Nitrogen turnover and N₂:N₂O partitioning from agricultural soils – a simplified incubation assay

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Abstract

Nitrogen turnover and related denitrification losses are a major uncertainty when estimating N loss and replacement from agro-ecosystems, due to methodological constraints quantifying N₂ and laborious analytical procedures. We present a novel, simplified incubation assay that combines the ¹⁵N gas flux method with the ¹⁵N pool dilution method, to quantify denitrification losses as a function of N turnover. This assay was tested using a pasture soil from sub-tropical Australia. N-fertiliser (35 µg g⁻¹ soil) was applied either as a single (NH₄¹⁵NO₃) or double (¹⁵NH₄¹⁵NO₃) labelled treatment at 10 atom %, with a third treatment (NH₄¹⁵NO₃) at 60 atom % to quantify N₂ emissions. Gross rates of N mineralisation, nitrification and related N₂ and N₂O emissions were measured during 48 hours of incubation at 80% WFPS. Gross N production and gross N consumption was consistent with the directly measured N pool sizes, with denitrification losses (N₂+N₂O) at 7.0 ± 1.4 µg N g⁻¹ soil accounting for 62% of the calculated NO₃⁻ consumption. N turnover was dominated by mineralisation and nitrification, increasing the NO₃⁻ pool by a factor of 3. High NO₃⁻ concentrations shifted the N₂:N₂O ratio towards N₂, with 60% of denitrification losses emitted as N₂O. More than 25% of the applied ¹⁵N fertiliser was lost via denitrification, showing the significance of denitrification as a major pathway of N loss from agro-ecosystems. The simplified incubation assay proved to be an efficient tool to quantify N pools and emissions, and as such is an effective method to establish comprehensive datasets of denitrification losses linked to N turnover from agro-ecosystems.

Key Words Denitrification; Dinitrogen; Nitrous oxide; ¹⁵N pool dilution; Mineralisation; Nitrification

Introduction

Denitrification losses in the form of dinitrogen (N₂) and nitrous oxide (N₂O) from agricultural soils are a major uncertainty when estimating nitrogen (N) loss and replacement, reflecting the difficulties of measuring N₂ against a high atmospheric background and laborious analytical procedures involved. Denitrification losses are tightly coupled to N-turnover in soil, where plant uptake, microbial N immobilisation, dissimilatory nitrate reduction (DNRA) and denitrification compete for the mineral N supplied into the NO₃⁻ pool. Net rates of N turnover show only the change of the mineral N pools over time, e.g. the increase or decrease of the ammonia (NH₄⁺) and nitrate (NO₃⁻) pool. In contrast, gross N production and consumption rates quantify the actual influx of N into and efflux from the respective mineral N pool, revealing the relation between production and consumption of NH₄⁺ and NO₃⁻. This is significant for both magnitude and N₂:N₂O partitioning of denitrification losses: N turnover controls the amount of NO₃⁻ available for denitrification; and high NO₃⁻ levels shift the N₂:N₂O ratio towards N₂O (Giles et al., 2012).

Denitrification is a major pathway of N-loss from upland soils, however the magnitude of N₂ and N₂O emissions and related gross rates of N turnover are still unknown for a wide range of agroecosystems (Butterbach-Bahl et al., 2013). The ¹⁵N gas flux method enables the direct quantification of N₂ and N₂O emissions after ¹⁵N fertiliser addition (Mulvaney, 1984). The ¹⁵N pool dilution method (Kirkham and Bartholomew, 1954) is based on the isotopic dilution of the ¹⁵N labelled N pool and quantifies gross rates of N turnover.

Combining both methods allows to address major uncertainties of the N-cycle: gross rates of mineralisation and nitrification, the fraction of N₂O derived from nitrification as a function of gross nitrification and the magnitude of denitrification including N₂:N₂O partitioning. The objective of this study is to develop and test an efficient method to obtain comprehensive datasets on N cycling, combining the ¹⁵N gas flux method with the ¹⁵N pool dilution method in a simplified soil incubation assay.

Material and Methods

Incubation

Bulk soil samples (0-10cm) were collected from an intensively managed pasture in subtropical Casino, New South Wales, Australia. The soil is a heavy clay Vertosol with a pH of 6.6. Soil samples were air dried, sieved to < 4 mm and stored in a cold room. Prior to incubation, soil water content was determined gravimetrically after drying the soil at 105°C for 24 hours. Soil microcosms were established in centrifuge
Gaseous N emissions and net and gross rates of N turnover are shown in Figure 1.

Results

Ambient air samples (n=4) were taken daily before closing the centrifuge tubes, to quantify ambient N₂O concentrations. Specific background samples were taken above the respective soil microcosms treated with NH₄NO₃ at 60 atom % (c) for ¹⁵N₂ analysis before closing the tubes. The entire headspace atmosphere was sampled 24 and 48 h after closure using a gas-tight syringe. After gas sampling, the Suba-seals were removed for 10 minutes, allowing the headspace atmosphere to equilibrate. Gas samples were transferred into pre-evacuated 12 ml Exetainer tubes with a double wadded Teflon/silicon septa cap (Labco Ltd, Buckinghamshire, UK) and stored until N₂O analysis by gas chromatography (Shimadzu GC-2014). Gas samples were analysed for ¹⁵N₂O and ¹⁵N₂ using an automated isotope ratio mass spectrometer (IRMS) (Sercon Limited, 20-20, UK).

Fluxes of N₂ and N₂O
The flux rates of N₂O were calculated from the slope of the linear increase in gas concentration during the closure period and were corrected for temperature and air pressure. Assuming that N₂ and N₂O originate from the same source pool undergoing denitrification, the ¹⁵N enrichment of the NO₃⁻ pool is calculated based on ¹⁵N-N₂O (Stevens and Laughlin, 2001). ¹⁵N-N₂O gas analysis enables the calculation of the fraction of N₂O (d/D) derived from denitrification, and the ¹⁵N enrichment of the NO₃⁻ pool undergoing denitrification (αD). Fluxes of N₂ were calculated using αD and the increase of ¹⁵N-N₂ in the chamber headspace following denitrification (Mulvaney, 1984). Flux calculations are described in detail in Friedl et al. (2016).

¹⁵N pool dilution and DNRA
Gross N mineralisation and gross NH₄⁺ consumption were calculated based on the isotopic dilution of the ¹⁵NH₄⁺ pool in treatment (b), with the ¹⁵N enrichment of treatment (b) corrected for ¹⁵N recovered in the NH₄⁺ pool of treatment (a). Gross nitrification and gross NO₃⁻ consumption were calculated based on the isotopic dilution of the ¹⁵NO₃⁻ pool in treatment (a), assuming nitrified NH₄⁺ at mean ¹⁵NH₄⁺ pool excess abundance (Barraclough, 1991). DNRA was calculated as described in Huygens et al. (2008).

Results

N₂ and N₂O emissions
Gaseous N emissions and net and gross rates of N turnover are shown in Figure 1. Over the 2-day incubation, 7.01 ± 1.39 µg N g⁻¹ were emitted via denitrification (N₂+ N₂O). The main product of denitrification was N₂O, with cumulative fluxes of 4.3 ± 0.9 µg N-N₂O g⁻¹, exceeding cumulative N₂ fluxes of 2.8 ± 1.1 µg N-N₂ g⁻¹ by a factor of 1.5. Nitrification contributed 1.3 ± 0.1 µg N-N₂O g⁻¹ to overall N₂O fluxes. The method detection limit (DL) for N₂ ranged from 0.014 µg g⁻¹ day⁻¹ with αD assumed at 50 atom % to 0.127 µg g⁻¹ day⁻¹ with αD assumed at 20 atom %.

N-turnover
The NH₄⁺ pool remained constant over the 2 day incubation, while the NO₃⁻ concentrations increased by a factor of 3 to 65.5 ± 1.5 µg N-NO₃⁻ g⁻¹, reflected in the respective net rates. Gross rates of mineralisation and
nitrification averaged at 55.6 ± 11.5 and 54.5 ± 6.5 µg N·g⁻¹ over two days respectively, and showed a high variation between soil microcosms. N₂ and N₂O emissions accounted for 67% of the calculated gross NO₃⁻ consumption.

**Recovery**

The fertiliser recovery for the single labelled treatment (NH₄¹⁵NO₃) is shown in Table 1. Recoveries were calculated based on treatment (a) and (c). Immediately after fertiliser addition, 88% percent of the applied fertiliser was recovered in the NO₃⁻ pool, with less than 1% recovered in the soil after extraction. At the end of the incubation, more than 50% of the fertiliser remained in the NO₃⁻ pool, with only minor amounts found in the NH₄⁺ pool. N₂ and N₂O emissions accounted for more than 25% of N fertiliser applied.

<table>
<thead>
<tr>
<th>N -fertiliser recovery in %</th>
<th>NH₄¹⁵NO₃ after fertiliser addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺ pool</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>NO₃⁻ pool</td>
<td>88.64 ± 0.96</td>
</tr>
<tr>
<td>Immobilised</td>
<td>0.90 ± 0.06</td>
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<tr>
<td>Sum</td>
<td>89.54 ± 1.00</td>
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<table>
<thead>
<tr>
<th>N -fertiliser recovery in %</th>
<th>NO₃⁻ pool at the end of the incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄⁺ pool</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>NO₃⁻ pool</td>
<td>51.15 ± 4.53</td>
</tr>
<tr>
<td>Immobilised</td>
<td>6.35 ± 0.31</td>
</tr>
<tr>
<td>N₂O emission</td>
<td>16.47 ± 1.19</td>
</tr>
<tr>
<td>N₂ emissions</td>
<td>10.34 ± 4.10</td>
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<tr>
<td>Sum</td>
<td>84.5 ± 6.2</td>
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**Discussion**

The simplified soil incubation assay directly quantified denitrification losses and N₂ and N₂O partitioning as a function of gross rates of N turnover. This novel method minimizes the workload in terms of the experimental setup, sampling and soil extraction, requiring only inexpensive equipment and small amounts of ¹⁵N labelled fertiliser. As such, it allows for large numbers of samples to be processed, recommending its use to establish comprehensive datasets of denitrification losses linked to N turnover.

At 80% WFPS, N turnover was dominated by mineralisation and nitrification (Figure 1), with gross rates of >45 µg g⁻¹ over 2 days for both processes. The gross mineralisation rate reveals the high N supply from the organic N pool in subtropical pasture soils, and the difference between gross nitrification and gross NH₄⁺ consumption indicates a potential contribution of heterotrophic nitrification to NO₃⁻ production. The relationship between these specific processes is not reflected in the respective net rates, emphasising the importance of gross rates of mineralisation and nitrification to accurately capture N cycling in the soil. Gross nitrification exceeded NO₃⁻ consumption by a factor of 5, which led to a sharp increase of the NO₃⁻ concentration in the soil. Denitrification was the main NO₃⁻ consuming process, and only minor amounts of N were recycled into the NH₄⁺ pool via DNRA, with both processes accounting for 74% of gross NO₃⁻ consumption. Significantly, the aerobic process of nitrification dominated at 80% WFPS over denitrification, which is typically favored by low O₂ availability. This can be explained by the occurrence of these processes at different microsites in the soil matrix, which is further evidenced by the substantial amount of N₂O emitted via nitrification.

Denitrification losses were dominated by N₂O emissions, exceeding N₂ emissions by a factor of 1.5. This is consistent with an incubation study using a subtropical pasture soil, reporting low product ratios of denitrification (N₂/(N₂+O₂)) in the first 3 days at 80% WFPS (Friedl et al., 2016). At 80% WFPS, the remaining oxygen (O₂) in the soil matrix is likely to inhibit the N₂O reductase, shifting the N₂/(N₂+O₂) ratio towards N₂O. High NO₃⁻ concentrations as observed at the end of the incubation may have also inhibited the reduction of N₂O to N₂ by decreasing the C/NO₃ ratio and/or as a consequence of preferential NO₃⁻ reduction (Giles et al., 2012).

The ¹⁵N recovery in the different N pools from the single labelled treatments of 85% of the applied fertiliser shows that the simplified incubation assay can provide reliable results for N₂ and N₂O emissions (Table 1). The high ¹⁵N enrichment of the fertiliser and a closure time of 24 hours resulted in a DL for N₂ between 0.014 and 0.127 µg g⁻¹ day⁻¹, depending on ¹⁵N enrichment of the NO₃⁻ pool. This low DL enables the detection of significantly lower N₂ fluxes than observed in this study, an important trait when obtaining datasets on denitrification over a range of agricultural soils with a different denitrification potential.
Conclusion

Combining the $^{15}$N gas flux method and the $^{15}$N pool dilution method, this novel incubation assay accurately quantified gross rates of N turnover in the soil-atmosphere system. This study confirms denitrification as a major pathway of N loss from subtropical pasture systems, and reveals the rapid N turnover in these agroecosystems. The simplified incubation assay allows large numbers of samples to be processed, and as such is an effective method to establish comprehensive datasets on N cycling in agro-ecosystems. These datasets, while improving a quantitative process understanding for denitrification, will enable biochemical models to accurately simulate the N cycle, revealing both short and long term effects of improved management practice on N use efficiency for fertilised agro-ecosystems.

References


