1985; Aulakh and Rennie 1986). Rainfall, as the next distal controller of soil moisture has also been shown to be a strong controller of denitrification at the field scale (Sexstone et al. 1985a; Duxbury and McConnaughey 1986).

The role of available C in controlling denitrification at the field scale is poorly characterized. Several models have been developed to predict the flux of available C in soil (Juma and Paul 1981; van Veen and Frissel 1981; Molina et al. 1983) but these have generally been considered to be too complicated to apply to denitrification studies (Rolston et al. 1984). The

relatively dramatic effects of soil water and NO<sub>3</sub> on denitrification activity have also inhibited study of available C control of denitrification. However, Sexstone *et al.* (1985a) suggested that lack of available C can limit denitrification in field soils at certain times. They observed a lack of denitrification response to rainfall during autumn in soils that had previously shown sharp responses to rainfall. Since soil NO<sub>3</sub> levels were always high, they concluded that the non-responsive soils were depleted of available C.

Recent research in our laboratories shows how seasonal patterns of C availability can affect denitrification at the field scale. In a temperate forest soil in Michigan (Table 2), denitrification rates showed a decreasing response to additions of water and NO<sub>3</sub> through the summer (from July to August), indicating that available C was becoming depleted. In November, following C input to soil by litterfall, water plus NO<sub>3</sub>-amended rates were about three orders of magnitude higher than they were in August. At least some tropical forest soils also show evidence of C limitation of denitrification (Table 2). High temperature, moisture, and NO<sub>3</sub> availability in tropical soils make conditions favorable for denitrification, which can be stimulated by C inputs as discussed previously. Patterns of C availability thus may be an important controller of denitrification at the field scale in both temperate and tropical forests. Studying available C control of denitrification by measuring the response of denitrification to different amendments appears to be useful for characterizing these patterns. Further understanding of distal control of available C levels in soil is dependent on increasing our understanding of decomposition and microbial turnover processes.

**Table 2:** Denitrification response to amendments in temperate and tropical forest soils.

Ecosystem	Denitrification			
	June	July	August	November
	<i>ng/cm<sup>2</sup>/h</i>			
Temperate upland forest <sup>1</sup>				
Unamended	5.2	1.5	0.4	5.9
Plus water	2.9	2.7	1.0	1.6
Plus water and nitrate	113.7	23.6	4.2	1068
Tropical lowland rainforest <sup>2</sup>				
Plus water	110.7			
Plus water and nitrate	91.6			
Plus water and glucose	1064			

1. Groffman and Tiedje (1987a); 2. Robertson and Tiedje (1987)

Nitrate can also be a key factor controlling denitrification in soils, especially in unfertilized, non-agricultural soils with actively growing vegetation, where vigorous competition for mineral N between plants and microorganisms can severely restrict NO<sub>3</sub> availability. Distal control of denitrification in these soils is often centered on nitrification. In the temperate forest soil in Table 2, moisture additions did not increase denitrification rates at any time, suggesting that NO<sub>3</sub> was limiting activity. Nitrification potential was the strongest predictor of denitrification in a range of forest soils in Michigan and North Carolina (Table 3). Because nitrification has been shown to be linked to patterns of forest community composition and landscape position (Robertson and Vitousek 1981; Pastor *et al.* 1984; Zak *et al.* 1986), understanding how nitrification exerts distal control over denitrification at the field scale allows us to understand increasingly distal controls of denitrification at landscape and regional levels.

**Table 3:** Predictors of denitrification in forest soils.

Site	Coefficient of determination <sup>1</sup>	Probability level
12 Michigan sites <sup>2</sup>		
Nitrification potential	0.356	0.041
CO <sub>2</sub> production	0.488	0.049
Percent moisture	0.531	0.094
8 clearcut North Carolina treatment plots <sup>3</sup>		
Nitrification potential	0.88	0.001
Nitrate pool size	0.55	0.05
Old-field (successional) site (238 samples) <sup>4</sup>		
Nitrate pool size	0.286	0.001
CO <sub>2</sub> production	0.433	0.001

1. Coefficient of determination ( $r^2$ ) values are additive going down the column for the Michigan and the old-field sites; 2. Robertson and Tiedje (1984); 3. Robertson *et al.* (1987); 4. Robertson *et al.* (1988)

One poorly characterized area of denitrification investigation in agricultural soils is activity during the non-crop season. Estimates of annual N loss to denitrification are generally considered to be non-significant in an agronomic context (Colbourn and Dowdell 1984; Mosier *et al.* 1986), but the vast majority of agricultural field studies have only measured activity between planting and harvest of a summer crop. This crop season-based sampling often eliminates the periods of highest soil water from the investigation. Available C is also likely to be high in the 'off' season, due to residue input from harvest, plowing, or disruption by freeze/thaw cycles.

Soil  $\text{NO}_3$  levels are likely to be high during this time due to a lack of plant uptake. Several studies have shown that most of the annual N loss to denitrification occurs during the non-crop season (Groffman 1985; Aulakh and Rennie 1986; Mahli and Nyborg 1986). Mahli and Nyborg (1986) from a number of experiments in Canada found an average of almost 50 kg N/ha were denitrified during winter. Clearly, studies of denitrification in agricultural field soils need to consider non-crop season activity.

## Landscape

There are few data on denitrification at scales of investigation larger than the field scale. Landscape and regional scale data are needed for calculation of large scale N budgets and for atmospheric chemistry questions affected by soil-atmosphere N gas flux (Robertson 1986). We have begun to experimentally address landscape scale dynamics of denitrification by focusing on distal, landscape scale factors that influence denitrification. Such studies form the basis for regional and global scale studies of denitrification.

We have studied landscape scale dynamics of denitrification using an approach based on soil classification. Soil texture and drainage are landscape scale parameters that have strong effects on denitrification, largely through their influence on soil water. Drainage class is a general indicator of soil wetness and soil texture has strong effects on the ability of soil to hold and transmit water. Texture also affects denitrification because fine textured soils have smaller pores that more easily become anaerobic than the large pores present in coarse textured soils (Papendick and Campbell 1980).

Soils are organized in coherent patterns across the landscape that are useful as guides for landscape scale experimental designs. Soil texture is controlled by parent material, which is related to broad scale geological and physiographic features. Soils of similar texture are often found in adjacent topographic positions and thus differ in soil drainage. The coherent patterns of soil type in the landscape, and the fact that soils are well mapped for large regions of the world, make soil texture and drainage potentially very useful parameters for study of denitrification at the landscape scale.

Table 4 presents estimates of annual N loss to denitrification for nine forest soils of different texture and drainage in Michigan. Soils of similar texture were located in adjacent slope positions in catenas. Catenas of different texture were associated with different glacial landforms - moraine, till plain, outwash plain. Annual N loss to denitrification was determined by repeated sampling using soil cores over the course of a year.

Soil texture and drainage appear to be very strong predictors of denitrification activity at the landscape scale. We quantified soil drainage by calculating a Drainage Index parameter developed by soil geographers (Hole

**Table 4:** Annual N loss to denitrification, soil drainage (Drainage Index) and soil texture (percent sand) for forested soils in Michigan.

Soil type	Denitrification N loss <sup>1</sup>	Drainage Index <sup>2</sup>	Soil texture <sup>2</sup>
	<i>kg N/ha/y</i>		<i>% sand</i>
<b>Loam</b>			
Well drained	10	40	26
Somewhat poorly drained	11	50	42
Poorly drained	24	70	18
<b>Clay loam</b>			
Well drained	18	44	34
Somewhat poorly drained	17	64	35
Poorly drained	40	74	15
<b>Sandy loam</b>			
Well drained	0.6	40	76
Somewhat poorly drained	0.8	60	74
Poorly drained	0.5	61	77

1. Groffman and Tiedje (1987a); 2. Groffman and Tiedje (1987b)

1978; Schaetzl 1986). Drainage Index is largely based on soil drainage class but is modified by soil great group, sub-group and textural factors. The index provides a measure of general soil wetness and is a continuous numerical variable suitable for regression analysis. Percent sand (from mechanical analysis) was a useful variable for regression analysis of soil texture. In a multiple regression model, we were able to explain 86% of the variability in annual N loss to denitrification with soil texture (% sand) and drainage (Drainage Index).

The results in Table 4 suggest that large scale studies of denitrification may be productive. We were able to explain a much higher percentage of the variability in annual N loss to denitrification at the landscape scale than we could at the field scale (within each of our 9 soils), for example, over 80% of the variability in annual N loss to denitrification at the landscape scale was explained by distal factors such as soil texture and drainage, whereas we were seldom able to explain more than 50% of the variability in daily or hourly rates of denitrification at the field scale using more proximal factors such as soil water, NO<sub>3</sub> or CO<sub>2</sub> production. Increasing the scale of investigation in both time (annual loss rather than hourly or daily rates) and space (landscape rather than field scale) may be useful for overcoming the variability problems frequently encountered in denitrification research (Folonoruso and Rolston 1984; Robertson and Tiedje 1984; Burton and Beauchamp 1985).

## Regional

To address regional dynamics of denitrification, we need to extrapolate data from landscape studies, and to investigate land use and plant community variables as distal controllers of denitrification. Few data address the effects of land use on denitrification, for example comparing adjacent forest and agricultural areas. There is however, much data on denitrification in different agricultural management systems that can be assembled for regional scale studies, or that can at least serve as a guide for the type of data needed. While there is relatively little data on denitrification in most natural communities (Bowden 1986), we have accumulated data on denitrification in successional communities in both tropical and temperate forests (Table 5) that can serve as a guide for the collection of data for regional scale studies.

**Table 5:** Denitrification rates in a temperate and a tropical forest sere. Values are means ( $\pm$  standard error).

Successional stage	Denitrification rate	
	Tropical <sup>1</sup>	Temperate <sup>2</sup>
	<i>ng/cm<sup>2</sup>/h</i>	
Early	24.3(2.2)	1100(370)
Mid	4.8(1.2)	15(8)
Late	15.0(3.5)	36(28)

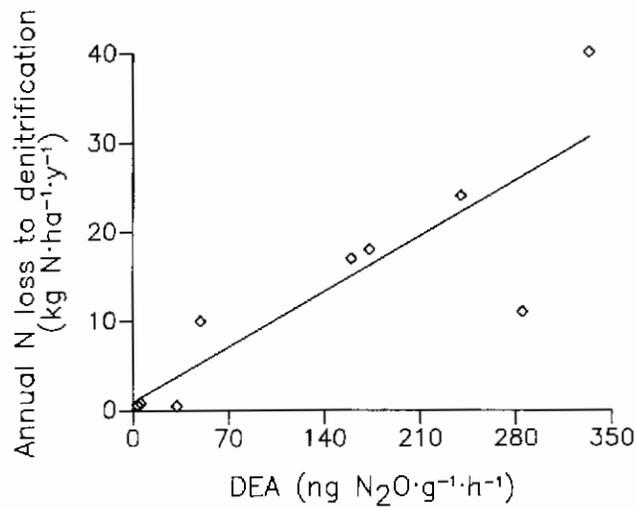
1. Robertson and Tiedje (1987); 2. Robertson and Tiedje (1984).

Studying denitrification in different plant communities within a successional sere appears to be a useful approach to characterizing regional scale dynamics of denitrification in forest communities. Table 5 shows that in both a temperate and a tropical sere, denitrification is relatively high in early and late successional communities and is low in mid-successional communities. This pattern of denitrification is consistent with overall successional patterns of nutrient cycling and loss as outlined by Vitousek and Reiners (1975) and Bormann and Likens (1979). Nitrogen losses and soil NO<sub>3</sub> levels in mid-successional, aggrading forests are often very low due to intense competition for N between and among plants and microorganisms. Early and late successional communities may have higher N losses than mid-successional communities due to less intense levels of competition and a more patchy distribution of vegetation, although patterns in early succession may vary with type of disturbance (Gorham *et al.* 1979). For regional scale studies, data on successional patterns of denitrification can be combined with data on the effects of soil type on denitrification to produce estimates of denitrification in forest components of a regional area.

Extrapolating data from the landscape to a regional scale can be done using geographic information systems (GIS). These systems contain information about soils, land use, hydrology and other factors for areas ranging from tens to thousands of square kilometers. Simple algebra can be used to incorporate biological functions into the GIS database to produce large scale estimates of biological processes like denitrification. We are currently working with a simple GIS for the State of Michigan that contains information about land use and soil type. We are using this GIS to extrapolate our landscape scale data to produce estimates of denitrification from forest soils for a large area of Michigan. To do this, all soils in the region are assigned to one of nine groups represented by the nine soils in our original study. Thus, each soil mapping unit is assigned one of nine estimates of annual N loss to denitrification. The GIS can calculate the areal extent of each soil type under forest cover and can thus calculate regional estimates of annual N loss to denitrification. While this estimate requires broad extrapolation of our landscape data, it is an improvement on previous regional scale studies of N cycling processes. Our nine groups contain two orders of magnitude of variability in annual N loss estimates and provide considerably higher resolution than any previous studies that have assigned single values to much larger study areas. Future work in this area will incorporate successional information to our models for forest soils and will address the effects of other land uses on denitrification within our study region.

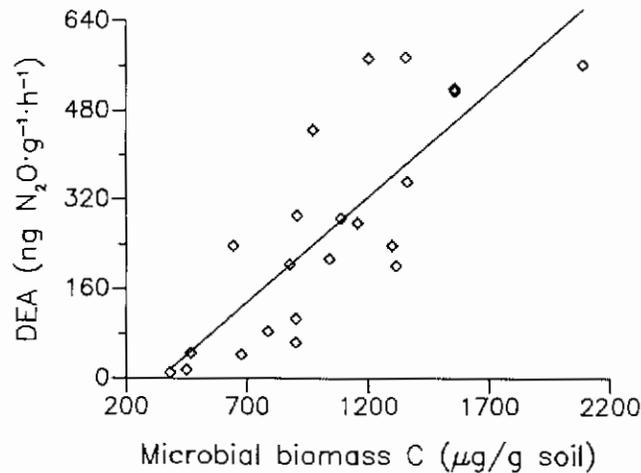
To test the validity of our broad scale extrapolations, we are developing methods to verify landscape or regional scale patterns of denitrification. Figure 3 shows that the annual mean (nine sample dates) of denitrification enzyme activity (DEA) was strongly related to annual N loss to denitrification among the nine soils in our landscape scale study. While it is quite laborious to measure annual N loss to denitrification, DEA can be quantified relatively easily since it varies very little in time and space relative to actual denitrification rate. Within any one soil in our landscape study, DEA varied by a factor of 2 or 3 over the course of the year while actual denitrification rate varied by 2 or 3 orders of magnitude. DEA can thus be measured only a few times, at a large number of sites, to verify extrapolation of landscape patterns of denitrification to a regional scale. We measured DEA (on one occasion only) in 18 soils taken over a wide area of Michigan similar to those in our landscape scale study. We found that the strong patterns of denitrification with soil texture and drainage that we observed at the landscape scale held up at a regional scale, and thus extrapolating results from our landscape scale studies to a regional level was justified.

Our success at characterizing landscape and regional scale dynamics of denitrification is encouraging for developing methods for characterizing large scale dynamics of microbial processes in general. Figure 4 shows that DEA was reasonably closely related to microbial biomass C in the group of 18 soils that we sampled across a wide area of Michigan, and in turn microbial biomass C was strongly related to soil texture (Fig. 5, see also Merckx *et al.* 1985; van Veen *et al.* 1985). Microbial biomass C and N content are also related to

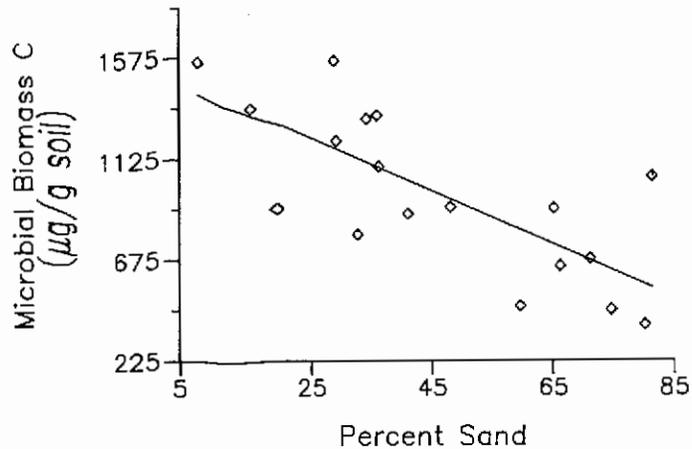


**Fig. 3:** Annual N loss to denitrification versus denitrification enzyme activity (DEA, phase I assay, Smith and Tiedje 1979) for nine forest soils of different texture and drainage classes in Michigan, USA. (Adapted from Groffman and Tiedje 1987b).

other landscape scale parameters such as land use, succession and soil drainage. Easily measured, low variance parameters like DEA and microbial biomass C and N content, that are strongly related to biological fluxes such as denitrification and soil respiration may be very useful for studying



**Fig. 4:** Denitrification enzyme activity (DEA) versus microbial biomass carbon content (chloroform fumigation-incubation method, Jenkinson and Powlson 1976) in 18 forest soils of different texture and drainage classes in Michigan, USA. (Adapted from Groffman *et al.* 1987).



**Fig. 5:** Microbial biomass carbon content versus percent sand in 18 forest soils of different texture and drainage classes in Michigan, USA. (Adapted from Groffman *et al.* 1987).

regional scale dynamics of biogeochemical processes in general.

A potentially useful tool for landscape and regional scale studies of microbial processes is remote sensing. Figure 6 shows a conceptual model of soil water-plant productivity-N cycling and N loss relationships and how remote sensing can be used to study these relationships. Soil water can be remotely-sensed by microwave radiometry (Schmugge 1983), and plant production (Goward *et al.* 1985) and N content (Spanner *et al.* 1985) can be remotely-sensed using space satellites. We are currently investigating the use of remotely-sensed soil water as a predictor of denitrification at the landscape scale. Remotely-sensed data may be especially useful for large scale studies when combined with GIS data on soil type and hydrology.

### Global

Global estimates of N cycle processes, including denitrification, have been compiled (Soderlund and Svensson 1976; Rosswall 1981; Banin *et al.* 1984). The approaches used assembled data from different ecosystem or biome types and matched these data with estimates of the areal extent of each biome to produce global estimates. Since the available sets of denitrification data are scattered in time and space, highly variable in methodology, and often very limited in spatial and/or temporal scope, the current global estimates of denitrification are highly uncertain. Global scale estimates of biogeochemical processes like denitrification are important for understanding the effects of land use changes and other anthropogenic effects on atmospheric chemistry and climate.

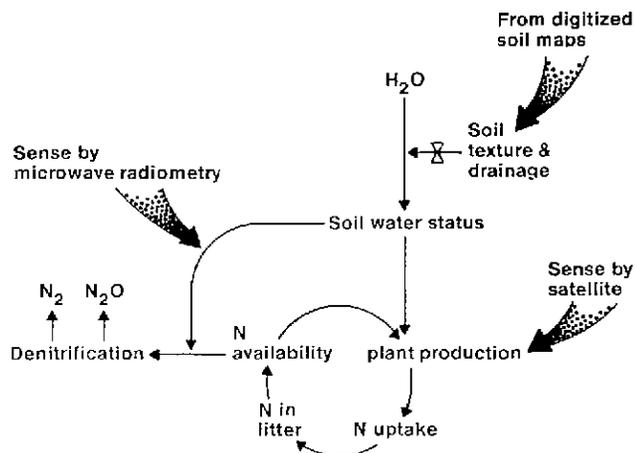


Fig. 6: Model of denitrification control and how remote sensing could improve large scale estimates of the process.

There are several obvious routes to improve our understanding of the global dynamics of denitrification. Fairly good data sets of relative comparisons of biome productivity have been assembled (Webb *et al.* 1983) and there are strong links between biomass productivity and climate and microbial processes (Heal and Ineson 1984). Our landscape scale studies have shown that there are strong relationships between microbial processes in general and denitrification. If enough landscape or regional scale denitrification data sets become available, then relatively accurate global N budgets could be produced. An organized effort towards producing these data sets, perhaps within the aegis of existing global scientific programs could be worthwhile.

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