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Research article

Biotic and abiotic controls on nitrogen leaching losses into waterways during successive bovine urine application to soil

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ABSTRACT

Cattle waste products high in nitrogen (N) that enter waterways via rainfall runoff can contribute to aquatic ecosystem health deterioration. It is well established that N leaching from this source can be reduced by plant assimilation, e.g. pasture grass. Additionally, N leaching can be reduced when there is sufficient carbon (C) in the soil such as plant litterfall to stimulate microbial processes, i.e. denitrification, which off-gas N from the soil profile. However, the relative importance of these two processes is not well understood. A soil microcosm experiment was conducted to determine the role of biotic processes, pasture grass and microbial activity, and abiotic processes such as soil sorption, in reducing N leaching loss, during successive additions of bovine urine. Pasture grass was the most effective soil cover in reducing N leaching losses, which leached 70% less N compared to exposed soil. Successive application of urine to the soil resulted in N accumulation, after which there was a breaking point indicated by high N leaching losses. This is likely to be due to the low C:N ratio within the soil profiles treated with urine (molar ratio 8:1) compared to water treated soils (30:1). In this experiment we examined the role of C addition in reducing N losses and showed that the addition of glucose can temporarily reduce N leaching. Overall, our results demonstrated that plant uptake of N was a more important process in preventing N leaching than microbial processes.

1. Introduction

Wide scale land-use change has resulted in increased river exports of nitrogen (N) from catchments to oceans (Downing et al., 1999; Howarth et al., 1996). This poses a problem, as the quantity and form of N washed into streams can contribute to the deterioration of water quality, e.g. algal blooms. One concern is that agricultural land use may leach more N than forested land use, through the use of fertilizers (Barraclough et al., 1992), livestock waste (Di and Cameron, 2002) and sedimentation from livestock hooves removing the soil cover (Bartley et al., 2014). Globally, grazed grasslands account one fourth of the earths unfrozen land (Steinfeld et al., 2006). Consumer demand for bovine food products, milk and meat, are predicted to nearly double between 2000 and 2050 (Steinfeld et al., 2006). Pressure to increase cattle stocking densities have prompted research in United States (Stout et al., 1997), Denmark (Hansen et al., 2012), New Zealand (Di and Cameron, 2002), and Australia (Eckard et al., 2004) to address concerns of N enrichment from agriculture on ground and surface waterways (Li et al., 2012; Smith and Frost, 2000).

Leaching losses of N occur when soil field capacity (soil-moisture tension) is met, and N has accumulated in the soil due to input exceeding plant and microbial demand. The soil under a urine patch (600–1000 kg N ha^{-1}, Haynes and Williams, 1992) can be ten-fold higher than the N load under clover ground cover or under fertilized soils. For example, N-fixation by clover results in a N load of 108 kg N ha^{-1} (Ledgard et al., 1990), while for arable and grazed pastures the maximum fertilizer application loads is 250 kg N ha^{-1} (Di and Cameron, 2002). Therefore, given these N loads it is reasonable that urine N is a considerable source of N to soils, and to the N cycle within soil.

Despite the negative effects of agriculture on N runoff to waterways, it is well established that pasture grass cover can reduce N leaching losses to groundwater systems, particularly from bovine urine. This can occur by direct N uptake for incorporation into the

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grass biomass (Di and Cameron, 2007; Malcolm et al., 2014), as well as indirectly by reducing water percolation through the soil (Loiseau et al., 2001). However, leaching loss can still occur because grass roots cannot necessarily assimilate the entire N load deposited from a bovine urination event (Haynes and Williams, 1992). In these cases soil microbial processes play an important role in N retention.

In the soil profile, sufficient carbon (C) relative to N is essential for microbial processes (i.e. the consumers) that effect the transformations and fate of N added to the soil (Sterner and Elser, 2002). Soils with low C:N ratios have been found to have increased sensitivity to N inputs resulting in N leaching losses (Mooshammer et al., 2014). Denitrification processes are more likely to be hindered if there is limited C as an energy source, rather than from a lack of denitrifying organisms (Clough et al., 1998). The C required for denitrification must be present in the soil at a C:N ratio >5:1 (Sobieszuk and Szewczyk, 2006). Soil C stock is a product of the vegetative cover. Specifically, grass-covered soils have low decaying biomass inputs and are often described as C limited (soil organic matter C:N <17:1) (Mooshammer et al., 2014). This C:N ratio may be further imbalanced by livestock grazing, which removes plant biomass (Barnett et al., 2014). Although, solid livestock excretal products (i.e. dung) may contribute C and organic forms of N, alongside inorganic N, back to the soil (Monaghan and Barraclough, 1992; Wachendorf et al., 2005). N under a dung patch may still result in N leaching losses of approximately 30% of the input N (Wachendorf et al., 2005). In comparison, C inputs to the soil from decaying litterfall, have been found to increase soil C pools to have a final C:N ratio of ~71:1 (Mooshammer et al., 2014). The specific ratios of C:N required for N transformation processes may be unclear; however, a number of studies have shown that N leaching losses can be predicted by measuring soil C:N ratios (Christ et al., 2002; Venterea et al., 2003).

As a resource, bovine urine would be considered C limited, as the concentration of C is similar to the concentrations of N (1:1, C:N) (Bristow et al., 1992). Consequently, soils amended with bovine urine can become stoichiometry imbalanced to be N-rich (i.e. comparison to C and P), which would be quite prominent in soils that are already C limited (e.g. such as grass covered soil). Recently, Riaz et al. (2011) showed that manipulations to the soil with low C (12:1 to 17:1) can be achieved though additions of litterfall to effectively reduced nitrate (NO_3^-) leaching. Alternatively, pyrolyzed organic material, termed biochar, may be applied to improve soil C sequestration and soil organic C (Farrell et al., 2015) and aid in soil N retention (Chen et al., 2012). The mechanisms of how biochar amendment effects N transformations and fate in soils is largely undescribed (Anderson et al., 2014; Clough and Condron, 2010). However, under a bovine urine patch, the microbial processes that transform N may not be a straight forward as describing the stoichiometry of the soil.

There are inconsistencies in the reported results of the effects of urine on soil microbial processes (Bertram et al., 2012; Kelliher et al., 2005). These include increased microbial respiration (Kelliher et al., 2005), reduced microbial biomass (Bertram et al., 2012; Orwin et al., 2010), and reductions in the capacity of microbial communities to use C (reviewed in Millard and Singh, 2010). Thus, further research is warranted to investigate the impact of urine on soil microbial biomass C and N pools, which along with grass, are important to reduced N from leaching.

In order to address these gaps in knowledge, the objectives of this research were to: (1) quantify N leaching losses from bovine-urine amended soils; (2) compare the effect of soil cover (grass, litterfall, exposed soil) on the amount and form of bovine urine N leached from soil; and (3) determine the effects of C amendment on reducing N leaching losses. To achieve this we compared the relative roles of soil sorption, microbial processes and plant uptake in reducing N leaching losses with sustained application of urine, with and without C addition. Three soil cover types were examined assuming that grass would store N and litterfall C would stimulate microbial activity and enhance microbial incorporation of N. We hypothesized that the grass-covered soil would leach less N compared to litterfall-covered and exposed soil, and that C addition to soils would reduce N leaching.

2. Material and methods

2.1. Study site

The study area was Lake Baroon catchment, southeast Queensland, Australia. This region is in the subtropics with a mean annual temperature and precipitation of 23 °C and 2000 mm, respectively (Bureau of Meteorology, (2014); www.bom.gov.au). The dominant soil type in the catchment is haplic, mesotrophic and red ferrosols (Isbell, 1996) classed as a nitisol according to US soil taxonomy (Gageler et al., 2014), with gentle to moderate hills derived from tertiary basalt flows. The catchment area is 74 km^2 and consists of dairy and beef farming, forested areas and peri-urban residential blocks.

The grassed study site is regularly grazed by beef cattle. The dominant grass species of the grazed area were kikuyu grass (*Pennisetum clandestinum*), narrow-leaved carpet grass (*Axonopus affinis Chase*) and Queensland blue couch *(Digitaria didactyla)*. Within the grazed area were also bare patches of mineral soil. The adjacent riparian area, isolated from cattle access, is dominated by swamp mahogany (*Eucalyptus Robusta*) and flooded gums (*Eucalyptus grandis*) that produced large quantities of litterfall that cover the associated riparian area (Saxton, *personal communication*).

The bovine urine used in our experiment was collected directly from lactating dairy cows (*Bos taurus*) at Maleny Dairies, southeast Queensland. The samples contained 7.4 g L^{-1} total dissolved C (TDC) and 5.1 g L^{-1} total dissolved N (TDN) (analyses as per section 2.5 Leachate analysis). This was similar to values reported by De Klein et al. (2003), i.e. 5.9 g N L^{-1} in synthetic and natural cow urine. The applied dosage to the soil equalled 57.93 kg C ha^{-1} as dissolved organic carbon (DOC) and 41.49 kg N ha^{-1} as TDN with 0.52 kg ha^{-1} NO_3^- and 0.002 kg ha^{-1} ammonium (NH_4^+). Dietary intake of forage and pasture grass will effect bovine urine N load (Edwards et al., 2014; Ledgard et al., 1999) and dilution (Li et al., 2012).

Intact soil core samples were obtained from a grazed pasture area and adjacent vegetated riparian area on Lawley Creek, a tributary of Bridge Creek (26°44′59.10″S, 152°51′22.21″E), over two sampling dates in the austral summer, 7th January 2014 and 11th February 2014. There were three soil cover treatments: litterfall-covered soil, grass-covered soil and exposed soil. Samples were collected from the grazed and litterfall covered areas described above. Additionally, there were two application treatments, i.e. water and urine, and water, resulting in a factorial design, i.e. 30 samples (3 cover treatments × 2 applications (urine, no urine) × 5 replicates). Treatment names were as follows; Grass-U, Grass-W, Litterfall-U, Litterfall-W, Soil-U and Soil-W. To reduce variability in soil type, the soil cores were collected in close proximity (within 15 m radius). Intact soil cores were collected in polyvinyl chloride (PVC) pipes (dimensions; 10 cm internal diameter, 15 cm depth) which were hammered into the ground and excavated with care taken to maintain the core integrity.

To reduce degradation and maintain field moisture, samples were stored on ice in bags, and transported to the glasshouse facilities within 8 h. The soil texture was determined as loamy sand, using particle size analysis with the hydrometer method (Black,