Evidence of soil microbial population acclimatisation to long-term application of winery wastewater

Kim P Mosse[A, B, C], Antonio F Patti[A, B] and Timothy R Cavagnaro[C, D]

[A]School of Applied Sciences and Engineering, Monash University, Churchill, Vic. 3842, Australia
[B]Centre for Green Chemistry, Monash University, Clayton, Vic. 3800, Australia
[C]School of Biological Sciences, Monash University, Vic. 3800, Australia
[D]Australian Centre for Biodiversity, Monash University, Vic. 3800, Australia

Abstract
The long-term treatment of soil with winery wastewater (WWW) leads to an acclimatised soil microbial population. The acclimatised soil displays greater biological activity when exposed to further irrigation with winery wastewater. In this study, soil which had been acclimatised to winery wastewater for about 30 years was compared to the same soil, which had not received the same water. Furthermore, many medium to large wineries have their own water treatment plants. The ability to reuse winery wastewater, treated or untreated, requires a fundamental understanding of the potential toxicity of the water at different times of the year, coupled with the effects of the water on soils and plants. Two matched sites on the same soils were both treated with WWW (treated and untreated) and the microbial activity in the soils was monitored over two weeks, by measuring CO_2 efflux and a range of other soil parameters. It was found that the acclimatised soil displayed greater microbial activity, particularly with the untreated winery wastewater. Soil ammonium levels showed overlapping effects, however, elevated ammonium levels peaking after 6 days were associated with the acclimatised soil.

Key Words
Winery wastewater, soil microbial activity, CO_2 efflux.

Introduction
Land disposal of agro-industrial wastewaters is a potentially sustainable management practice, and could be of significant benefit in water-poor winegrowing regions of the world, including South Africa, California and south-eastern Australia. Currently, this practice is limited to designated 'dumping areas' and is generally considered to be unsuitable for irrigation of vines or crops; one of the key concerns is the long-term implications such a practice might have on soil health. Winery wastewater arises mostly from cleaning operations and spillage within the winery, and therefore is likely to consist of wine, grape juice and solids (vintage season only) and cleaning agents. The wastewater in most wineries is high in organics , containing predominantly sugars, followed by organic acids (acetic, tartaric, propionic), esters and polyphenols (Malandra *et al.* 2003). Inorganic ions present are predominantly potassium and sodium, with low levels of calcium and magnesium (Chapman *et al.* 1995). Whilst the constituents appear to be reasonably consistent across all wineries, the volumes generated and exact values are variable; for example, the Spanish wine industry generates six times the volume of wastewater than Italy or France (Bustamante *et al.* 2005), therefore meaning that direct comparisons of waste streams are largely insignificant. It is also important to note that the variability in wastewaters applies both spatially and temporally; the wastewater quality within a particular winery has been shown to vary on an hourly basis (Chapman 1995). As mentioned above, a major concern with wastewater reuse is the long term sustainability of such a practice. Whilst the effects of many contaminants are often not evident for a number of years, microbial populations have been shown to be an early warning signal of ecosystem perturbations (Wakelin *et al.* 2008; Friedel *et al.* 2000), and therefore of potentially unsustainable practices. Microorganisms play vital roles in nutrient and energy cycling, and are therefore are a critical component of any functioning ecosystem. This study aimed to assess the effects that long-term winery wastewater application upon vineyard soils has on the response to subsequent winery wastewater applications. A selection of preliminary results is presented in this paper.

Methods
Field Studies
Field studies were conducted in Coldstream, Victoria, Australia, in May-June 2009. Plots were located in two sites; 'acclimatised' soil, which had received winery wastewater and solid waste over a 30 year period, and 'non-acclimatised' soil, which had no history of wastewater application. The two sites were adjacent to

each other, separated by a man-made creek/drain. The soils at both sites were grey-brown silty clay loams of uniform texture formed on the Yarra River alluvial plain. The vegetative cover of the soils were predominantly weeds and pasture species. For each water treatment (winery wastewater, WWW; treated winery wastewater, TWWW; pure water dH_2O), 8L of water was applied to each of four replicate plots (1.5mx1.5m) over an eight-hour period. This was equivalent to application of 36ML/Ha, and consistent with industrial application rates.

Soil Sampling
From each plot, four soil samples were collected using a Dutch auger to collect the surface soil (0-7 cm), and the samples pooled to provide a replicate sample. Samples were collected 24 hours prior to water application, and 1, 3, 7 and 16 days after water application. Soil respiration was measured at each of these time points using a LiCor 6400 in conjunction with 10cm PVC collars inserted into each plot.

Soil physicochemical analyses
Inorganic N was extracted in the field, using 2M KCl. Extracts (three replicates) were frozen, and later used to determine nitrate (not reported here) and ammonium using the a modification of the methods of Miranda *et al.* (2001) for NO^{3-} (plus NO^{2-}) and Forster (1995) for NH_4^+
All spectrophotometric analyses were performed in Greiner 96 well plates, and analysed using a TeCan Evo Spectrophotometer.

Microbial Respiration and PLFA Analysis
Soil respiration was measured at each sampling time point using a LiCor 6400 in conjunction with 10cm PVC collars inserted into each plot. PLFA analysis is currently being conducted on sub-samples of fresh soil, but the data is not yet available for inclusion in this paper.

Statistical analysis
Results were analysed using a mixed model analysis in SPSS 16.0.

Results and discussion

Respiration
The application of wastewater resulted in significant increases in soil microbial activity, as shown by an increase in CO_2 efflux (Figure 1). This increase in CO_2 efflux was particularly evident the day after application, and was most noticeable in the acclimatised soils, with the application of untreated WWW. In general, the microbial response to water addition was greater in the acclimatised soil than the non-acclimatised soil, although the initial CO_2 efflux values were quite similar. This suggests that the microbial population in the acclimatised soils has altered, and responds more quickly when WWW is applied.

The application of untreated winery wastewater to the acclimatised soil gave the greatest CO_2 efflux (~8.3 umol/cm^3/sec) effect, one day after the water treatment. In contrast, the non-acclimatised soil also showed a peak in CO_2 efflux after one day, but somewhat lower ((~3.3 umol/cm^3/sec). The acclimatised soil subjected to TWWW showed a slight increase in CO_2 efflux, whereas, the non-acclimatised soil showed little change with TWWW. The distilled water treatment of both acclimatised and non-acclimatised soils did show some difference at day one, with the greater CO_2 efflux in the acclimatised soil. In all cases, the CO_2 efflux had dropped to "baseline" levels after 7 days. The results indicated that the soil which had a long history of winery wastewater application is likely to have different microbial populations, which have adapted to the water, hence the greatest response. The non-acclimatised soil did respond but to a lesser extent. The treated wastewater contained lower levels of total dissolved carbon and other nutrients, hence the lower responses, but nevertheless, TWWW still showed some response in the acclimatised soil, peaking at day 1.

Soil ammonium levels were also significantly affected by WWW addition, although the trends were not as consistent as in the case of respiration (Figure 1). Once again, the largest spike occurred in the combination of WWW and acclimatised soil; there was also a large spike for the combination of TWWW and non-acclimatised soil. Interestingly, the timing of these two spikes is separated by six days. These observations reflect two different effects are operating. The initial spike in ammonium levels observed one day after the addition of different water treatments to the soils may be associated with solubilisation of existing ammonium ions held in the soil. Interestingly, the distilled water treatment of the non-acclimatised soil shows no spike, suggesting pre-existing ammonium ion is negligible or below detection in the non-acclimatised soil. At the same time, the stimulated microbial populations will be accessing this liberated nitrogen where it is available. The TWWW sample contained lower levels of dissolved organic carbon and may also contain additional ammonium ions, thus giving the largest spike after day 1, but not at day 6. At day 6, the ammonium levels are primarily associated with microbial activity, with the acclimatised soil showed the highest levels with untreated wastewater. Unfortunately data for the water nitrogen analysis is currently unavailable, but will be investigated.

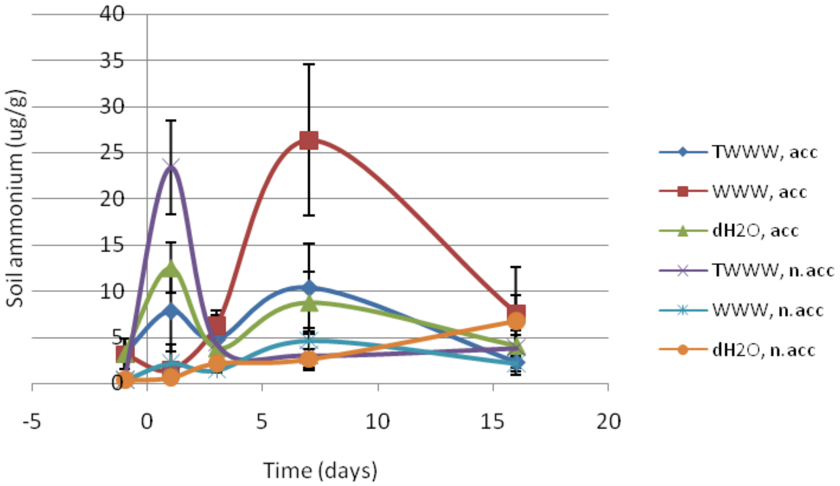

Figure 1. Effect of winery wastewater application on soil ammonium levels.

Conclusion

The data obtained to date shows that the application of WWW to soils over an extended time period causes changes in the response to subsequent WWW additions. It is likely that this altered response is mediated by a change in the soil microbial community; this change may be predominantly in the numbers of microorganisms present, or may also be in the types of microorganisms present. This area is therefore one of significant interest, and may be able to provide information relating to the sustainability of long-term winery wastewater application to soil. The PLFA analysis results will assist in providing evidence for the different microbial populations present.

Acknowledgements

This project was supported with funding from the Cooperative Research Centre for Irrigation Futures, and the Australian Grape and Wine Research and Development Council. Kim Mosse is in receipt of an Australian Postgraduate Award.

References

Bustamante MA, Paredes C, Moral R, Moreno-Caselles J, Perez-Espinosa A, Perez-Murcia MD (2005) Uses of winery and distillery effluents in agriculture: characterisation of nutrient and hazardous components. *Water Sci. Technol*. **51**(1), 145-51.

Chapman J (1995) PhD Thesis: Land Disposal of Winery and Distillery Wastewaters.

Chapman JA, Correll RL, Ladd JN (1995). Removal of soluble organic carbon from winery and distillery wastewaters by application to soil. *Australian Journal of Grape and Wine Research* **1**(1), 39-47.

Forster JC (1995) Soil nitrogen. *In* Methods in Applied Soil Microbiology and Biochemistry. (Eds. Alef K, Nannipieri P), pp. 79-87. Academic Press, San Diego, CA.

Friedel JK, Langer T, Siebe C, Stahr K (2000) Effects of long-term waste water irrigation on soil organic matter, soil microbial biomass and its activities in central Mexico. *Biology and Fertility of Soils* **31**(5), 414-421.

Malandra L, Wolfaardt G, Zietsman A, Viljoen-Bloom M (2003) Microbiology of a biological contactor for winery wastewater treatment. *Water Research* **37**(17), 4125-4134.

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous determination of nitrate and nitrite. *Nitrc. Oxide* **5**, 62-71.

Wakelin SA, Colloff MJ, Kookana RS (2008) Assessing the effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. *Applied and Environmental Micro* **74**, 2659-2668.