Urine patch size and nitrogen load: effects on nitrogen uptake from the urine patch in plantain and ryegrass/white clover pastures

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Abstract
Field measurements from micro-plots (0.20 - 0.36 m²) of perennial ryegrass/white clover and of pure plantain were used to mimic a urine patch (UP) and to test the effects of UP nitrogen (N) load and size on pasture N offtake. Urine N offtake was greater with plantain than with standard pasture; however, the relative contribution to uptake from the wetted area and surrounding edge was the same for both species. Most (>90%) of the apparent offtake of urine N by plantain and standard pastures was within 20 cm of the edge of the UP. For the two urine patch sizes tested, edge contribution to urine N offtake was on average about 30% of the total from the UP, but was higher for at 600 kg N/ha urine N (45%) than at 300 kg N/ha (18%). Understanding this edge contribution is important for model improvement, and for the development of mitigations to decrease N leaching.

Keywords: urine patch edge, wetted area, nitrogen dynamics, leaching

Introduction
The urine patch (UP) area can be divided into a wetted area (where urine was directly voided) and the immediate surrounding area. Pasture plants outside of the wetted area can access urinary N via their roots and nitrogen (N) diffusion/convection through the soil (Lantinga et al. 1987; Tinker & Nye 2000; Cichota et al. 2018). An understanding of pasture growth and N uptake through this ‘edge contribution’ is important (Moir et al. 2011). Firstly, lysimeters used for measuring N leaching losses have confined edges, but the contribution to uptake of urinary N by pasture over the entire effective area should be considered because N leaching is generally inversely related to N uptake (Moir et al. 2013). Secondly, some mitigation options under development look to alter the size and shape of UPs and this can change the contribution of the UP edge to uptake (Balvert & Shepherd 2015). Furthermore, research has focused on standard perennial ryegrass (Lolium perenne) and white clover (Trifolium repens) pastures, whereas there is currently interest in alternative pasture species as forage.

The aim of this paper is to report some data from a larger study that compared offtake from wetted and edge areas of pasture micro-plots mimicking UPs. The hypotheses were that the edge component of the UP makes a substantial contribution to overall N offtake and that plantain (Plantago lanceolata) and standard pastures, would perform similarly in terms of edge effects. Understanding this edge contribution is important for model improvement, and for the development of mitigations to decrease N leaching.

Approach
A replicated experiment was established at Scott Farm, Hamilton, New Zealand, in the spring of 2015 to compare growth and N offtake from micro-plots of pure plantain and standard perennial ryegrass/white clover pastures (clover about 15% of DM) representing urine patches to (a) determine the relative contribution of wetted area and outside area to N removal from the urine patch and (b) to test if these differed between the pasture species. The overall design of the experiment was based on previous experiments with ryegrass/white clover pastures (Balvert & Shepherd 2015; Buckthought et al. 2016); the novelty in this experiment was the comparison of species. Square micro-plots, were used to mimic urine patches and to make harvesting easier. Each comprised a ‘wetted area’ and two concentric zones 20 cm wide denoted as the ‘inner’ and ‘outer’ edge (Figure 1). Adjacent Nil-N strips (2 m²) acted as control areas.

Two square UP areas (0.20 and 0.36 m² typical of the range of cattle urine patches were compared, Selbie et al. 2015) and two urinary N rates (300 and 600...
kg N/ha equivalent) in three paddock replicates on a Horotiu silt loam soil. Artificial urine was applied in a single application to UPs at a rate of 10 L/m², with N concentration of the urine adjusted to achieve the desired loadings of either 300 or 600 kg N/ha, i.e. 3 or 6 g N/L. Urine analysis for total N concentration before use confirmed that the target concentrations were achieved. Urine treatments were applied after the forage had been mown to simulate a residual height of about 5 cm. Applications dates were 9th September (plantain) and 16th September (standard), with final harvests on 15th January (plantain, five harvests, 128 days in total) and 19th January (standard, four harvests, 124 days in total). Application dates differed by a week to synchronise with separate experimental work on the same site. Number of harvests differed between species because of differential growth rates.

Pasture was harvested by mower to determine pasture response and by harvesting the ‘zones’ of the plot separately (wetted area and the edges). Control strips (1.8 m²) were also harvested from where no treatment or N fertiliser had been applied. Herbage samples were taken from each plot to determine dry matter (DM%) and nitrogen contents (N%). Sample DM% was determined by drying a subsample at 65°C for 48 hours; N content was determined by Kjeldahl analysis.

Expression of results
The combination of UP size and N load/UP meant that the four UPs received different amounts of N (Figure 1). This and the differences in areas of each zone as affected by UP size (Figure 1) needed to be factored into calculations of relative N offtake from each zone; expression of results on a /ha basis was meaningless for this comparison, given the difference in the area of each zone.

The following calculation procedure was therefore followed:
1. Calculate net (‘apparent’) N offtake (kg N/ha) from zones by subtracting control N offtake:
   \[ \text{Net Noff (kg N/ha)}_{\text{zone}} = \text{Noff (kg N/ha)}_{\text{zone}} - \text{Noff (kg N/ha)}_{\text{control}} \]
2. Calculate apparent N offtake (g N) from each zone:
   \[ \text{Net Noff (g N)}_{\text{zone}} = \text{N offt (g N/ha)}_{\text{zone}} \times \text{Area}_{\text{zone}} \]
3. Calculate total apparent N offtake from a UP by summing zones:
   \[ \text{Total Net Noff (g N)} = \text{Noff (g N)}_{\text{wetted zone}} + \text{Noff (g N)}_{\text{inner zone}} + \text{Noff (g N)}_{\text{outer zone}} \]
4. Express total apparent N offtake as a proportion of urine N applied:
   \[ \text{Net Noff (% applied)} = \frac{\text{Net Noff (g N)}}{\text{N applied (g N)}} \times 100 \]

Statistical analysis
Analysis of variance was performed on all the data using the GenStat statistical program (13th Edition). The Least Significant Difference (LSD) was calculated at P<0.05.

Results
Climate
Air temperatures from September - December 2015 were close to the long-term average, but January and February were about two degrees warmer than average (Table 1). Rainfall from July-September 2015 was near normal, and soil conditions were moist at the time of the treatment applications to the plantain and standard pastures. Heavy rain (16 mm) fell in the night after the plantain treatment application. October was drier than average. Higher than average rainfall provided for uninhibited pasture growth in November, while December was dry followed by average rainfall in January.

Pasture production and nitrogen offtake – wetted area
There was no additional observable response by the pastures to the applied N beyond the first three harvests (data not shown). Over the total measurement period, more pasture DM was produced and more N taken up after urine application for plantain than for standard pasture (Table 2). There was also an effect of N rate, but not for urine patch size on pasture production and N offtake.

Contribution of edge areas to N offtake
Total apparent N offtake of urine N (as a total of the three zones, i.e. the ‘effective area’) was greater for plantain than for standard, and greater for 600N than 300N (Table 3). Average apparent N recovery (as a total of the effective area) was 45%, i.e. about half of the applied N was recovered by the pasture. However, the apparent N recovery was greater for plantain than for standard

Table 1

| Month     | Rain (mm) | LTA  | Temperature (°C) | LTA  |
|-----------|-----------|------|------------------|------|
| Sep       | 119       | 101  | 11.6             | 11.9 |
| Oct       | 36        | 91   | 13.7             | 13.2 |
| Nov       | 106       | 58   | 15.1             | 14.9 |
| Dec       | 25        | 91   | 17.3             | 17.2 |
| Jan       | 82        | 82   | 20               | 18.9 |
| Feb       | 68        | 43   | 21.5             | 19.4 |
pasture (67 versus 25%; P<0.01); there were no urine patch size or N load effects on the N recovery.

In terms of the contribution to apparent N offtake by each zone, there were differences in absolute amounts of N taken up by the two pastures, but there was no difference between species in the relative contribution of each zone. More than 90% of total apparent N offtake occurred in the wetted zone and within 20 cm of the wetted zone for both pastures (Table 4). However, there was an indication that the edge contribution became more important at the higher N rate (Table 4).

**Discussion**

The average N recovery of 25% is low for a ryegrass/white clover pasture. For example, Moir *et al.* (2013) measured on average 30 and 50% recovery in harvested DM from 13 grass species at N rates of 750 and 300 kg N/ha (mean 40%), respectively. Nil N yield of the standard pasture was about 90% of the plantain, but only around 70% of plantain when urine was applied (Table 2). The low growth and low recoveries by the standard pasture were consistent across the three separate paddocks and are difficult to explain. The extra 16 mm rain after urine application to plantain was the only important difference in growing conditions, but it is difficult to see why this would have such a large effect. Critically, however, the proportions in each zone were similar between the two pasture types despite the differences in absolute amounts of N taken up.

Moir *et al.* (2011) noted a seasonal pattern in UP size (smaller in winter than in spring), and also noted that research is required to establish a clear relationship between the area wetted by urine and the associated pasture growth response (and hence, N offtake). Limited research suggests that extra N uptake from the wetted area is mainly restricted to pasture in the zone 150-200 mm from the edge (e.g. Deenan & Middelkoop 1992; Decau *et al.* 2003). Data from this experiment support this finding, although there was an indication at high urinary N rates, of pasture access or diffusion/convection of N resulting in some uptake.

**Table 2**  Total dry matter (DM) production and nitrogen (N) offtake from the wetted urine patch area for plantain and standard pastures (mean of both urine patch sizes).

| Urine N rate (kg N/ha) | Plantain | Standard | Mean |
|------------------------|----------|----------|------|
| DM production (t/ha)   |          |          |      |
| 300                    | 8.20     | 6.62     | 7.41 |
| 600                    | 10.69    | 7.11     | 8.90 |
| Mean                   | 9.44     | 6.87     |      |
| LSD<sub>5%</sub>       | 1.41     |          |      |

**Table 3**  Apparent N offtake and N recovery (as a total of the three urine patch zones) for plantain and standard pastures.

| Urine N rate (kg N/ha) | Plantain | Standard | Mean |
|------------------------|----------|----------|------|
| Apparent N offtake     |          |          |      |
| (g N)                  |          |          |      |
| 300                    | 6.6      | 0.5      | 7.9  |
| 600                    | 10.2     | 5.6      | 3.6  |
| Mean                   | 8.4      | 3.1      | 3.6  |
| LSD<sub>5%</sub>       | 4.6      | P<0.05   |      |

**Table 4**  The proportion of total apparent N offtake in each zone, as affected by N application rate.

| Urine N rate (kg N/ha) | Mean |
|------------------------|------|
| Patch                  | 69   |
| Inner edge             | 25   |
| Outer edge             | 6    |
| LSD<sub>5%</sub>       | 18.0 | P<0.01 |
| LSD<sub>5%</sub>       | 12.7 | P<0.001 |
The effect of urine patch size (assumed to be circular) on the area of the edge zone (calculated assuming the width of edge zone was 20 cm, solid line) and the edge zone area relative to the wetted area (broken line).

Figure 2

Beyond 20 cm, Table 4 shows that, as an average of the two N rates, an amount of N equivalent to 44% of the wetted area offtake occurred outside the wetted area (31 as proportion of 69 = 45%). Phillips & Shepherd (2013) estimated that N offtake outside of the UP was equivalent to around 50% of the offtake within the wetted area. Buckthought et al. (2016), using $^{15}$N labelled urine, reported similar results. The implications are that surplus N within a UP is depleted more rapidly if pasture outside of the wetted area is able to access urinary N. Accounting for this in models is important to correctly estimate N losses (leaching and gaseous) from urine patches, paddocks and farms, since urine patches are the major source of N loss. The lateral spread of urinary N beyond the patch area can reduce the effective N load of urine patches, enabling more plants to access the deposited N and consequently reduce N leaching losses; in the case of modelling of N leaching from a UP 60 cm in diameter (Cichota et al. 2018), this decreased by 8-37% when edge contribution to uptake was included in the model.

The 20 cm band around a urine patch represents an important additional area of pasture for N removal from the UP (Figure 2). However, more striking than the changes in absolute area with change in UP size, was the change in relative area between wetted and edge areas as the UP size decreased. Figure 2 shows the change in relative areas was small for the UP sizes that were tested (2.2 for a UP size 0.2 m$^2$ and 1.5 for a UP 0.36 m$^2$). This small difference in relative edge areas between the two UP sizes is perhaps why there was no statistically significant size effect on the relative edge contribution to urine N recovery. Figure 2 sets up the hypothesis that UPS have to be <0.2 m$^2$ for the edge effect to substantially increase relative to the wetted area, although this calculation can provide only a possible approximation of the response, it suggests a hypothesis for further evaluation. The challenge is that testing this by field experimentation is fraught with difficulty given the practical aspects of accurate measurement at this plot scale. The use of process-based models of urine patch dynamics such as that reported by Cichota et al. (2018), allows a much wider range of scenarios to be evaluated in terms of UP size, shape and N load than can be undertaken in field experiments, provided that the model is adequately verified against field data.

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