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THE EFFECTS OF CROP RESIDUES (TWO LEGUMES, TWO GRASSES AND CORN)
ON DENITRIFICATION, DISSIMILATORY NITRATE REDUCTION,
MINERALIZATION AND IMMOBILIZATION OF N IN SOIL

by

Wang Shi Wei

A Thesis
Submitted to the Faculty of Graduate Studies and Research through the Department of Chemistry and Biochemistry in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor
Windsor, Ontario, Canada
1993
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ABSTRACT

THE EFFECTS OF CROP RESIDUES (TWO LEGUMES, TWO GRASSES AND CORN) ON DENITRIFICATION, DISSIMILATORY NITRATE REDUCTION, MINERALIZATION AND IMMOBILIZATION OF N IN SOIL.

Since cover crops and intercrops are increasingly used to reduce soil erosion, N loss, and to provide efficient N utilization, it is important to evaluate the consequences of crop residues to N cycling processes. The objectives were to determine effects of incorporation of *Vicia villosa* L. Hairy vetch (HV); *Trifolium pratense* L. Red clover (RC); *Lolium temulentum* L. Annual ryegrass (ARG); *Phalaris arundinacea* L. Reed canarygrass (RCG); and *Zea mays* L. corn residues on denitrification, dissimilatory nitrate reduction, and N mineralization/immobilization in a Brookston clay loam (Typic Agriaquoll). A gas flow system was used to measure rates of NO and N$_2$O production for soils amended with dry residue or water soluble extract of residue. Some samples were incubated at 30.5% moisture for 2-days under anaerobic-only conditions. Most samples were incubated at 16% moisture. With anaerobic-only incubation and when soils were not amended with NO$_3^-$, all residues stimulated denitrification resulting in NO+N$_2$O production 2 times greater than that in the control soil. There was no response in NH$_4^+$ production. The initial addition of NO$_3^-$ not only increased denitrification losses of NO+N$_2$O on average by 55% but also stimulated NH$_4^+$ production. This was probably due to dissimilatory nitrate reduction to ammonium which thereby decreased soil NO$_2^-$.
concentration in all treatments. During a 5-day aerobic and during subsequent anaerobic periods significant mineralization occurred with incorporated HV. Net immobilization was observed in RC, ARG, RCG and corn treatments during the 5-day aerobic phase. This was particularly evident in the RC treatment where 34 and 85% of the initial NO₃⁻ content with and without NO₃⁻ addition, respectively, was immobilized. During 2-days under anaerobic conditions following a 5-day aerobic preincubation, without NO₃⁻ amendment. NO₃⁻+N₂O production in RC and corn were almost the same as that in the control soil, indicating that denitrification was not stimulated by residue under all conditions. The losses of NO₃⁻+N₂O increased with the NO₃⁻ amendment in all residue treatments, and particularly in legume treatments, amounting to 5 to 7 times that in the control soil. An increase of NH₄⁺ production was also obtained in the legume and ARG treatments which had relatively high initial residue N content, but the production of NH₄⁺ in RCG and corn which had low initial residue N content did not respond to the NO₃⁻ amendment. Although initial denitrification, over the 5d aerobic / 2d anaerobic period, with ARG was less than with HV, cumulative losses over the 5d aerobic / 6d anaerobic period did not differ significantly. The quality of the crop residue, rather than only C/N ratio, appears to be the most important factor regulating these microbial processes under otherwise similar conditions.
DEDICATION

To all my family,
for their continued love and encouragement.

To Zhuo and Fang,
for the great joy they have brought me.
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ABBREVIATIONS

ARG  annual ryegrass
$f_{\text{NO}}$  net production rate of nitric oxide
$f_{\text{N$_2$O}}$  net production rate of nitrous oxide
HV  hairy vetch
RC  red clover
RCG  reed canarygrass
CHAPTER 1

Introduction

Intensive soil and crop management systems have depended upon large inputs of agricultural fertilizers to maintain or increase crop yields. The increasing cost of fertilizer has concerned farmers. Nitrogen losses from cropland represent an economic loss and a potential environmental hazard when transported to ground or surface waters as NO₃⁻, or when denitrified to N₂O (Legg and Meisinger, 1982). Increasing public concern about environmental quality and the long-term productivity of agroecosystems has emphasized the need to develop and implement management strategies that maintain and protect soil and water resources. Both issues are related to maintaining the quantity of soil organic matter, which are strongly influenced by management (Janzen, 1987). Therefore, alternative production practices such as cover crops and intercrops are increasingly employed to reduce production costs, control or decrease erosion, improve soil structure and fertility, maintain or increase crop yields, and address some of the aforementioned environmental concerns.

Crop residues influence soil quality, nutrient cycling, microbial processes, and can protect soil against erosion and contribute significant amounts of N to the main crop (Power and Doran, 1988; Collins et al., 1992). Among the apparent advantages is the possible reduction in loss of N with run-off and by leaching. Nitrogen release from crop
residues depends on microbial degradation of the crop residue as influenced by residue type, placement, degree of incorporation in soil, soil temperature, and water/aeration regimes (Aulakh et al. 1991). In addition to assimilation by the cover crop or intercrop, retention of N may occur if NH$_4^+$ or NO$_3^-$ is immobilized by the microbial biomass or if NO$_3^-$ is converted to NH$_4^+$ by dissimilatory nitrate reduction under anoxic conditions. Thus, increased inputs of plant residue may be expected to enhance N conservation. In contrast, crop residues supply available carbon to microbes via mineralization from plant materials (Collins et al., 1990). Moreover, localized areas of high crop residue concentrations may stimulate intense microbial activity, resulting in O$_2$ consumption at rates sufficient to produce anaerobic microsites (Parkin, 1987). Therefore, denitrification may be increased with the addition of crop residues as a direct result of available C supply and as an indirect result of inducing anoxic conditions. Losses as gaseous NO, N$_2$O and N$_2$ or as dissolved N$_2$O in run off and leachate can occur which may have environmental consequences (Dowdell et al., 1979).

Although the influence of crop residue type, placement and environmental conditions (soil temperature and moisture) on microbial processes that affect residue decomposition have been well documented (Christensen, 1986; Collins et al., 1990; Douglas and Rickman, 1992), data on their effects on denitrification and mineralization in soil are sparse (Aulakh et al., 1991; Walters et al., 1992). A study comparing different crop residues having a wide range of C/N ratio with different initial NO$_3^-$ nitrogen concentration in soil is not available in the literature. Denitrification refers to anaerobic
respiration and reduction of NO$_3^-$ and NO$_2^-$ to N gases (NO, N$_2$O, N$_2$) by organisms that normally use O$_2$ for respiration (Groffman, 1991). The potential for denitrification in soils involves a complex interaction between aeration, nitrate availability, carbon substrate availability and other soil factors (moisture, pH and temperature) (Paul and Clark, 1989). Numerous and diverse C substances from plant tissue are usually available in soil. Although denitrifying microbes may use a wide variety of organic compounds as carbon and energy sources under aerobic conditions, a more limited number may be used under anaerobic conditions (Beauchamp et al., 1989). Thus, under certain soil moisture conditions, denitrification potential and rates in crop residue-amended soils are mainly controlled by the amount of NO$_3^-$ and C susceptible to mineralization from crop residues.

Nitrogen mineralization is defined as the transformation of N from the organic state into the inorganic forms of NH$_4^+$ or NH$_3$ (Jansson and Persson, 1982). The lower the C/N ratio and the higher the amount of N in the crop residue, the higher was the amount of N mineralized (Norman et al., 1990). N immobilization is defined as the transformation of inorganic N compounds (NH$_4^+$, NH$_3$, NO$_3^-$, NO$_2^-$) into the organic state (Jansson and Person, 1982). Since nitrogen immobilization is closely tied to the availability of substrate carbon and abiotic parameters (Paul and Clark, 1989), plant residues could also affect immobilization. The higher the C/N ratio and the lower the amount of N in the crop residue, the greater was the amount of residue N recovered in the soil (Norman et al., 1990). This finding was confirmed by the results obtained by Aulakh et al., (1991).

In contrast to denitrification, dissimilatory NO$_3^-$ reduction to NH$_4^+$ conserves soil
nitrogen. However, dissimilatory nitrate reduction can result in massive accumulation of nitrite (Cole, 1988). Griffiths and Cole (1987) pointed that addition of nitrate stimulates rather than represses nitrite reduction by *E. coli*.

The objectives of this study were to determine effects of various crop residues (two legumes, two grasses and corn) with alteration of soil moisture and aeration, with and without NO$_3^-$ addition on denitrification, dissimilatory nitrate reduction, mineralization and immobilization of N in soil.
Chapter 2

Materials and Methods

A Brookston clay loam (Orthic Humic Gleysol) soil was selected for this study. On average the soil contained 360 g clay kg\(^{-1}\), 350 g silt kg\(^{-1}\), 290 g sand kg\(^{-1}\) and 21 g organic C kg\(^{-1}\). Field-moist soil was air dried to a gravimetric moisture content of 40 to 80 g kg\(^{-1}\), sieved (4mm) and stored at 4°C. Two legumes, hairy vetch (HV) and red clover (RC), two forage grasses, annual ryegrass (ARG) and reed canarygrass (RCG), and corn residue (stalks and leaves) were used. These were obtained from field sites at the Eugene Whelan experimental farm, Agriculture Canada, Research Station, Woodslee, Ontario. The above-ground portion of these plants were harvested at the end of the corn-growing season, dried and ground in a Wiley mill fitted with a 20-mesh (0.841mm) screen. Some samples were incubated at 30.5% moisture under a 2-day anaerobic-only condition. Most samples were incubated at 16% moisture. In most of the experiments, ground dry residue was mixed with soil at 5 or 10 g kg\(^{-1}\) (oven-dry basis, 104°C). In some experiments only the water soluble material extracted from the residues was added. In these cases, 1.0 g of dry residue was shaken with 20 ml distilled deionized water for 2 h, and then filtered through a Whatman No. 40 filter paper, and added to 100 g of soil (oven-dry basis, 104°C). No attempt was made to ensure that the extraction and transfer of water soluble materials was quantitative, although in trials in which extraction times
were increased up to 24 h there was no significant difference in results (data not presented). The initial residue N content and C/N ratio of dry residue and water soluble extract of residue are shown in Table 1. Total C was determined by combustion followed by CO₂ analysis using a LECO carbon analyzer (model no. CR 12; St. Joseph, MI). The Kjeldahl method was employed for determination of total N by digesting the sample with K₂SO₄ and Se catalyst at 375°C to convert organic N to NH₄⁺-N (Bremner and Mulvaney, 1982). Ammonium was determined in the digest using a TRAACS 800 auto analyzer (Bran and Luebbe, Buffalo Grove, IL, USA) with NaOH added to the EDTA solution to neutralize the H₂SO₄ in the digest.

Soil was thoroughly mixed with each of the dry residues, then KNO₃ (100 mg N kg⁻¹) solution and distilled deionized water added to achieve either 160 g H₂O kg⁻¹ or 305 g H₂O kg⁻¹ (oven-dry basis, 104°C). In the case without KNO₃ addition, distilled deionized water was added to a soil moisture content of 160 g H₂O kg⁻¹. Experiments were also carried out using soil amended with water-soluble extracts of the crop residues, KNO₃ (100 mg N kg⁻¹) solution and distilled deionized water added to achieve a moisture content of 160 g kg⁻¹. Each residue treatment and a control (no C addition) was typically replicated three times. Each amended sample contained 100 g soil for the 2 d, 110 g for the 4 d and 5 d, 140 g for the 7 d, and 180 g for the 11 d experiments to allow for more soil sub-sampling with the longer incubation times. Each soil sample was placed into a thermostatted (20±0.1°C) column. A maximum of ten columns in parallel could be run simultaneously using a gas-flow system similar to that described by McKenney et al.
Table 1. Characteristics of crop residues.

<table>
<thead>
<tr>
<th>Residue</th>
<th>N content g kg⁻¹</th>
<th>C/N ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(d.r.)</td>
<td>(w.s.e.)</td>
</tr>
<tr>
<td>Hairy vetch</td>
<td>37.0(0.4)</td>
<td>11.3(0.1)</td>
</tr>
<tr>
<td></td>
<td>28.1(0.4)</td>
<td>12.3(0.1)</td>
</tr>
<tr>
<td>Red clover</td>
<td>27.1(0.6)</td>
<td>15.1(0.3)</td>
</tr>
<tr>
<td></td>
<td>18.0(0.3)</td>
<td>19.4(0.4)</td>
</tr>
<tr>
<td>Annual ryegrass</td>
<td>24.7(0.3)</td>
<td>15.6(0.5)</td>
</tr>
<tr>
<td></td>
<td>24.9(0.3)</td>
<td>12.3(0.2)</td>
</tr>
<tr>
<td>Reed canarygrass</td>
<td>14.6(1.3)</td>
<td>28.3(3.6)</td>
</tr>
<tr>
<td></td>
<td>16.9(0.4)</td>
<td>20.4(0.3)</td>
</tr>
<tr>
<td>Corn</td>
<td>6.2(0.1)</td>
<td>65.5(0.8)</td>
</tr>
<tr>
<td></td>
<td>11.5(0.2)</td>
<td>30.4(0.6)</td>
</tr>
</tbody>
</table>

d.r.: Dry residue.

w.s.e.: Water-soluble extract.
(1982) (Fig. 1). A constant flow (412 cm$^3$ min$^{-1}$) of humidified N$_2$ sparged evolved gases from the anaerobic soil columns. The carrier gas with soil gas was analyzed for NO and N$_2$O at selected times over periods ranging up to 11 d. Several series of runs were carried out following initial aerobic incubations by constantly flowing compressed air at 412 cm$^3$ min$^{-1}$ through the soil columns at 20°C for periods ranging up to 5 days. Anoxic conditions were established by replacing the compressed air with N$_2$. Nitric oxide was analyzed with a NO/NO$_2$/NO$_x$ analyzer (Model 14B/E, Thermo Electron Corp., Hopkinton, MA). Continuous monitoring over a range of 0.001 to 10 ppm of NO can be achieved. Gas samples were taken from T (Fig. 1) and analyzed for N$_2$O using a Hewlett Packard (Model 5880 A, Avondale, PA) gas chromatograph fitted with a Porapak Q column (Water Assoc., Milford, MA) and electron capture detector. Precise injection to the G.C. was accomplished through a 1.0 mL Hamilton Microliter Syringe (Hamilton Company). Net production rates were calculated as $f_{NO} = f_c [NO] / m$ and $f_{N_2O} = f_c [N_2O] / m$, where $f_c$ is the total flow rate (mL min$^{-1}$) through the column, $m$ is the mass of soil in the column (kg), [NO] is determined from the instrument reading, and [N$_2$O] is calculated from sample's peak area (counts) and calibration of known amount of N$_2$O. [NO] and [N$_2$O] are concentrations (parts per million by volume) in the effluent gas stream (McKenney et al., 1982, 1984).

Analyses for NO$_3^-$, NO$_2^-$ and NH$_4^+$ concentrations as a function of time were also obtained. Soil samples (10 g) were taken from the column at the start and each subsequent day during a run. In the case of the 11 d runs, after the 5 d aerobic
Fig. 1. Schematic diagram of apparatus. C, soil column; W, water column for controlling moisture level in carrier gas; J₁-J₆, six needle valves for carrier gas flow control; M, copper pipe manifold; R₁ and R₂, rotameter flow meters; A, zero gas (N₂) flask; V, vents. NO, NO/NOₓ/NO₂ analyzer; G.C., gas chromatograph; P, pump; T, plastic T for gas sampling; B, Neslab model TE 45 constant-temperature circulating bath for temperature control (±0.1°C) of C and W (From D.J. Mckenney, et al., 1982).
incubation, samples were taken every 12 h. Each sample was weighed into a 300 Erlenmyer flask, 100 ml of 2 M KCl added, and then shaken for 1 h on a rotary shaker. The extractant was filtered through a Whatman No. 40 filter paper and stored at 4°C. The extracts were analyzed on a TRAACS 800 auto analyzer (Bran and Luebbe, Buffalo Grove, IL, USA) for NH₄⁺ using the Berthelot reaction, through the formation of a blue-coloured compound apparently related to indophenol from the reaction of ammonium and sodium phenoxide, followed by the addition of sodium hypochlorite (Tel and Heseltine, 1990). Nitrate and nitrite are analyzed using a colorimetric method based on the formation of an azo dye. The reaction involves the reduction of NO₃⁻ to NO₂⁻ with a copper-cadmium coil and the subsequent reaction of NO₂⁻ with sulphanilamide and N-1-naphthylethylenediamine dihydrochloride (Tel and Heseltine, 1990). Nitrate content was calculated by difference. All the extracts were analyzed by Agriculture Canada, Research Station, Harrow, Ontario.
CHAPTER 3

Results

3.1 Denitrification

3.1.1 Denitrification: with an initial addition of KNO₃ and without an aerobic preincubation

Net NO production rates for the anaerobic-only incubation for all treatments increased to a maximum of approximately 10.5 mg N kg⁻¹ d⁻¹ compared to the control rate of about 3.6 mg N kg⁻¹ d⁻¹ over 48 h (Fig. 2). Similar results were obtained in runs extending up to 148 h with only a very gradual decrease in rates over the longer period (data not shown). Net N₂O production rates followed similar patterns (Fig. 3), and negligible differences in rates (maximum fₙ₂ₒ = 1.6 mg N kg⁻¹ d⁻¹) were obtained among treatments. Dry residue added to the soil increased rates and the total quantity of NO+N₂O produced to roughly the same extent in all treatments (Table 2). Doubling the quantity of added residue increased NO+N₂O production by approximately 70% in all residue treatments. The proportion, N₂O / (NO+N₂O), was not significantly changed. Increasing soil moisture content increased the total denitrification products, NO+N₂O by a factor of ~4-5 and the proportion of N₂O to NO+N₂O more than 90% in agreement with the observation mentioned by Drury et al., (1992). When the soil at a moisture content of 160 g H₂O kg⁻¹ was amended with 10 g dry residue kg⁻¹, approximately equal amounts
Fig. 2. Net rates of soil NO production versus time under 2-day anaerobic conditions without an aerobic preincubation and with a 5-day aerobic preincubation. The soil at a moisture content of 160 g H₂O kg⁻¹ was amended with 100 mg NO₃-N kg⁻¹ and 10 g dry residue kg⁻¹.
Fig. 3. Net rates of soil N\textsubscript{2}O production versus time under 2-day anaerobic conditions without an aerobic preincubation and with a 5-day aerobic preincubation. The soil at a moisture content of 160 g H\textsubscript{2}O kg\textsuperscript{-1} was amended with 100 mg NO\textsubscript{3}\textsuperscript{-}N kg\textsuperscript{-1} and 10 g dry residue kg\textsuperscript{-1}. 
Table 2. Effect of quantity of dry residue and moisture content on NO + N₂O and the proportion of N₂O produced over 48 h under anaerobic conditions without an aerobic preincubation. The soil was amended with 100 mg NO₃-N kg⁻¹.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NO + N₂O Produced</th>
<th>N₂O/(NO + N₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg N kg⁻¹</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>160 g H₂O kg⁻¹</td>
<td>305 g H₂O kg⁻¹</td>
</tr>
<tr>
<td></td>
<td>5 g residue kg⁻¹</td>
<td>10 g residue kg⁻¹</td>
</tr>
<tr>
<td>Hairy vetch</td>
<td>11.3 (0.1)</td>
<td>18.8 (0.1)</td>
</tr>
<tr>
<td>Red clover</td>
<td>11.7 (0.2)</td>
<td>20.4 (0.1)</td>
</tr>
<tr>
<td>Annual ryegrass</td>
<td>12.0 (0.1)</td>
<td>19.4 (0.2)</td>
</tr>
<tr>
<td>Reed canarygrass</td>
<td>9.6 (0.1)</td>
<td>18.1 (0.1)</td>
</tr>
<tr>
<td>Control</td>
<td>7.2 (0.1)</td>
<td></td>
</tr>
</tbody>
</table>

† The numbers in parentheses are standard errors. For the 10 g residue kg⁻¹ data n = 3; for the 5 g residue kg⁻¹ data n = 2; for the Control n = 3.
of NO+\textsubscript{2}O were produced in dry residue treatments. 18.0 to 20.4 mg N kg\textsuperscript{-1}, compared to 7.2 mg N kg\textsuperscript{-1} for the control over the 2-day anaerobic-only incubation (Table 2).

3.1.2 Denitrification: with an initial addition of KNO\textsubscript{3} and a 5-day aerobic preincubation

During a 2 day anaerobic incubation following a 5-day aerobic incubation, rates were greater than the anaerobic-only incubation and differences in rates were observed among the residue treatments (Fig. 2). The highest maximum rates were produced by the legume-treated soils. 25.5 mg N kg\textsuperscript{-1} d\textsuperscript{-1} with HV and 22.1 mg N kg\textsuperscript{-1} d\textsuperscript{-1} with the RC treatment. The ARG and RCG treatments produced rates of 13.1 mg N kg\textsuperscript{-1} d\textsuperscript{-1}, the corn treatment formed 9.8 mg N kg\textsuperscript{-1} d\textsuperscript{-1} whereas the control showed a maximum rate of only 2.1 mg N kg\textsuperscript{-1} d\textsuperscript{-1}. Similar to the f\textsubscript{\textsubscript{NO}} results, significant differences in f\textsubscript{\textsubscript{\textsubscript{N}_{2}O}} were obtained following the 5-day aerobic incubation (Fig. 3). The maximum observed rates were as follows: HV, 9.7 mg N kg\textsuperscript{-1} d\textsuperscript{-1}; RC, 7.3 mg N kg\textsuperscript{-1} d\textsuperscript{-1}; ARG, 3.0 mg N kg\textsuperscript{-1} d\textsuperscript{-1}; RCG, 2.4 mg N kg\textsuperscript{-1} d\textsuperscript{-1}; corn, 1.9 mg N kg\textsuperscript{-1} d\textsuperscript{-1}; and the control soil, 0.3 mg N kg\textsuperscript{-1} d\textsuperscript{-1}.

When an aerobic incubation preceded anaerobic conditions, significant differences in NO+\textsubscript{2}O production during the anaerobic phase occurred among residue treatments (Table 3). Differences increased with increasing aerobic incubation time and soil treated with HV always produced the greatest quantity of NO+\textsubscript{2}O. For example, after the 5-day aerobic incubation followed by the 2-day anaerobic period, losses of N as NO+\textsubscript{2}O varied from 59.4 mg N kg\textsuperscript{-1} for HV to 3.5 mg N kg\textsuperscript{-1} for the control soil (Table 3). The proportion of \textsubscript{N_{2}O} in the evolved gas also increased with increasing incubation time in
Table 3. Accumulated NO + N₂O and the proportion as N₂O from Brookston clay loam over 48 h under anaerobic conditions with and without aerobic preincubation with incorporated dry residue or water-soluble extracts (10 g residue kg⁻¹). The soil at a moisture content of 160 g H₂O kg⁻¹ was amended with 100 mg NO₃⁻N kg⁻¹.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NO + N₂O production mg N kg⁻¹</th>
<th>N₂O / (NO + N₂O) %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0d † 2d 3d 5d</td>
<td>0d 2d 3d 5d</td>
</tr>
<tr>
<td>Hairy vetch</td>
<td>18.8(0.1) 19.0(0.2) 33.1(1.1)</td>
<td>10.8(0.2) 16.4(0.5) 23.3(2.1) 27.9(1.3)</td>
</tr>
<tr>
<td>d.r. w.s.e.</td>
<td>23.8(0.1)</td>
<td>8.6(0.3)</td>
</tr>
<tr>
<td>Red clover</td>
<td>20.4(0.1) 14.5(0.5) 19.4(0.4)</td>
<td>9.9(0.2) 14.0(0.9) 12.9(0.5) 25.6(1.4)</td>
</tr>
<tr>
<td>d.r. w.s.e.</td>
<td>24.6(0.1)</td>
<td>8.3(0.3)</td>
</tr>
<tr>
<td>Annual ryegrass</td>
<td>19.4(0.2) 16.1(0.9) 19.6(1.4)</td>
<td>12.2(0.6) 14.7(2.4) 11.6(2.3) 16.9(0.6)</td>
</tr>
<tr>
<td>d.r. w.s.e.</td>
<td>21.2(0.3)</td>
<td>6.5(3.1) 6.1(4.0)</td>
</tr>
<tr>
<td>Reed canarygrass</td>
<td>18.1(0.1) 14.5(0.3) 22.0(0.5)</td>
<td>11.0(0.3) 14.9(0.5) 11.2(0.6) 14.2(1.2)</td>
</tr>
<tr>
<td>d.r. w.s.e.</td>
<td>12.3(0.1)</td>
<td>10.3(0.3) 9.6(0.1)</td>
</tr>
<tr>
<td>corn</td>
<td>18.0(0.1) 10.1(0.1) 16.3(0.5)</td>
<td>10.8(0.2) 13.3(0.1) 12.0(1.1) 11.2(1.5)</td>
</tr>
<tr>
<td>d.r. w.s.e.</td>
<td>12.6(0.1)</td>
<td>11.0(0.1) 9.5(0.2)</td>
</tr>
<tr>
<td>control</td>
<td>7.2(0.1) 4.9(0.1) 5.8(0.1) 3.5(0.1)</td>
<td>13.1(1.3) 6.8(1.0) 9.2(0.9) 9.3(3.5)</td>
</tr>
</tbody>
</table>

d.r.: Dry residue (10g residue kg⁻¹) added.
w.s.e.: Water-soluble extract from 10 g residue kg⁻¹ added.
†: Duration of aerobic preincubation.
‡: The numbers in parentheses are standard errors. n=3 except for corn where n=2.
the legume-treated soils, from 10.8 to 27.9% for HV, and 9.9 to 25.6% for RC.

Aerobic incubation appeared to have little effect on the $\text{N}_2\text{O}/(\text{NO}+\text{N}_2\text{O})$ ratio obtained from soils treated with water-soluble extract (Table 3). The proportion ranged from 6.1 to 11.0%, with the highest proportion obtained by the corn treatment. Relatively large quantities of NO+$\text{N}_2\text{O}$ were produced with HV, ARG and RC in the water-soluble extract treated soils. In all treatments using water soluble extracts, the aerobic incubation led to a decrease in the total NO+$\text{N}_2\text{O}$ produced. This was especially evident with the RC and the control treatment.

In the case of 5d aerobic/ 2d anaerobic / 2d aerobic/ 2d anaerobic conditions, rates of NO production were negligible during the first aerobic period (Fig. 4). When anaerobic conditions were introduced, rates in HV and ARG amended soils immediately increased. When aeration changed from anaerobic to aerobic conditions, these rates dropped quickly; but once anaerobic conditions were restored, the rates again increased and then decreased. Although the net NO production rate for HV was higher than that for ARG during most of the anaerobic period, the rate in the former treatment reached only half of the latter treatment at day 11. Similar results for $\text{N}_2\text{O}$ production rates were observed (Fig. 4). In the case of 5d aerobic/ 6d anaerobic conditions (Fig. 4), the rate of NO production had a steep decrease in HV treatment and more gradual decrease in ARG treatment from day 8.5. After day 9.5, the HV treatment produced a rate of only 1 mg N kg$^{-1}$, even lower than the control. Net $\text{N}_2\text{O}$ production rates were lower than NO rates but the rates followed similar patterns, Fig. 4. A comparison of results from 5d aerobic/ 2d anaerobic
Fig. 4. Net rates of soil NO and N₂O production versus time under 5 days aerobic, 6 days anaerobic conditions, and under alternating aerobic, anaerobic regimes. The soil at a moisture content of 160 g H₂O kg⁻¹ was amended with 100 mg NO₃⁻N kg⁻¹ and 10 g dry residue kg⁻¹.
Table 4. Nitrate consumption compared to production of $\text{NH}_4^+$, $\text{NO}_2^-$ and $\text{NO}+\text{N}_2\text{O}$ in soil during 7 and 11-day incubations. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 100 mg NO$_3^-$-N kg$^{-1}$ and 10 g dry residue kg$^{-1}$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Consumption of NO$_3^-$</th>
<th>Production</th>
<th>Net Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\text{NH}_4^+$</td>
<td>$\text{NO}_2^-$</td>
<td>NO+$\text{N}_2\text{O}$</td>
</tr>
<tr>
<td>Hairy vetch</td>
<td>65.6(1.3) †</td>
<td>47.4(1.4)</td>
<td>37.4(0.3)</td>
</tr>
<tr>
<td></td>
<td>118.0(9.8)</td>
<td>78.0(7.0)</td>
<td>6.7(4.4)</td>
</tr>
<tr>
<td></td>
<td>129.6(0.3)</td>
<td>85.2(0.8)</td>
<td>&lt;0.1(&lt;0.1)</td>
</tr>
<tr>
<td>Annual ryegrass</td>
<td>40.6(1.0)</td>
<td>11.0(1.6)</td>
<td>42.5(1.5)</td>
</tr>
<tr>
<td></td>
<td>86.9(16.9)</td>
<td>31.2(6.5)</td>
<td>25.2(6.5)</td>
</tr>
<tr>
<td></td>
<td>117.4(5.7)</td>
<td>22.0(1.3)</td>
<td>17.4(4.4)</td>
</tr>
<tr>
<td>control</td>
<td>2.1(1.2)</td>
<td>0.8(0.2)</td>
<td>4.0(0.1)</td>
</tr>
<tr>
<td></td>
<td>12.3(1.5)</td>
<td>1.8(0.3)</td>
<td>1.6(0.1)</td>
</tr>
<tr>
<td></td>
<td>12.9(2.2)</td>
<td>2.4(0.6)</td>
<td>4.5(0.5)</td>
</tr>
</tbody>
</table>

A: 5-day aerobic preincubation + 2-day anaerobic conditions.
B: 5-day aerobic preincubation + 2-day anaerobic + 2-day aerobic + 2-day anaerobic conditions.
C: 5-day aerobic preincubation + 6-day anaerobic conditions.
†: The numbers in parentheses are standard errors. for A n=6, for B and C n=3.
/ 2d aerobic/ 2d anaerobic and 5d aerobic/ 6d anaerobic conditions (Table 4) shows that
the intervening aerobic phase (2-day) after the first 2-day anaerobic incubation did not
affect the total NO+N₂O production in HV treatment but had some effects on ARG and
treatment respectively (Table 4).

3.1.3 Denitrification: without an initial addition of KNO₃ and without an aerobic
preincubation
Without an initial addition of KNO₃ and without an aerobic preincubation net NO
production rates for residue-amended soils (Fig. 5) were lower than those with KNO₃
addition (Fig. 2). The maximum rates for all residue treatments were approximately 8 mg
N kg⁻¹ d⁻¹. Nitrous oxide production rates were similar (Fig. 6) for all treatments. For
all residue treatments, the total NO+N₂O production was about 12 mg N kg⁻¹,
approximately 65% of that produced with an initial addition of KNO₃ (Table 5).

3.1.4 Denitrification: without an initial addition of KNO₃ and with a 5-day aerobic
preincubation
Temporal patterns of net rates of NO and N₂O production, shown in Fig. 5 and 6
respectively, were similar to those obtained with initial KNO₃ addition (Fig. 2 and 3),
although the magnitudes were different and there was a more dramatic decrease in NO
and N₂O rates after 24 h with the HV treatment. Differences in NO and N₂O production
rates among the residue treatments were also large. The maximum rates for net NO
production were 16.1, 3.6, 11.2, 9.1 and 5.8 mg N kg⁻¹ d⁻¹ for HV, RC, ARG, RCG and
corn respectively. Both rates of NO and N₂O in HV-treated soil decreased rapidly after
Fig. 5. Net rates of soil NO production versus time under 2-day anaerobic conditions without an aerobic preincubation and with a 5-day aerobic preincubation. The soil at a moisture content of 160 g H₂O kg⁻¹ was amended with 10 g dry residue kg⁻¹.
Fig. 6. Net rates of soil N$_2$O production versus time under 2-day anaerobic conditions without an aerobic preincubation and with a 5-day aerobic preincubation. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 10 g dry residue kg$^{-1}$. 
Table 5. Production of NH$_4^+$, NO$_3^-$ and NO+NO$_2$ with and without NO$_3^-$ amendment (100 mg N kg$^{-1}$) over 48 h under anaerobic conditions without an aerobic preincubation. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 10 g dry residue kg$^{-1}$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial NO$_3^-$</th>
<th>Final NO$_3^-$</th>
<th>Production</th>
<th>Total N Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>NH$_4^+$</td>
<td>NO$_3^-$</td>
</tr>
<tr>
<td>Hairy vetch</td>
<td>34.0(0.5)†</td>
<td>140.4(1.6)</td>
<td>24.0(0.2)</td>
<td>1.4(1.7)</td>
</tr>
<tr>
<td>Red clover</td>
<td>34.0(0.3)</td>
<td>139.8(1.0)</td>
<td>23.4(0.2)</td>
<td>-1.3(0.8)</td>
</tr>
<tr>
<td>Annual rye-grass</td>
<td>36.0(1.0)</td>
<td>143.7(3.1)</td>
<td>27.6(1.1)</td>
<td>0.6(0.3)</td>
</tr>
<tr>
<td>Reed canary-grass</td>
<td>36.7(1.6)</td>
<td>145.5(1.7)</td>
<td>25.3(1.3)</td>
<td>0.2(0.3)</td>
</tr>
<tr>
<td>Corn</td>
<td>32.8(0.5)</td>
<td>142.5(2.7)</td>
<td>24.3(0.4)</td>
<td>0.5(0.1)</td>
</tr>
<tr>
<td>Control</td>
<td>33.2(0.4)</td>
<td>128.9(0.9)</td>
<td>28.7(0.1)</td>
<td>0.4(0.2)</td>
</tr>
</tbody>
</table>

A: Without NO$_3^-$ amendment.
B: With NO$_3^-$ amendment.
†: The numbers in parentheses are standard errors (n=3).
24 h, and by 48 hours were only approximately 1 mg N kg$^{-1}$ d$^{-1}$ for NO and 0.2 mg N kg$^{-1}$ d$^{-1}$ for N$_2$O respectively, even lower than the control. At hour 48, rates varied from 0.5 to 4.0 mg N kg$^{-1}$ d$^{-1}$ for NO and from 0.3 to 0.5 mg N kg$^{-1}$ d$^{-1}$ for N$_2$O with the other residue treatments. These rates were all very much lower compared to the rates with an initial addition of NO$_3^-$ (Fig. 2 and 3). The amounts of NO+N$_2$O were 19.7, 4.1, 16.6, 15.1 and 6.6 mg N kg$^{-1}$ for HV, RC, ARG, RCG and corn treatments respectively. These losses were considerably lower compared to those produced with an initial addition of NO$_3^-$, particularly with the legume treatments (Table 6).
Table 6. Nitrate consumption compared to production of NH$_4^+$, NO$_2^-$ and NO+NO$_2$ over 48 h under anaerobic conditions with a 5-day aerobic preincubation. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 10 g dry residue kg$^{-1}$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Consumption of NO$_3^-$</th>
<th>Production</th>
<th>Net Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg N kg$^{-1}$</td>
<td>NH$_4^+$</td>
<td>NO$_2^-$</td>
</tr>
<tr>
<td>Hairy vetch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>18.3(0.3)$^\dagger$</td>
<td>14.3(5.2)</td>
<td>0.1(0.1)</td>
</tr>
<tr>
<td>B</td>
<td>79.5(5.8)</td>
<td>16.7(1.8)</td>
<td>20.0(1.1)</td>
</tr>
<tr>
<td>Red clover</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4.7(1.0)</td>
<td>0.7(0.8)</td>
<td>0(0)</td>
</tr>
<tr>
<td>B</td>
<td>67.4(8.0)</td>
<td>10.3(0.3)</td>
<td>16.4(1.7)</td>
</tr>
<tr>
<td>Annual ryegrass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>17.5(2.4)</td>
<td>0.9(0.2)</td>
<td>2.5(2.0)</td>
</tr>
<tr>
<td>B</td>
<td>21.2(1.1)</td>
<td>9.7(0.6)</td>
<td>55.6(0.8)</td>
</tr>
<tr>
<td>Reed canarygrass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>17.1(1.1)</td>
<td>1.3(0.8)</td>
<td>1.8(1.4)</td>
</tr>
<tr>
<td>B</td>
<td>46.0(7.6)</td>
<td>1.3(1.2)</td>
<td>20.6(0.1)</td>
</tr>
<tr>
<td>corn</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>10.4(2.1)</td>
<td>0.3(0.1)</td>
<td>0.2(0.2)</td>
</tr>
<tr>
<td>B</td>
<td>31.5(7.9)</td>
<td>-2.5(5.7)</td>
<td>15.6(0.1)</td>
</tr>
<tr>
<td>control</td>
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<td></td>
</tr>
<tr>
<td>A</td>
<td>2.8(0.4)</td>
<td>0(0.3)</td>
<td>1.5(0.2)</td>
</tr>
<tr>
<td>B</td>
<td>3.2(1.0)</td>
<td>-0.7(0.5)</td>
<td>2.2(0.2)</td>
</tr>
</tbody>
</table>

$^A$: Without NO$_3^-$ amendment (100 mg N kg$^{-1}$).

$^B$: With NO$_3^-$ amendment (100 mg N kg$^{-1}$).

$^\dagger$: The numbers in parentheses are standard errors (n=3).
3.2 Mineralization

3.2.1 Mineralization: with an initial addition of KNO₃

Without a prior aerobic incubation, the net decrease in NO₃⁻ was similar in all dry residue treatments, about 25 mg N kg⁻¹ (Fig. 7). Some loss of NO₃⁻ with dry residue treatments occurred during the aerobic incubation (Figs. 8 to 10). In each case, the decrease of NO₃⁻ in RC-amended soil was greatest as compared to the other residue treatments. The total loss of NO₃⁻ reached 26.6, 31.1 and 42.7 mg N kg⁻¹ during the 2d, 3d and 5d aerobic incubations, respectively. Over the 5-day aerobic incubation, however, the loss of NO₃⁻ in the HV amended soil was only 17.0 mg N kg⁻¹. The corresponding values were 24.3, 27.1 and 29.8 mg N kg⁻¹ for ARG, RCG and corn respectively. With the 5-day aerobic preincubation, for dry residue treatments, the net decrease in NO₃⁻ concentration from day 5 to day 7 (Fig. 10) during the anaerobic phase was about 3 times greater for the legumes and 2 times greater for RCG compared to the rates observed without the aerobic pre-incubation (Fig. 7). This decrease in NO₃⁻ concentration for HV and RC corresponded to the increase in denitrification (Table 3). For the ARG and corn treatments, the decrease in NO₃⁻ concentration were comparable with/without aerobic preincubation. During the 6-day anaerobic incubation period, the NO₃⁻ concentration decreased rapidly in both HV and ARG treatments (Fig. 11) with the highest rate in HV, corresponding to the increase of NO and N₂O (Fig. 4). The residual NO₃⁻ was consumed by day 9.5 in HV-amended soil. In the case of 5d aerobic / 2d anaerobic / 2d aerobic /
Fig. 7. Nitrate, $\text{NO}_2^-$ and $\text{NH}_4^+$ (mg N kg$^{-1}$ oven-dry soil) versus time under 2-day anaerobic conditions without an aerobic preincubation. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 100 mg NO$_3^-$-N kg$^{-1}$ and 10 g dry residue kg$^{-1}$. 

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Fig. 8. Nitrate, NO\textsubscript{3}\textsuperscript{-}, and NH\textsubscript{4}\textsuperscript{+} (mg N kg\textsuperscript{-1} oven-dry soil) versus time under 2-day anaerobic conditions following a 2-day aerobic preincubation. The soil at a moisture content of 160 g H\textsubscript{2}O kg\textsuperscript{-1} was amended with 100 mg NO\textsubscript{3}\textsuperscript{-}-N kg\textsuperscript{-1} and 10 g dry residue kg\textsuperscript{-1}.
Fig. 9. Nitrate, NO$_3^-$ and NH$_4^+$ (mg N kg$^{-1}$ oven-dry soil) versus time under 2-day anaerobic conditions following a 3-day aerobic preincubation. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 100 mg NO$_3^-$-N kg$^{-1}$ and 10 g dry residue kg$^{-1}$. 
Fig. 10. Nitrate, NO$_3^-$ and NH$_4^+$ (mg N kg$^{-1}$ oven-dry soil) versus time under 2-day anaerobic conditions following a 5-day aerobic preincubation. The soil at a moisture content of 100 g H$_2$O kg$^{-1}$ was amended with 100 mg NO$_3^-$-N kg$^{-1}$ and 10 g dry residue kg$^{-1}$. 
Fig. 11. Nitrate, $\text{NO}_3^-$ and $\text{NH}_4^+$ (mg N kg$^{-1}$ oven-dry soil) versus time under 6-day anaerobic conditions following a 5-day aerobic preincubation. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 100 mg NO$_3^-$-N kg$^{-1}$ and 10 g dry residue kg$^{-1}$.
Fig. 12. Nitrate, NO$_3^-$ and NH$_4^+$ (mg N kg$^{-1}$ oven-dry soil) versus time under alternating aerobic, anaerobic regimes. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 100 mg NO$_3^-$-N kg$^{-1}$ and 10 g dry residue kg$^{-1}$. 
2d anaerobic incubation (Fig. 12), the concentrations of NO\textsubscript{3}\textsuperscript{-} in the HV and ARG treatments decreased rapidly during the anaerobic phase and considerably slower during the aerobic phase, consistent with the change in rates of NO and N\textsubscript{2}O production (Fig. 4). Under the anaerobic-only condition, the NH\textsubscript{4}\textsuperscript{+} produced in dry residue and NO\textsubscript{3}\textsuperscript{-}-amended soils over the 2-day period was 5.0, 4.5, 11.2, 5.2 and 9.2 mg N kg\textsuperscript{-1} for HV, RC, ARG, RCG and corn respectively (Table 5). In the cases with an aerobic preincubation, the concentration of NH\textsubscript{4}\textsuperscript{+} decreased during the first 2 days of the aerobic period (Figs. 8, 9 and 10). With the 5d aerobic / 2d anaerobic incubation (Fig. 10), there was an increase in NH\textsubscript{4}\textsuperscript{+} in the HV treatment from day 3 resulting in a net gain of 31 mg N kg\textsuperscript{-1} over the 5-day aerobic period. Only a slight increase was observed for the other residues. Ammonium levels increased continuously in HV-amended soils under anaerobic conditions, with more being produced following the 3 and 5 day aerobic incubation, resulting in a net accumulation of 26.2 and 16.7 mg N kg\textsuperscript{-1} over the 2-day anaerobic incubation and reaching concentrations of 32.3 and 58.4 mg N kg\textsuperscript{-1} at day 5 and day 7, respectively (Fig. 9 and 10). In the case of 5d aerobic / 6d anaerobic incubation, the ammonium concentration showed a steady increase from day 3 for HV and from day 5 for ARG with 90.8 mg NH\textsubscript{4}\textsuperscript{+}-N kg\textsuperscript{-1} obtained with the HV treatment and 24.6 mg NH\textsubscript{4}\textsuperscript{+}-N kg\textsuperscript{-1} with the ARG treatment by day 11 (Fig. 11).

In all cases, NO\textsubscript{2}\textsuperscript{-} concentrations increased under anaerobic conditions (Figs. 7 to 10). Following the 2-day aerobic treatment (Fig. 8), NO\textsubscript{2}\textsuperscript{-} concentrations of 49 for HV, 46 for ARG, 38 for both RC and RCG and 30 mg N kg\textsuperscript{-1} for corn were obtained after 2
days anaerobic incubation. Following the 3-day aerobic incubation, concentrations were lower after the 2d anaerobic period (Fig. 9). After the 5-day aerobic incubation, the NO₃⁻ concentration was 55.6 mg N kg⁻¹ for the ARG treatment, and considerably lower in all other treatments compared to concentrations found following the 2-day incubation, although still well above the level in the control soil (Fig. 10). In the 5d aerobic / 6d anaerobic incubation, nitrite increased through maxima of 48.9 mg N kg⁻¹ for ARG and 39.7 mg N kg⁻¹ for HV (Fig. 11), in inverse correspondence to the f_NO and f_N₂O maxima (Fig. 4).

Consideration of total mineral N content NO₃⁻+NO₂⁻+NH₄⁺ after subtracting values for the control soil over the 5-day aerobic plus 2-day anaerobic trials provides a daily summary of the effect of the dry residue treatments (Fig. 13). By day 7 the net decrease of N in the RC treated soil was about 69% of the total NO₃⁻ initially present. With the RCG and corn treatments 37% and 32% respectively of initial NO₃⁻ was lost. The HV treated soil showed a net gain by day 5 as a result of mineralization. However, rapid denitrification from day 5 to day 7 depleted the nitrogen resulting in a net overall loss over the 7 days of 29.6 mg N kg⁻¹, approximately 24.0% of NO₃⁻ initially present. The ARG treated soil however showed a net increase of 13.4 mg N kg⁻¹, about 10% of the initial NO₃⁻.

3.2.2 Mineralization: without an initial addition of KNO₃

Under the anaerobic-only condition, the losses of NO₃⁻ were 10.0, 10.6, 8.4, 11.4 and 8.5 mg N kg⁻¹ for HV, RC, ARG, RCG and corn respectively (Table 5). In the case
Fig. 13. Ammonium+NO$_3^-$+NO$_2^-$ content (mg N kg$^{-1}$ oven-dry soil) versus time under 2-day anaerobic conditions following a 5-day aerobic preincubation. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 100 mg NO$_3^-$-N kg$^{-1}$ and 10 g dry residue kg$^{-1}$.
Fig. 14. Nitrate, NO₃⁻ and NH₄⁺ (mg N kg⁻¹ oven-dry soil) versus time under 2-day anaerobic conditions following a 5-day aerobic preincubation. The soil at a moisture content of 160 g H₂O kg⁻¹ was amended with 10 g dry residue kg⁻¹.
of the 5-day aerobic / 2d anaerobic incubation, concentrations of \( \text{NO}_3^- \) decreased rapidly after the first day of the aerobic incubation (Fig. 14). Over the total 5-day aerobic period, the \( \text{NO}_3^- \) losses amounted to 14.8, 28.2, 16.2, 13.6 and 20.8 mg N kg\(^{-1}\) for HV, RC, ARG, RCG and corn, respectively. Although RC had a low C/N ratio (Table 1), it produced the greatest loss of \( \text{NO}_3^- \), even more than that produced by corn which had the highest C/N ratio. In the following 2-day anaerobic period, the \( \text{NO}_3^- \) was rapidly depleted in all residue treatments resulting in the \( \text{NO}_3^- \) being used up or almost exhausted in HV, RC and corn treatments (Fig. 14).

Negligible changes of \( \text{NH}_4^+ \) concentration were observed in all treatments under the anaerobic-only incubation without \( \text{NO}_3^- \) amendment (Table 5). Under the 5-day aerobic incubation, concentrations of \( \text{NH}_4^+ \) decreased in all residue treatments during the first 3 days (Fig. 14). Over this period the total nitrogen losses (\( \text{NO}_3^- \) and \( \text{NH}_4^+ \)) for the HV treatment was 18.1 mg N kg\(^{-1}\), comparable to that lost (17.6 mg N kg\(^{-1}\)) during the 2 first days with an initial \( \text{NO}_3^- \) addition (Fig. 10). After day 3, \( \text{NH}_4^+ \) continued to decrease in RC, ARG, RCG and corn treatments but increased in the HV-amended soil. This increase of \( \text{NH}_4^+ \) continued during the following 2-day anaerobic incubation, obtaining another net gain of 14.3 mg N kg\(^{-1}\) (Table 6). Compared to the 1.4 mg N kg\(^{-1}\) observed in anaerobic-only incubation (Table 5), 12.9 mg N kg\(^{-1}\) more \( \text{NH}_4^+ \) was formed in the 5-day aerobic preincubation for HV, whereas, there was no significant change in \( \text{NH}_4^+ \) production for RC, ARG, RCG and corn treatments. The influence of initial \( \text{NO}_3^- \) amendment on \( \text{NH}_4^+ \) production was shown in Table 6.
As shown in Table 5, NO$_2^-$ production over the 2-day anaerobic incubation ranged from 13.6 to 16.0 for all residues resulting in at least a 2-fold increase as compared to the control treatment. It was notable that accumulation of NO$_2^-$ decreased with initial NO$_3^-$ addition. Under the 2-day anaerobic incubation with a 5-day aerobic preincubation (Fig. 14), there was no accumulation of NO$_2^-$ in the RC-treated soil, probably because of the very low concentration of NO$_3^-$ (4.7 mg N kg$^{-1}$) at the beginning of anaerobic incubation. For the other residue treatments, the NO$_2^-$ increased in the first day and then decreased rapidly, presumably due to the limited NO$_3^-$ substrate. The effect of initial KNO$_3$ addition on NO$_2^-$ accumulation was apparent (Table 6). An analysis of total mineral nitrogen, NO$_3^-$ $+$ NO$_2^-$ $+$ NH$_4^+$, is represented in Fig. 15. The sum of NO$_2^-$ $+$ NO $+$ N$_2$O was comparable to the consumption of NO$_3^-$ in all treatments, and there was a net gain of NH$_4^+$ only in HV treatment (14.3 mg N kg$^{-1}$)(Table 6).
Fig. 15. Ammonium+NO$_3^-$+NO$_2^-$ content (mg N kg$^{-1}$ oven-dry soil) versus time under 2-day anaerobic conditions following a 5-day aerobic preincubation. The soil at a moisture content of 160 g H$_2$O kg$^{-1}$ was amended with 10 g dry residue kg$^{-1}$. 
CHAPTER 4

Discussion

4.1 Denitrification

Denitrification losses were increased when legume, grass and corn residues were added to soil if sufficient NO$_3^-$ was present. With initial NO$_3^-$ amendment, the greater the amount of dry residue added, the more NO+$N_2O$ was produced, and the greater the soil moisture, the greater were the nitrogen losses confirming the results of Drury et al., (1992). Compared to the effect of added dry residue, the influence of moisture was more important (Table 2). Although denitrification was stimulated by the addition of dry residue, there were no significant differences in rates, total amount of NO+$N_2O$ produced, or in the proportion, $N_2O/(NO+N_2O)$, among the various residue treatments when there was no prior aerobic incubation. Presumably this was due to comparable C availability to denitrifying organisms in these soils, since there was almost identical availability of nitrate levels when the anaerobic incubation was started (Figs. 2, 3, 5 and 6; Table 2 and 5). With initial addition of NO$_3^-$, the legumes and grasses significantly increased denitrification under anaerobic conditions when preceded by a 5-day aerobic incubation (Table 3). During the subsequent 2-day anaerobic incubation, the HV-treated soil resulted in a 3.2-fold increase in NO+$N_2O$ produced as compared to the anaerobic-only condition, whereas there was a 2.3-fold increase for the RC treatment and a 1.3-fold increase for
both the ARG and RCG treatments. Similar increases in the proportion of $N_2O / (NO+N_2O)$ occurred. However, no significant change was observed for the corn treatment. Comparisons of the effects of dry residue addition with those obtained by addition of water soluble extracts of the residues (Table 3), suggest that introduction of the dry residue to the soil promoted denitrification mainly by supplying water-soluble organic carbon under the anaerobic-only condition, and by supplying not only soluble but also easily decomposable components under anaerobic conditions with sufficient time of aerobic preincubation.

Douglas et al. (1992) suggested that residue decomposition occurs in two phases. Phase 1 is relatively rapid occurring over 134 days and is dependent on initial residue N content. Phase 2 decomposition, which is slower than phase 1, is regulated by lignin decomposition and shows little difference in residue decomposition rate regardless of initial N content because soluble, easily decomposable components have already been utilized by microorganism or lost by leaching. The decomposition of all dry residues in the 5-day aerobic incubation presumably occurred during phase 1. Aulakh et al. (1991) found that initial rates of residue decomposition were inversely related to residue C/N ratio. Because of their relatively high initial residue N content, the decomposition of HV and RC could occur earlier and comparatively more rapidly than the other species probably resulting in the accumulation of available C and thereby enhancing the denitrification losses as NO and $N_2O$ when the $NO_3^-$ was sufficiently available. This would account for the highest denitrification losses being produced by the legumes.
Without initial addition of NO$_3^-$, HV resulted in the greatest production of NO+N$_2$O, while the lowest losses were found in the RC treatment although both HV and RC are legumes. These results suggest that the effect of initial residue N content on denitrification should be considered together with the influence of availability of NO$_3^-$ under anaerobic conditions. When sufficient initial NO$_3^-$ was supplied, denitrification production of NO+N$_2$O mainly depended on residue type, probably due to the initial residue N content. However, when nitrate concentration was very low, the most important regulator for denitrification was the availability of NO$_3^-$ in the soil. This would explain why NO+N$_2$O production in residue-amended soils increased in the order HV > RC > ARG > RCG > corn when NO$_3^-$ was added, but in the order HV > ARG > RCG > corn > RC when NO$_3^-$ was not added (Table 6). It is noteworthy that NO+N$_2$O production in RC and corn treatments were almost the same as that in the control treatment. This fact indicates that it is possible to eliminate the stimulation of denitrification by residue.

Tables 5 and 6 show that under the same soil conditions (moisture, quantity of dry residue added, preincubation and anaerobic incubation time), the initial addition of KNO$_3$ stimulated denitrification whether without or with aerobic preincubation. In the former case, the NO$_3^-$ amendment increased denitrification losses of NO+N$_2$O by an average of 55%. The influence of NO$_3^-$ amendment on HV and RC was much greater in the latter case (Table 6). The total production of NO+N$_2$O increased 10.5-fold and 2-fold for RC and HV with the initial addition of NO$_3^-$, respectively. Therefore, in order to reduce
denitrification losses, the NO$_3^-$ concentration after longer aerobic preincubations should be maintained at relatively low levels, especially with legume-amended soils, since legumes have higher initial residue N contents. The HV treatment resulted in 1.8-fold higher denitrification NO+N$_2$O production, compared with the ARG treatment over the 5d aerobic/2d anaerobic period. However, as anaerobic incubation proceeded to 6 days, the total denitrification losses as NO+N$_2$O over the 5d aerobic / 6d anaerobic incubation for the HV and ARG treatments did not differ significantly (Table 4).

4.2 Mineralization

During the 5-day aerobic incubation, in the first 2-3 days, both NO$_3^-$ and NH$_4^+$ concentrations decreased without an increase in NO$_2^-$ or significant production of NO+N$_2$O in all dry residue treatments, presumably due to the microorganism’s requirement of nitrogen for growth. From day 2 to day 5 and from day 3 to day 5 in the cases with and without KNO$_3$ addition respectively, NH$_4^+$ concentration in HV-treated soil increased without a change in NO$_3^-$, NO$_2^-$ or NO+N$_2$O production, indicating that mineralization exceeded immobilization (Fig. 10 and 14). Over the whole 5-day aerobic period, when KNO$_3$ was added initially, the NO$_3^-$ loss was 17 mg N kg$^{-1}$ and the net gain of NH$_4^+$ was 31 mg N kg$^{-1}$. Without KNO$_3$ addition, the NO$_3^-$ loss was 14.8 mg N kg$^{-1}$ and there was no gain of NH$_4^+$. Since the NO$_3^-$ losses were comparable in these two cases, the great difference in net gain of NH$_4^+$ implied that the rate of mineralization for HV may be related to the initial concentration of NO$_3^-$ at the start of aerobic incubation. With RC, ARG, RCG and corn treatments, gaseous NO+N$_2$O production could not account
for the decrease in mineral N (Fig. 13 and 15) during the 5-day aerobic incubation. Therefore, net immobilization occurred. This observation agrees with the point made by Aviva Hadas et al., (1992), that immobilization was primarily due to the microbial biomass drawing N from the inorganic pool, rather than utilizing N directly from the decomposing soil organic matter. Among these treatments, most immobilization was observed in RC-amended soil whether NO$_3^-$ was added or not. A total of 34% and 85% of initial NO$_3^-$ in the soil, corresponding to the initial NO$_3^-$ concentrations of 125.8 and 32.9 mg N kg$^{-1}$ was apparently immobilized (Fig. 10 and 14), respectively. In the latter case, since the NO$_3^-$ concentration dropped to 4.7 mg N kg$^{-1}$ at the end of 5-day aerobic incubation, only a total of 4.1 mg N kg$^{-1}$ was lost as NO$\text{+N}_2\text{O}$ in the following 2-day anaerobic incubation. These results suggest that RC may help reduce nitrate losses by immobilization during aerobic conditions and thus conserve N within the agricultural ecosystem. In addition RC may be the best choice for reducing denitrification losses in the aforementioned conditions when fertilizer nitrogen was not added to the soil or when residual NO$_3^-$ level is low. Norman et al., (1990) suggested that the higher the C/N ratio and the lower the amount of N in the crop residue, the greater was the amount of residue N recovered in the soil. Aulakh et al. (1991) also pointed that incorporation or surface placement of residues with high C/N ratios could cause significant immobilization of mineral N for several weeks. Although the results obtained with the corn treatment in this work supported their suggestion, the data obtained with the RC treatment did not as immobilization also occurred.
Under the anaerobic-only condition, in contrast to NO+\(\text{N}_2\text{O}\) and \(\text{NH}_4^+\) production, \(\text{NO}_2^-\) production decreased with the addition of \(\text{NO}_3^-\) in all dry residue treatments (Table 5). Comparison of \(\text{NO}+\text{N}_2\text{O}, \text{NH}_4^+\) and \(\text{NO}_2^-\) production between with and without \(\text{NO}_3^-\) amendment, revealed that for each treatment the lower the initial \(\text{NO}_3^-\) concentration, the lower was both the \(\text{NO}+\text{N}_2\text{O}\) production and the \(\text{NH}_4^+\) production and the higher was the accumulation of \(\text{NO}_2^-\); the higher the initial \(\text{NO}_3^-\) concentration, the higher was both the \(\text{NO}+\text{N}_2\text{O}\) and \(\text{NH}_4^+\) production and the lower was the accumulation of \(\text{NO}_2^-\). These results suggest that the addition of \(\text{NO}_3^-\) not only increased denitrification losses of \(\text{NO}+\text{N}_2\text{O}\), but also stimulated dissimilatory nitrate reduction thereby decreasing soil \(\text{NO}_2^-\) concentration. The latter observation is similar to that reported by Griffiths and Cole, (1987), whereby addition of \(\text{NO}_3^-\) stimulated rather than repressed \(\text{NO}_2^-\) reduction by \(E.\text{coli}\).

Under anaerobic conditions with a 5-day aerobic preincubation, similar to \(\text{NO}+\text{N}_2\text{O}\) production, \(\text{NO}_2^-\) accumulation was significantly stimulated by the addition of \(\text{NO}_3^-\) in all dry residue treatments (Table 6). Although the initial addition of \(\text{NO}_3^-\) did not affect the \(\text{NH}_4^+\) production in soils amended with RCG and corn, which had low initial residue N contents, an increase in \(\text{NH}_4^+\) was observed in the HV, RC and ARG treatments. Similar to \(\text{NO}+\text{N}_2\text{O}\) production, \(\text{NH}_4^+\) production among residue treatments ranged in the order \(\text{HV} > \text{RC} > \text{ARG} > \text{RCG} > \text{corn}\), reflecting the relative initial N content of the respective residues. These results suggest that \(\text{NO}_2^-\) loss appeared to be mainly through denitrification and the effect of dry residue on mineralization was evident in legumes and
ARG treatments. Since anoxic conditions repressed nitrification, denitrification losses of NO+\(N_2O\) should be mainly attributed to the consumption of available NO\(_3^-\). Without NO\(_3^-\) amendment, the loss of NO\(_3^-\) in the HV-treated soil was almost the same as the NO+\(N_2O\) production over the 2-day anaerobic incubation with a 5-day aerobic preincubation. Meanwhile, NH\(_4^+\) accumulated to 14.3 mg N kg\(^{-1}\) (Table 6). Since most of the NO+\(N_2O\) was produced by NO\(_3^-\) depletion, the NH\(_4^+\) production was mainly caused by mineralization of HV. Furthermore, comparison of NH\(_4^+\) production between with and without a 5-day aerobic preincubation (Table 5 and 6) shows that the difference in NH\(_4^+\) concentration caused by 5-day aerobic preincubation was 12.9 mg N kg\(^{-1}\). Since the total NH\(_4^+\) production in the latter case was just 14.3 mg N kg\(^{-1}\), most NH\(_4^+\) production should be attributed to mineralization of HV. In HV treatments, Figs 10, 11, 12 and 14, mineralization occurred during the aerobic preincubation period and appeared to continue at more or less the same rate during the anaerobic periods. Hence, once mineralization of HV was stimulated by the aerobic preincubation, the rate of mineralization was not significantly affected by alternating aerobic and anaerobic conditions. Similar results were observed in ARG treatments (Fig. 12).

Table 4 shows that over 11-day incubations, HV resulted in a considerable production of NO+\(N_2O\) and NH\(_4^+\) production. In the ARG treatments, however, the net gains of NH\(_4^+\) were much less than the losses of NO+\(N_2O\). The intervening aerobic phase (2-day) after the first 2-day anaerobic incubation reduced denitrification losses and increase NH\(_4^+\) production in the ARG treatment.
CHAPTER 5

Conclusions

Although laboratory studies of the type described here cannot fully simulate actual field situations, the results obtained in this work offer several conclusions which seem to have useful agronomic implications. The data show that the legume residues provide definite advantages over the grass and corn residues in terms of N-conservation, depending on the aeration status of the soil. For example, HV provides significant NH$_4^+$-N production whether fertilizer N is supplied or not during aerobic conditions or during anaerobic conditions preceded by a several-day aerobic preincubation. While HV is a valuable cover crop in terms of providing N for a following main crop, it may be also useful from an environmental stand point when grown in low residual N situations. Since NO$_3^-$ is rapidly immobilized under aerobic conditions in the RC treatment, RC may be the preferred choice as a cover crop whether initial NO$_3^-$ levels are high or low but particularly when soil NO$_3^-$ concentrations are relatively low so that denitrification losses during anaerobic episodes would be minimized.

With ARG residue, which has a substantial N content (24.7 g kg$^{-1}$), less mineralization occurs that with HV. With this residue the possibility of substantial losses by denitrification exists, particularly if frequent anaerobic periods occur. Immobilization with the RCG and corn occurred. The RCG and corn treatment are not likely to be of
much benefit except for the environmental advantages such as reducing soil erosion and NO$_3^-$ losses via runoff and by leaching.

The different effects observed with these various residues can not be explained simply on the basis of variation in C/N ratio since some residues have similar C/N ratio, e.g., 11.3 for HV, 15.1 for RC, and 15.6 for ARG. The quality of crop residue therefore appears to be the most important factor regulating these microbial processes under otherwise similar conditions.
REFERENCES


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