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Original Research Paper

Inhibition of Nitrification in Soil Under Pastures in Western Australia

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ABSTRACT

Nitrification processes can play a key role in the functioning of Western Australia natural ecosystems. It is directly involved in plant nitrogen losses through leaching and denitrification. Suppression of this process by pastures is poorly understood. The research was conducted on the deep-sand at Mingenew in the Northern Agricultural Region, WA. Nitrification rate was determined under annual, perennial pastures and tagasaste fodders grown *in situ*. Nitrification and inhibition rates were calculated based-on measurements of NH_4-N, NO_3-N over a period of 6 months. Under natural conditions (control) the nitrification rates of ammonium-N (NH_4-N) were rapid from 80 to 97% during the season. Nitrification rates under annuals, perennial pastures and tagasaste plants were 35 to 80%, 58 to 58%, and 30 to 75% respectively. It was found that there is a high negative correlation (R^2= -0.84) between biomass and nitrification rate, and a high positive correlation between biomass and inhibition rate (0.74). Such results suggest that pastures species can have important consequences for nitrogen cycling at the permanent growth and population density.

INTRODUCTION

Nitrogen in fertilizers and organic components is mineralized into NH_4-N by soil microbes. In the nitrification processes, NH_4-N is converted into NO_2-N by ammonium-oxidizing bacteria and NO_2-N converted into NO_3-N by nitrite-oxidizing bacteria. Since plant can absorb only NH_4-N and NO_3-N, nitrification markedly influences the nitrogen absorption efficiency by plants. Nitrate-N that is not absorbed by plants can be easily leached in sandy textured soil and can pollute the groundwater. During the process of nitrification, nitrous oxide, one of the greenhouse gases, is emitted from the soil into the atmosphere. By controlling nitrification, it is possible not to increase the nitrogen absorption efficiency by plants but also to minimize nitrogen loss by leaching and volatilization. In order to decrease the nitrogen loss, chemical fertilizers with nitrification inhibition have been developed but their application is very limited in grasslands because of their high costs. If plant could inhibit the nitrification process, their nitrogen absorption efficiency would be considerably enhanced. It was reported that the amount of NO_3-N accumulated in a soil where perennial pasture and tagasaste were growing, seemed to be smaller than in the soil where annual pasture grew (Hasson et al. 1988, Hasson et al. 2008).

Previous studies reported that low nitrification rates were found under tree-grass mix paddlocks (De Rham 1973, Bate 1981, Verstraete 1981, Haynes 1986, Abbadie & Lensi 1990, Lensi et al. 1992, Serca et al. 1998).

It has been hypothesized that grasses can inhibit nitrification through the exudation of allelopathic compounds by roots (Munro 1966, Meiklejohn 1968, Rice & Pancholy 1972). This hypothesis has been at the centre of the controversy from many years because of the absence of *in situ* evidence for plant involvement in nitrification inhibition (Stiven 1952, Munro 1966, Meiklejohn 1968, Rice & Pancholy 1972, Purchase 1974, Lodhi 1978, Jordan et al. 1979, Robinson 1984, Donaldson & Henderson 1990, McCarty et al. 1991, Stienstra et al. 1994, Lata et al. 1999, Lata et al. 2000).

Inhibition of nitrification under perennial and tagasaste grasses is of major interest. The NAR of Western Australia has 2.36 million ha of poor sands that are suitable for perennial pastures. Currently there is about 1.43 million ha under pasture with 25000ha being perennial pasture. NAR is generally nutrient-poor ecosystem, especially for nitrogen as an essential element for productivity. Nitrification, therefore, plays an important role in the functioning of the region as it is directly involved in plant nutrition and nitrogen losses through leaching and denitrification (Huber et al. 1977, Jordan et al. 1979, Vitousek et al. 1979, Keeney 1986, Robertson 1989).

The objective of this study is to assess the inhibitory effect of annual, perennial and tagasaste pastures on the nitrification of ammonium and to compare it with the bare soil.

METHODS AND MATERIALS

Site description: The experimental site is located 37 km west of Mingenew, WA (29°18'57.350"S, 115°8'46.568" E). The soil was yellow/brown deep sandy duplex. The study site has three paddocks (annuals, perennial and tagasaste), about 10 ha each. The annual plot consisted of clover, capeweed, and annual grass adjoining the tagasaste and perennial paddocks consisted of Rhodes, Patterson curse, Signal and Kikuyu.

During 2008, temperature was relatively constant through the growing season (15°C on average). The cumulative rainfall was 396 mm. The rate of evapotranspiration was around 210mm. An amount of 50 kg ammonium sulphate, 100kg of super phosphate plus 30 kg of potash were applied on the break of season 2008.

Soil sampling and analysis: Each month thirty soil cores, (0-5, 5-10, 10-20, 20-30, 30-70, 70-90, 90-120, 120-160 cm depths) were collected from annual, perennial and tagasaste plots and analysed for inorganic nitrogen, nitrate-N and ammonium-N (Searle 1984). At each soil sample site, the total amount of soil from each depth was collected in polyethylene bags, sealed, frozen and delivered to the soil analysis laboratory for analysis immediately. Initial soil analysis of pastures is listed in Table 1. The monthly total of NH_4-N and NO_3-N are shown in Figs. 1 and 2.

The volumetric soil moisture values were taken on monthly basis from 0-160 cm (eight sub-samples). The water content at each depth interval was determined using a Neutron Soil Moisture Meter. Soil moisture was between 0.08 and 0.14 cm^3/cm^3.

Fifty randomized plants from each plot, (annuals, perennials and tagasaste pastures) were weighed for fresh and dry weights before grazing, and analysed for NH_4-N, NO_3-N and total N.

Nitrate leaching: Cumulative nitrate leaching was determined using pipe drain at 1.8m depth back filled gravel to the surface across all the studied pasture paddocks. The pipes drain trenches were inserted to the gravel mole drains entered. Sub samples of drainage water were collected at the time of sampling and frozen until analysed for NO_3-N.

The concentration of NO_3-N in each layer was interpolated from measured NO_3 and NO_2 concentrations in the soil. Leaching from soil surface was set at zero when evapotranspiration was greater than rainfall.

Calculations

All statistical analysis was performed based on SAS (SAS 1989) at the 0.05 significant level. Type III of squares were used for ANOVA and covariance. Values have been given as mean ± SE.

The amount NO_3 leached from soil at nine depths was calculated (Nz) as follows:

$$Nz = [Ns - Nt / Ws + R + d] \times D$$

Where Ns is amount of soil nitrate (kgN/ha), Nt is amount of leached and uptake by plants nitrate; Ws is soil water content (mm); R is amount of rainfall (mm); d is the preceding drained water (mm); and D is the amount of drained water (mm).

For the amount of mineral nitrogen control has been subtracted in each sample. Nitrification rate, NR % and inhibition of nitrification rate, I.N.R % were calculated as below (Hasson 1989).

$$N.R.\,\% = \{NO_3\text{-}N / (NH_4 + NO_3)\text{-}N\} \times 100$$

$$I.N.R.\,\% = [\{(N.R \text{ of } (NH_4)_2SO_4 \text{ alone}) - (N.R \text{ of planted } (NH_4)_2SO_4)\}/(N.R \text{ of } (NH_4)_2SO_4 \text{ alone})] \times 100$$

RESULTS

Ammonium sulphate in bare soil showed that the nitrification rate increased markedly. On the other hand, the nitrification rate was decreased by annuals, perennials and tagasaste plots (Fig. 3). In the pastures paddocks, there are significant differences between nitrification rates of annuals, perennials and tagasaste during the growing season. On the other hand, a significant decrease in the nitrification rate was observed when annuals and control were compared, especially in the season break and increase at late of the season (September and October). These results agree with those reported by Lata et al. (2004).

The inhibition of nitrification under perennial pastures and tagasaste fodder was significantly higher than annuals in May through to August, but not significant in September. The effect of pastures on inhibition of nitrification is shown in Fig. 4. The maximum inhibition of nitrification in the paddock growing perennial and tagasaste pasture reached 72%

Table 1: Initial soil analysis for Mingenew site.

Soil depths	Colour	NO_3-N mg/kg	NH_4-N mg/kg	P mg/kg	K mg/kg	S mg/kg	OC mg/kg	EC dS/m	pH pH-ca	pH H_2O	TN %
0-5 cm	BRGR	14.33	1.94	13.03	81.21	2.27	1.12	0.10	5.90	6.53	0.10
5-10 cm	BRGR	15.47	6.67	5.99	39.73	6.80	0.77	0.04	5.10	6.10	0.06
10-20 cm	LTGR	0.41	0.98	4.54	27.36	6.60	2.26	0.03	4.83	5.97	0.02
20-40 cm	YWGP	0.40	0.53	3.86	21.23	3.00	0.15	0.02	4.67	5.63	0.02

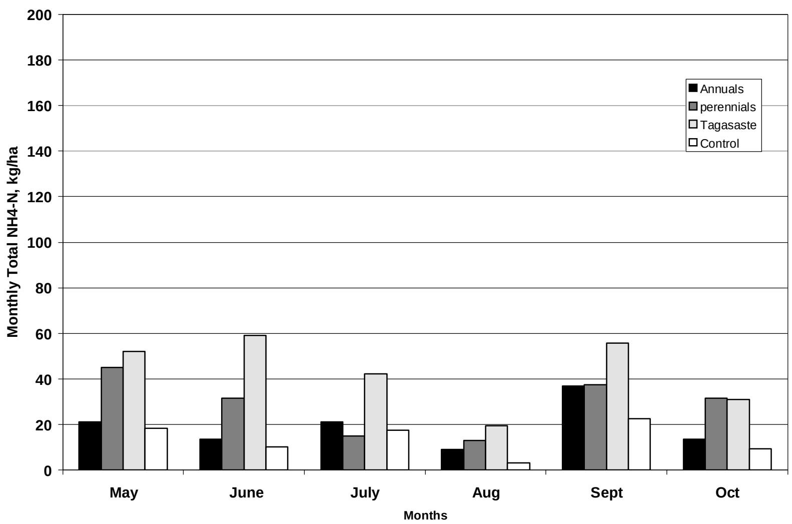

Fig. 1: Monthly total of NH_4-N in the soil profile at the experiment site.

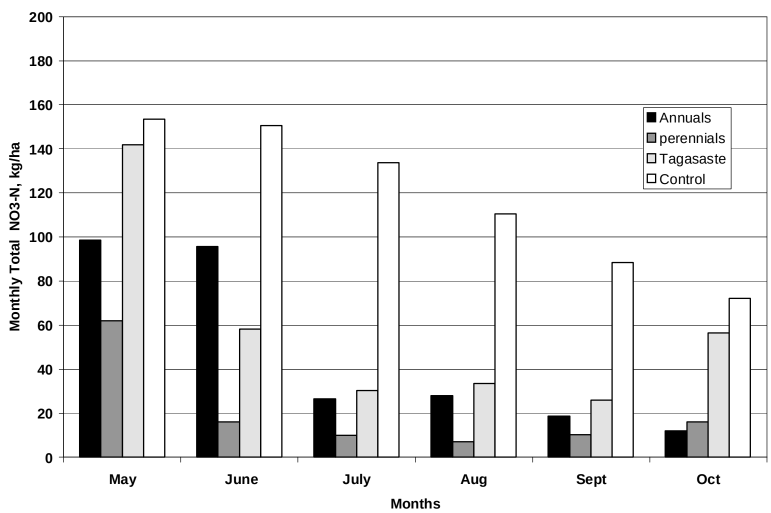

Fig. 2: Monthly total of NO_3-N in the soil profile at the experiment site.

and 60% in September, respectively (Fig. 5).

In case of annual pasture, the corresponding values were between 6% (June) and 57% (September). In general, the effectiveness of perennial pasture and tagasaste fodder as nitrification suppress decreased significantly in order: perennials > tagasaste > annuals.

The seven pastures were all negatively correlated between nitrification rate and plant biomass (R^2 = -0.84) (Fig. 5), while there was a positive correlation between biomass and inhibition of nitrification rate (R^2 = 0.74) (Fig. 6). These results were confirmed by measurements in other two seasons and location (data not shown).

DISCUSSION

The core objective of this study was to demonstrate the inhibition of nitrification by pastures. In Mingenew, soils can be found with high nitrate leaching and nitrification. In this case, the effectiveness of perennials was studied at the plant ecotype level as well as species level to control on NH_4 and NO_3-N behaviour under pastures and fodder (Hasson 2008).

Pastures paddocks showed that there was a clear effect on nitrification. However, there was a hypothesis that there is heterotrophic mineralization of organic matter, which accumulated significantly under pastures, and production of high organic and inorganic (such NH_4-N) compounds, which are concentrated close to roots (Hasso 2008, Abadie et al. 1992). Thus, the increased biomass could potentially increase NH_4-N availability and decrease NO_3-N in soil through an increase of root-derive organic carbon.

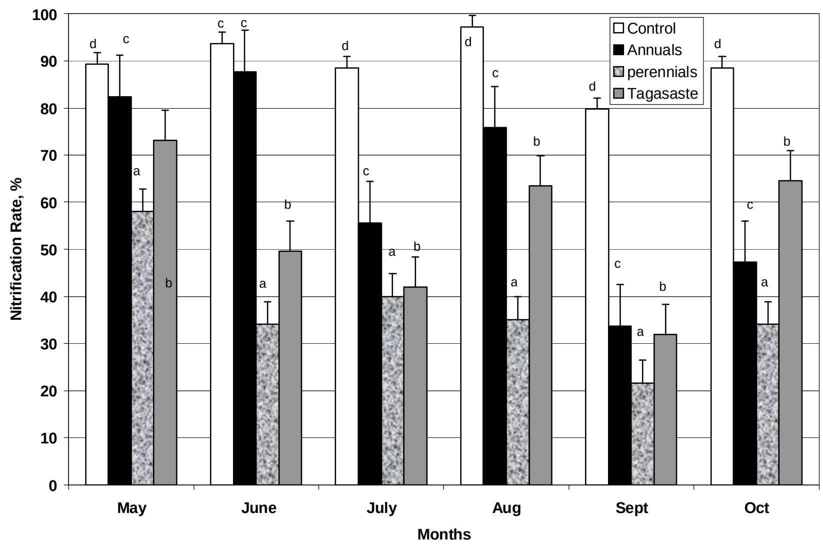

Fig. 3: Effect of pastures on nitrification rate during the season.

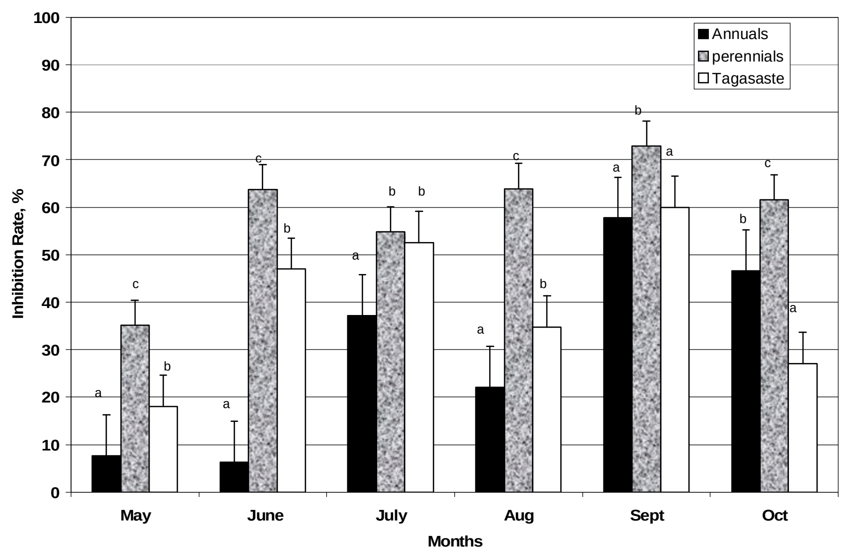

Fig. 4: Effect of pastures on nitrification inhibition rate during the season.

Depending on the competition for NH_4-N uptake between the pasture roots and soil microbes, this could increase the biomass. Moreover, because nitrifiers can grow in mixotrophic or heterotrophic media (Bock et al. 1983), the increase of nitrification with plant biomass could be caused by heterotrophic nitrification (Nemergut & Schmidt 2002). The present data can not distinguish between the competition and inhibition hypothesis.

Under natural conditions (bare soil) the nitrification rate of ammonium-N was rapid during the season from 80% to 97% during the season. In the soils with annuals, perennials and tagasaste plants, nitrification processes were at a rate of 35 to 80%, 58 to 58% and 30 to 75%, respectively.

As maximum concentration of NO_2-N in all the samples never exceeded 2 ppm, the data have not been reported. Varieties that slow nitrification to a level that is still consistent with good plant growth would not only help reduce greenhouse gas emissions but also lower water pollution, while enhancing productivity through more efficient use of fertilizer.

Strong negative correlations were found between nitrification rates and biomass originating from the grown annuals and perennials plots. This may be due to mostly autotrophic character of nitrification, as this process is

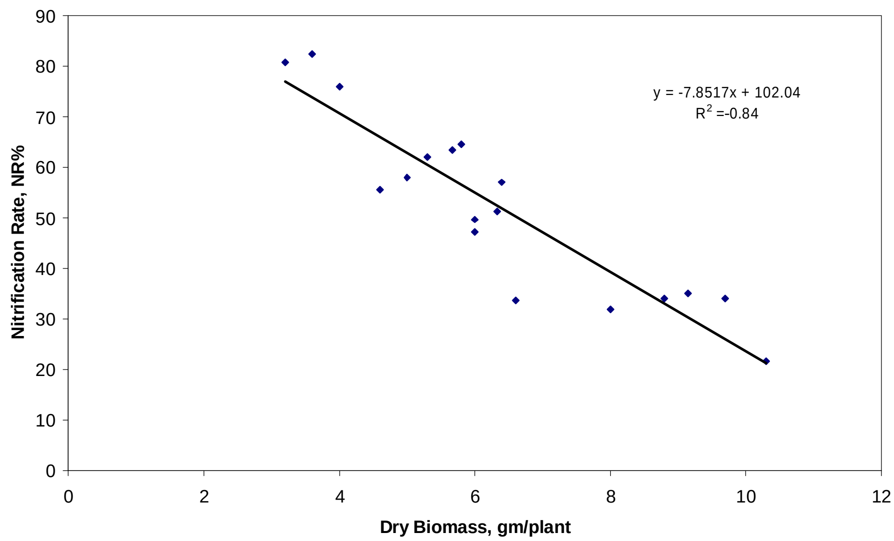

Fig. 5: Relationships between pasture biomass and nitrification rate.

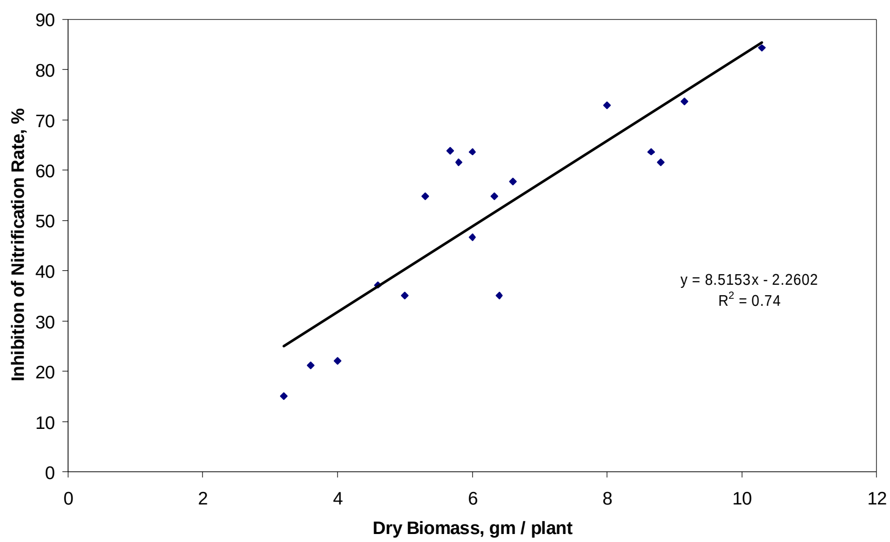

Fig. 6: Relationships between pasture biomass and inhibition rate.

generally considered not to be positively affected by root exudates (Bock et al. 1989). However, the substrate for nitrification is ammonium produce by the heterotrophic mineralization of organic matter.

Thus increases in pasture biomass could potentially increase ammonium availability in the soil through an increase of root-derived carbon (Hasson & Wily 2008, Degrange et al. 1997). Moreover, nitrifiers can also grow in mixotrophic or heterotrophic media (Bock et al. 1983).

Native and exotic annual and perennial pastures Rhodes, grass, radish, rye-grass, Patterson's curse and double gee are distributed in various areas in the northern region of Western Australia but their cultivated land is very low. In order to increase livestock production, if perennials could be increased, it would be possible to decrease the nitrogen input for agriculture by decrease of NO_3-N leaching (Hasson et al. 2008) in addition to preserving the environment and ecosystems.

Of the two nitrification processes, it is assumed that the process in which ammonium-N is changed into nitrite-N is a rate-determining process. By the suppression of multiplication of ammonium-oxidizing bacteria, ***Brachiaria humidicola*** suppresses nitrification in soil and nitrous oxide emission to the atmosphere.

In this case, the mechanism involved in the inhibition of nitrification by pastures remain unknown, but the hypothesis of an allelopathic inhibition through exudation of product(s) by the pastures roots.

In conclusion, the ability to inhibit nitrification, either through an inhibition factor or superior competitiveness of plants compared with microorganisms could give a strong adaptive advantage to pastures, especially perennials. Pastures could create better local availability of N by decreasing losses from NO_3-N leaching and denitrification. Carbon and nitrogen mineralization potentials were not significantly occurring in the experimental site.

Generally, it is considered that tropical grasses preferentially use nitrate-N compared to ammonium-N. Among the perennial species, it is assumed that only perennials can utilize both forms of nitrate-N and ammonium-N and that this function of perennials may lead to an efficient use of nitrogen in soil. Such results suggest that pasture species can have important consequences for nitrogen cycling.

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