

APPENDIX H

NITROGEN AVAILABILITY

H.1 INTRODUCTION

Nitrogen is undoubtedly the most intensively studied plant nutrient in both natural and managed ecosystems. Despite its importance to plant growth and ecosystem productivity, there is still no reliable soil test for nitrogen availability (Stanford, 1982). The reasons for this are numerous (see Stanford, 1982 and Stevenson, 1986 for reviews). Soil nitrogen for the most part is in the organic form (Stevenson, 1986) and unavailable to plants.

Organic nitrogen is mineralised to ammonium, a plant available form, by microbial processes. The rate of mineralisation depends on many factors including temperature and rainfall, the quality of the soil organic nitrogen, and the quality of organic inputs to the system. Once in the mineral form, nitrogen, like other plant nutrients, can be taken up by plants or microbes (immobilisation) or lost from the system by leaching. In addition, nitrogen can undergo various transformations, many of them biologically mediated. Many of these transformations such as nitrification, volatilisation, and denitrification can ultimately lead to losses from the system.

In natural ecosystems nitrogen losses are generally assumed to be small because of efficient nutrient cycling, mineralisation occurring in synchrony with plant uptake. In managed systems however, this synchrony may be disrupted leading to a build up of mineral nitrogen in the soil.

This nitrogen is susceptible to losses via the various pathways discussed. Leaching losses can be large especially in the humid tropics where rainfall is high and a majority of the soils have little retention capacity for cations or anions. Losses of nitrogen through denitrification may be as large, or larger, than through leaching in disturbed or managed systems. Little is known however, about denitrification in tropical ecosystems, natural or managed. The potential for denitrification should be high in tropical systems where nitrification rates are high and high rainfall can lead to anaerobic conditions in the soil. There are indications that denitrification is higher in undisturbed tropical forest ecosystems than those in temperate regions (Keller et al, 1986; Mooney et al, 1987) and that these rates increase with disturbance of the system (Keller et al, 1986; Matson et al, 1987).

One of the major objectives of the TSBF Programme is to find ways of minimising losses of nitrogen (as well as other nutrients) in managed systems by synchronising mineralisation with plant uptake. This will require an understanding of the controls of the various transformations and fluxes of nitrogen and how these are affected by management. Part of the problem in understanding nitrogen cycling in ecosystems has been the

lack of methodologies for measuring the different processes. In this section methodologies for estimating net mineralisation and denitrification and the problems associated with them are presented. The collection of leachate is covered in Appendix E, 'Soil Solution Sampling and Lysimetry'.

H.2 MINERALISATION

The concentration of mineral nitrogen in soil reflects a balance between inputs through mineralisation of organic N and outputs through microbial immobilisation, plant uptake, leaching, and denitrification. Most methods for estimating nitrogen availability assume it to be equivalent to net nitrogen mineralisation (gross mineralisation minus immobilisation); they are designed to prevent (or quantify) root uptake, leaching, and denitrification by incubating soil in the absence of active roots. Any method however, which involves either severing roots or removing them from soil alters the soil environment to a greater or lesser extent. Hence, any such effort to measure soil nitrogen availability interferes with the process, and a broad-scale measurement programme must seek a comparable and realistic index of nitrogen availability rather than a direct measurement of the process.

No single method for estimating nitrogen availability has gained universal acceptance, and indeed it is unlikely that any single method will prove applicable to all sites and conditions (Keeney, 1980). Moreover, relatively few of the extant methods have been tested in tropical soils. The TSBF Programme initially adopted a field incubation method (below) as the standard, but workers in high-rainfall areas have reported problems with flooding of soil cores. Other studies have reported substantial losses of field-incubated cores to soil fauna (Vitousek and Denslow, 1986). It was therefore decided to use aerobic incubations in the laboratory as the simplest standard method, and to suggest optional use of more realistic field incubations where practical (see (b) below).

Laboratory methods for estimating N-mineralisation were reviewed by Brown (1982), who showed that a wide range of incubation times, temperatures and conditions, and extraction procedures have been employed with little attempt at standardisation. Many authors have incubated soil samples over long time periods assuming that mineralisation is linear over time. Myers (1975) however, reported for tropical soils that ammonification rates in the first seven days of incubation were about six times higher than between 14 and 28 days.

It is also difficult to control soil moisture while maintaining aerobic conditions over long periods of incubation. Quartz sand may be used to disperse soil, but ambient field moisture conditions cannot be maintained using this method. As a compromise on the various limitations of laboratory incubations, the simplest aerobic incubation procedure will be used as a baseline for TSBF studies (see Section 5.5.2 for details).

Details are also given (Brown, 1982) for an anaerobic (waterlogged) laboratory incubation method of Waring and Bremner (1964). This method is rapid, and it may have potential as an index of the labile pool of plant-available N; it does not approximate field mineralisation processes in any way.

Methods used to estimate patterns of N mineralisation under field conditions include:

- a) Incubation of disturbed (sieved or mixed) soil in plastic bags buried in the field (eg Westermann and Crowthers, 1980).
- b) Incubation of relatively undisturbed soil columns (cores) enclosed in plastic bags or columns under field conditions (Nadelhoffer et al, 1985; Matson et al, 1986; Raison et al, 1987). Rapp et al (1979) performed in situ incubations after isolating the soil within thin metal cans pushed into the soil.
- c) Measurement of mineral-N collected by ion exchange resins placed in the field for extended periods (eg Hart and Binkley, 1985).
- d) Determining the effects of varying temperature and moisture on N mineralisation in disturbed soils in the laboratory, and then assuming that these relationships can be linked to variations in these parameters in the field - ie a modelling approach (eg Cameron and Kowalenko, 1976; Marion et al, 1981; Macduff and White, 1985).

In addition to changes induced by severing or removing roots (Hendrickson and Robinson, 1984) the following potential difficulties may apply:

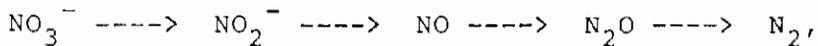
- i) soil disturbance markedly affects mineralisation (eg Runge, 1974; Nordmeyer and Richter, 1985). This effect will be significant for methods (a) and (d); it may be quite small for method (b);
- ii) rapid fluctuations in soil water content markedly affect mineralisation in many environments. With methods (a) and (b) the soil moisture content at the time of sampling is maintained throughout the incubation period;
- iii) method (c) estimates the amount of nitrogen in percolating water (Binkley, 1984), not net nitrogen mineralisation. It is insensitive to any N either absorbed by roots or exchanged on soil surfaces; and
- iv) for modelling approaches to be useful, temperature and moisture response surfaces must be determined for undisturbed soils, and account needs to be taken of seasonal variations in the pools of mineralisable organic N in soils (Popovic, 1971; Ellis, 1974; Theodorou and Bowen, 1983; Nordmeyer and Richter, 1985).

Of the above methods, (b) appears most suitable for a wide range of sites.

H.3 DENITRIFICATION

Denitrification is the biological reduction of soil nitrate to nitrous oxide (N_2O) and dinitrogen (N_2) gases. The process is carried out by a diverse group of bacteria using nitrogen oxides as terminal electron acceptors in lieu of oxygen under anaerobic conditions. In well-aerated soils these conditions most often exist within soil aggregates or where high concentrations of organic matter create strong, localised O_2 sinks.

The reductive pathway for denitrification is generally accepted to be:



with the ratio of N_2O : N_2 liberated by the denitrifying community highly inconstant, under the control of a wide suite of environmental factors (Firestone et al, 1980). Though denitrification is in most soils the largest single source of nitrogen gas, N_2O may additionally arise by a variety of other pathways. These include the chemical decomposition of hydroxylamine and nitrite reduction during nitrification (Blackmer et al, 1980; cf Poth and Focht, 1985), dissimilatory nitrite and nitrate reduction to ammonium (Bleakley and Tiedje, 1982), microbial nitrate assimilation (Sato et al, 1981), and other pathways as yet poorly described (Robertson and Tiedje, 1987).

Experimental methods for quantifying denitrification are reviewed by Focht (1982), Tiedje (1982), and Tiedje et al (1989), including ^{15}N tracer techniques. The simplest way to measure denitrification in field studies is the acetylene inhibition technique (Duxbury 1986, Tiedje et al 1989). Acetylene at relatively low concentrations (<10 kPa or <10% v/v at 1 atmosphere) blocks the expression of N_2O reductase in most soils, so that the N_2O which might otherwise have been reduced to N_2 remains as N_2O , easily measured against background atmospheric concentrations.

Ryden and Dawson (1982) report a field method where a constant stream of acetylene gas was injected 1 m below a pair of boxes covering a pasture sward while a second pair of boxes were untreated. Gas products from the two series of chambers were collected over 3-4 hr and scrubbed for N_2O using a molecular sieve; N_2O was then recovered and determined by gas liquid chromatography. The estimates of N losses by denitrification using this method at intervals over the growing season were in close agreement with estimates obtained from N budgets for the pasture system.

The main limitations on the acetylene inhibition technique are ensuring (i) that acetylene diffuses to the soil microsites where denitrification is taking place, and (ii) since acetylene also inhibits nitrification, that denitrification rates are quantified before nitrate becomes limiting. Both chamber and

soil core techniques have been used to quantify denitrification using the acetylene inhibition method; both techniques yield similar results (Ryden and Skinner, 1987; Tiedje et al, 1989) but the static core technique has the advantages of being relatively inexpensive and amenable to large sample sizes, important considerations given the high degree of spatial heterogeneity typical for this process in a variety of environments. The static core's principal disadvantage is that great care must be taken during sampling and subsequent analysis to avoid disrupting anaerobic microsites in soil by disrupting soil structure. The recommended TSBF procedure is given in Section 5.7.2.

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