Effects of polyphenolic rich biomaterials on transformation of nitrogen in soils

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Abstract
Laboratory based studies showed that polyphenolic rich plant biomaterials (PRPBs) have the potential to slow down mineralisation of recently immobilised nitrogen (N) in soils. Five PRPBs were tested in this study and their rate of N immobilisation was compared with glucose as a standard C source. Among the range of PRPBs tested, PRPB2 was the most effective. Even at 0.5 or 1% rate of C (w/w) added, the PRPB2 showed prolonged immobilisation of N added at 340 kg N/ha. Using ^{15}N as a tracer, we measured over 85% of the recently immobilised N in PRPB2 being retained within soil organic matter until 98 days. Also, it was identified that addition of the PRPB2 to soil changed the fatty acid composition. Ongoing research is looking at the effectiveness of this plant material in protecting soil organic matter and the underlying mechanisms.

Key Words
Tannin, ^{15}N, N cycling, NO_3 and NH_4, dissolved organic matter

Background
Increased nitrogen (N) flow from agricultural land to waterways is now one of the major threats to water quality across the globe. Some of the N in lakes and rivers comes directly from the mineralisation of the N bound in soil organic matter (SOM). The rate of mineralisation of resident or recently added organic matter can be affected by manipulating enzyme and microbial characteristics in soils. It has been suggested that N cycling in grazed pasture system can be manipulated by increasing the tannin content in pasture plants. This can lead to greater partitioning of N from animal excreta into dung which is slowly mineralised over time. The tannins are polyphenolic compounds with the ability to form stable complexes with proteins and other compounds. Tannins in litter may slow decomposition rates and thereby nutrient cycling (Bradley *et al.* 2000). Tannins from various plant species have been shown to affect N mineralisation, induce toxicity in microbes and affect enzyme activities in soils (Schimel *et al.* 1996; Bradley *et al.* 2000). The aims of this study were to determine the effects of tannin rich biomaterials on the mineralisation and immobilisation of N in pastoral soils, and to evaluate if these materials affect the dissolved organic matter in soils. Results are reported from a series of laboratory experiments which were conducted to evaluate proof of concept, which then can be applied to and tested further in field studies.

Methods
Five polyphenolic rich plant biomaterials (PRPB) were used to examine the effects of their input on N cycling in two pasture soils varying in mineralogy and organic matter contents (Table 1). Four sets of experiments were carried out to evaluate a; the proof of concept in terms of effects of PRPB's on N cycling using a closed incubation system, b; efficacy testing at lower rates of application of the PRPB, c: use of ^{15}N tracer to study the incorporation of labelled N into soil organic matter as influenced by PRPB and its subsequent mineralisation, and d; characterisation of soil organic matter after the addition of PRPB application.

In the first experiment, four PRPBs that were characterised for tannin, C and N concentration (Table 2) were added to Bruntwood (allophanic) and Rangiatea (pumice) soils at the rates equivalent to 1.5 and 3% soil C. Glucose was used as a standard C source for comparison. Urea-N was applied @ 340 kg/ha. All treatments and a control with added urea-N were replicated (4), incubated at 20°C and 70% water holding capacity. A factorial design was used and sub-samples of soil from all treatments were collected at 1, 3, 14, 28, 42, 63 and 77 days intervals and analysed for extractable NO_3, NH_4, DON and DOC following the methods described by Ghani *et al.* 2007.

In the second experiment, rates of application of PRPB were reduced to the equivalent of 0.25, 0.50 and 1% soil C and an additional citrus based PRPB was added following the method described above. Subsamples of soil from each treatment were collected at 1, 3, 14 and 28 day intervals and analysed for extractable NO_3, NH_4, DON and DOC.

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Table 1. Soil characteristics used in the incubation studies.

Soil properties	Bruntwood	Rangiatea
Soil Order	Volcanic ash	Pumice
% Total C (w/w)	8.4	4.6
% Total N (w/w)	0.8	0.4
Nitrate-N (µg/g soil)	7.5	5.5
Ammonium-N (µg/g soil)	0.5	0.5
Hot-water extractable C (µg/g soil)	2300	1550
Hot-water extractable N (µg/g soil)	350	170

Table 2. Some physical and chemical characteristics of the PRPB used in this study.

PRPB	% Moisture	% C*	% N*	Water-soluble C* (% of total C)	% Total tannin*
PRPM 1	5.1	52.9	1.3	1.0	7.1
PRPM 2	2.4	47.7	1.0	5.0	5.4
PRPM 3	13.5	50.4	2.5	2.5	0.4
PRPM 4	2.7	49.4	3.7	4.5	4.6
PRPM 5	3.0	48.0	2.5	7.0	5.2

*On dry weight basis

In the third experiment, ^{15}N-urea (99 atom %) was applied @ 200 kg N/ha to trace N incorporation into soil organic matter. A combination of closed and open incubation methods were used to examine N immobilisation and its subsequent mineralisation. The most promising PRPB treatment identified in the first two experiments was used along with glucose as a standard treatment. A control treatment did not receive any C source. All treatments were incubated in a closed incubation for 21 days to enhance immobilisation of the labelled N. At 7, 14 and 21 day intervals, sub-samples from each treatment were analysed for NO_3 and NH_4 to assess the level of incorporation of applied N. Also, ^{15}N in these soil samples was measured to get the accurate measure of incorporation of the applied N. At day 21, subsamples (30 g on oven dry wt. basis) from each of the treatments were packed into leaching columns. These columns were leached on a weekly basis and leachates were analysed for DOC, DON and NO_3 and NH_4. After each leaching, three replicate columns were destructively sampled for ^{15}N, total C and N analysis. At the conclusion of day 21 after the closed incubation system, soils organic matter fatty acids (C16-C20) from control, PRPB and glucose treatments were characterised using GCMS.

Results and discussion

Part 1. Effects of biomaterials

In contrast to glucose, the PRPBs had a small proportion (1-7%) of their total carbon in a water soluble form. Most of the C added as glucose was rapidly respired by soil microbes by day 14 when added at the lower rate i.e. 1.5% C (w/w basis) and by day 28 at the 3% C application rate (Figure 1). There was no difference in the rate of utilisation of added glucose between Bruntwood or Rangiatea soils. Application of PRPBs and glucose caused immobilisation of the added urea-N. The rate of immobilisation of added N was faster in glucose and PRPB2 treatments compared to other treatments (Figure 2). The range of immobilisation in the PRPB treatments illustrated the potential of using combination these treatments to retain more applied N in soils. PRPB2 was effective in lowering the concentration of DOC and DON in both soils (results not included). Effects of treatments for all other variables were similar in both soils therefore results from only one soil (Bruntwood) are presented.

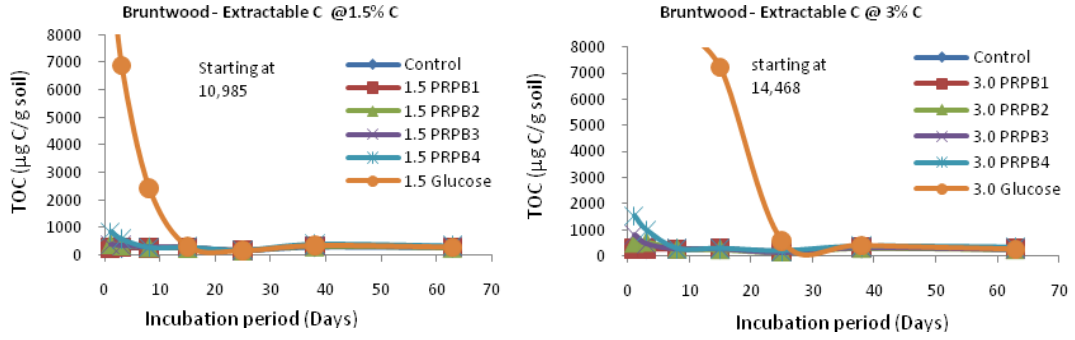

Figure 1. Influence of PRPB and glucose application on extractable C in soils.

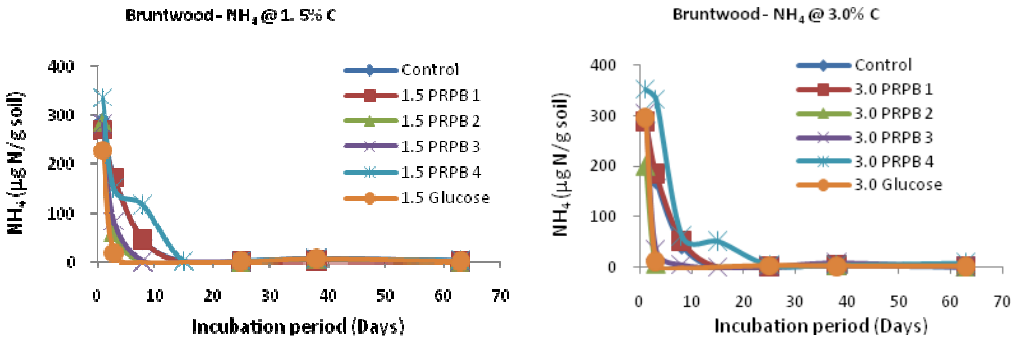

Figure 2. Effects of PRPB and glucose on the immobilisation of applied N in soils applied at 1.5. and 3% C (wt basis).

With the exception of glucose and PRPB2 treatments, none of the other PRPB treatments were effective in keeping the immobilised N in soil organic matter for any significant period of time at either application rate (Figure 3). Towards the end of the incubation period, PRPB 1, 3 and 4 treatments encouraged more mineralisation as the amounts of NO_3 was greater than the added N (Figure 2 and 3). The mode of action for immobilisation of applied N in glucose and PRPB2 is most likely to be through different mechanisms. In the glucose treatment, enhanced microbial activity due to availability of soluble C would be dominant but in the case of PRPB2, which has 25-40 times less soluble C (Table 2), complexing of N in protein may also be a reason for the prolonged immobilisation period.

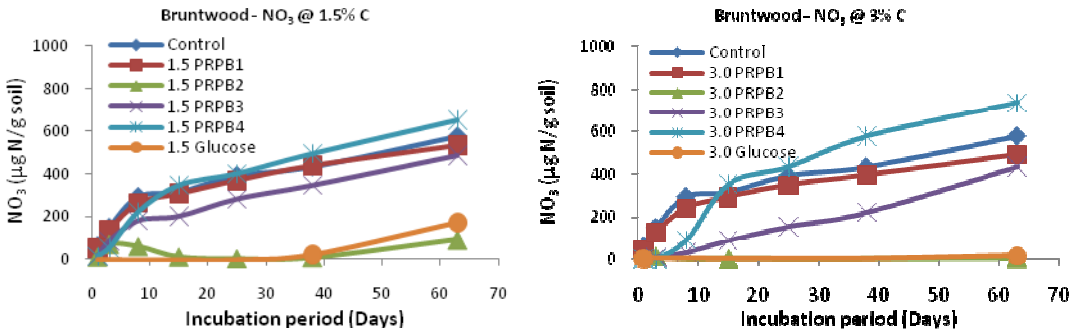

Figure 3. Effects of PRPB and glucose on the remineralisation of N in soils applied at 1.5 and 3% C (wt. basis).

Part 2. Efficiency of biomaterials

In the first experiment, rate of application for all treatments were too high for any practical use. Therefore, immobilisation efficacy of applied N caused by PRPBs was tested at lower rates of C application. The PRPB2 and glucose applied at lower rates of C (0.5 and 1% C) were equally effective in keeping the immobilised N into soil organic matter for a 4-8 week period. Addition of the citrus based PRPB treatment in the second experiment was least effective in causing immobilisation of added N (results not presented).

Part 3 and 4: How long was the recently immobilised N retained in soil organic matter?

Over 75% of the recently-incorporated Nin the control treatment was re-mineralised during the open incubation of 98 days. In comparison, only 12% of the recently incorporated N in soil organic matter from PRPB2 and glucose treatments was mineralised during the 98 days open incubation. For the first 75 days, N mineralisation from PRPB2 treatment was less than that of glucose treated soils (result not included in the text). The GCMS analysis showed that soil organic matter in the PRPB2 treatment had a slightly different make up of fatty acid compared to both control and glucose treatments (Figure 4).

Conclusions

The PRPBs can influence N cycling in soil systems. The concentration of tannin in the PRPB is not a good indicator of the potential of the specific PRPB to increase the period of immobilisation of the applied N in soil organic matter. Other chemical characteristics may help to indentify the efficacy of the PRPB in 'tightening' the N cycle. The PRPB2 showed considerable promise in immobilising the added N as well as protecting the immobilised N against microbial mineralisation for a longer period of time. Further work is required to evaluate the efficacy of PRPB2 in field experiments. Also, identification of the key chemical compounds that are responsible for protection of recently immobilised soil organic N is required.

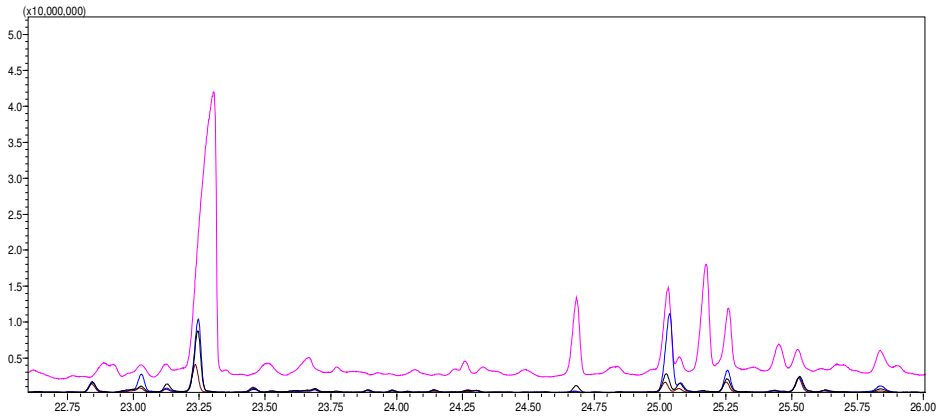

Figure 4. GCMS spectra showing chemical nature of soil organic matter when amended with PRPB2 (pink) and Glucose (blue) additions. The brown colour lines are for control treatment.

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