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Short communication

The potential in-situ antimicrobial ability of Myrtaceae plant species on pathogens in soil

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ABSTRACT

Concerns that land application of organic waste may introduce microbial contaminants into the environment could be mitigated by growing plants with antiseptic properties in waste-amended soil. We investigated the potential for two myrtaceous plants, manuka (*Leptospermum scoparium*) and kanuka (*Kunzea robusta*) with antiseptic properties to reduce numbers of the pathogen indicator *Escherichia coli* in soil. Pots containing perennial ryegrass (*Lolium perenne*), manuka and kanuka, were spiked with *Escherichia coli* and a rainfall event was simulated. Decimal reduction times (DRT) showed *E. coli* numbers were reduced under kanuka and manuka compared to a pasture control (8, 5 and 93 days respectively). Potentially, these myrtaceous species could mitigate the threat of microbial contamination of soil, while producing valuable biomass for fuel, essential oils or honey.

In many countries farmers traditionally cycle large quantities of animal and human wastes back onto the land for practical and economic reasons (Albihn, 2001; Hutchison et al., 2004; Petersena et al., 2007; Miaomiao et al., 2009). Such wastes can contain harmful elements such as heavy metals, antibiotics and disease causing organisms (Sarmaha et al., 2006; Elving et al., 2009; Miaomiao et al., 2009; Sidhu and Toze, 2009) that require strategies to control the associated environmental problems. The use of vegetative buffers for intercepting and mitigating contaminants are one such well-established on-farm technique for managing pollutants such as nitrogen and phosphorus.

Coupling the properties of plants as natural biofilters, with additional bioactive producing capabilities may offer enhanced ecosystem protection against potentially pathogenic organisms that re-cycled wastes can contain.

Manuka (*Leptospermum scoparium*) and kanuka (*Kunzea ericoides*) are pioneer species in the Myrtaceae family that colonise disturbed environments in New Zealand and South Australia (Mohan et al., 1984; Bergin et al., 1995; Stephens et al., 2005) and have known antiseptic properties (Stephens et al., 2005). Prosser et al. (2014) hypothesised that the components responsible for the antimicrobial ability of these plants may enter soil and ameliorate pathogen-contaminated sites. We aimed to determine the potential of manuka and kanuka to reduce the risks of pathogen contamination associated with recycling of organic waste to land. A silt loam soil (top 20 cm) was sourced from the Lincoln University Dairy Farm (43°38′11.70″S, 172°26′17.00″E); pH 5.6, C content 2.0%, and N content 0.18% (Table 1) (Knowles et al., 2011). The soil was homogenised, large roots removed and 6.25 g lime L^{-1} added to raise the pH to ~6. Nine of each of three plant species were used; Perennial ryegrass – *Lolium perenne* grown from seed (approx. 12 months old); Manuka – *L. scoparium*; Kanuka – *K. ericoides*, seedlings, approx. 2 years old.

Pots were adjusted to field capacity and inoculated with 1×10^9 cfu *Escherichia coli* (ATCC 13706; New Zealand Reference Culture Collection Medical Section, ESR, Wellington) (1×10^6 cfu *E. coli* g^{-1} dry soil) in 100 ml sterile PBS (phosphate buffered saline). Immediately following application, a rainfall event was simulated, one pore volume (1.375 L) of sterilised water was applied at the rate of 15 mm h^{-1}. Three of each plant species were chosen at random to be destructively harvested on days: 1 (T_0), 3 (T_1), and 7 (T_2) following *E. coli* inoculation. Soil samples were obtained by removing the bulk unattached soil and subsequently shaking the remaining soil from the plant roots. Soil was homogenised by mixing and analysed immediately. Soil pH was determined

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Table 1
Physicochemical profile of the soil used for the current study. Values represent averages ± standard error. Adapted from Knowles et al. (2011).

Property	Mean result (n = 3)
pH	5.6
CEC ($cmol_c\ kg^{-1}$)	12.4 ± 0.5
C (%)	2.0 ± 0.1
N (%)	0.18 ± 0.01
P ($mg\ kg^{-1}$)	518 ± 25
S ($mg\ kg^{-1}$)	193 ± 15
Ca ($mg\ kg^{-1}$)	3005 ± 101
Mg ($mg\ kg^{-1}$)	855 ± 11
K ($mg\ kg^{-1}$)	1401 ± 119
Na ($mg\ kg^{-1}$)	136 ± 4

according to Blakemore et al. (1987), and moisture content was determined by overnight drying at 105 °C. Enumeration of *E. coli* in soil was carried out using a five-tube Most Probable Number (MPN) (Feng et al., 2002). Microsoft Excel was used for the calculation of averages, standard deviations (SDs) and standard errors (SEs) for *E. coli* MPN and soil pH. MPN for *E. coli* were calculated using prepared MPN tables (Alexander, 1982; Woomer, 1994), and significance of difference was determined by comparison of 95% confidence intervals ($p < 0.05$). For pathogen data, log averages and associated SDs were calculated from each set of three replicates for each sample time. After fitting lineal trend lines to the data, decimal reduction times (DRT) were determined as described in Horswell et al. (2009). The hypothesis that all the trend lines had the same slope was tested using Analysis of Covariance (ANCOVA) $p = 0.031$. A p-value for each individual curve was calculated using the t-test.

Total *E. coli* declined in all treatments over the trial period but there was a significant ($p \leq 0.05$) difference in the rate of decline between plant species (Figs. 1 and 2). At day 7 (T_2) the number of *E. coli* (CFU) were significantly reduced under kanuka when compared to pasture; manuka also exhibited a considerable reduction in *E. coli* (Fig. 1). Analysis of Covariance (ANCOVA) undertaken on the lineal trend lines (Fig. 2) indicated that the trend lines had different slope ($p = 0.031$). Decimal reduction times (DRT) indicated soil under kanuka and manuka facilitated 90% reduction of *E. coli* after just 5 and 8 days respectively (Fig. 2), whilst DRT for pasture was 93 days. Results for soil pH indicated a difference between plant species in the order of kanuka < manuka < pasture (Fig. 3), however by day 3 this was not significant. Although pH has previously been shown to influence soil bacterial populations (Bååth, 1996) it is unlikely to be the mechanism by which manuka and kanuka affect the survival of *E. coli* in this study.

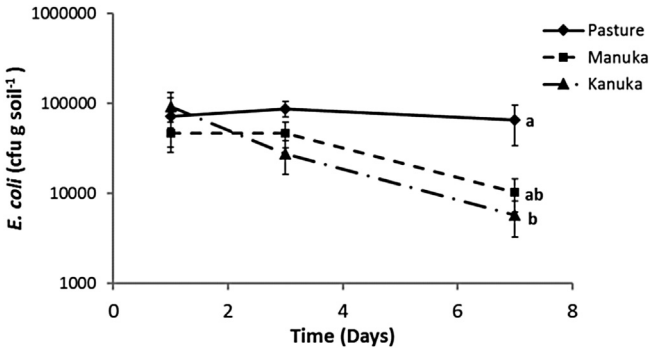

Fig. 1. Survival ($cfu\ g\ soil^{-1}$) of *E. coli* in soil samples from underneath pasture, manuka and kanuka over a seven day experimental period. Error bars represent standard error.

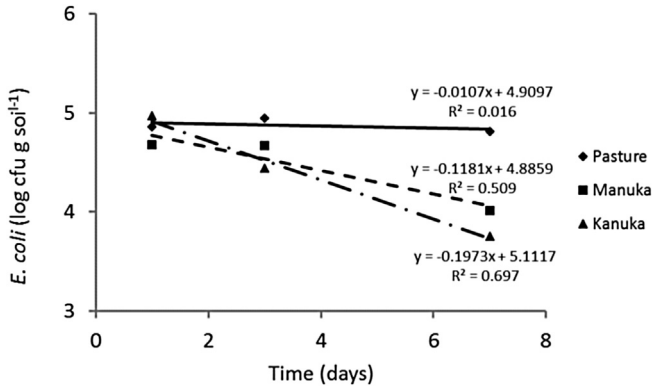

Fig. 2. Lineal trend lines applied to log cfu mpn data, used to calculate decimal reduction times (DRT) of *E. coli* under pasture, manuka and kanuka. DRT values represent the time (days) required for one log decline in *E. coli* numbers.

Decimal reduction times (DRT) indicated that soil under kanuka and manuka facilitated a more rapid reduction in total *E. coli* than the pasture control.

It has been suggested that the antibacterial agents from manuka may enter the soil via rhizodeposition (Prosser et al., 2014), thus altering rhizosphere soil through the release of root exudates, mucilage and sloughed cells (Castaldi et al., 2009). Studies have found that root exudates can remain stable in soils for significant lengths of time (Quartacci et al., 2009), and through altering soil conditions plant roots may be able to select for favourable microbial populations (Cheng et al., 2004; Quartacci et al., 2009). The antimicrobial agents responsible for the antimicrobial activity observed in this study are unknown. However, Cornes (2005) found that leptospermone (thought to be responsible for antimicrobial activity in manuka oil) produced by the roots of bottle brush (*Callistemon citrinus*) suppressed weeds around the plant's base. The phytochemical composition of manuka varies considerably between species, population, age and seasons (Porter et al., 1998; Porter and Wilkins, 1999). In manuka essential oil, triketones are reported to give manuka its antimicrobial properties. The major triketones are flavesone, iso-leptospermone and leptospermone, however, this varies with region and these triketones were not detected in manuka from some regions (Porter and Wilkins, 1999). The effect of antimicrobial plants will probably be different in different soils. This will be due to differences in the sorption of the phytochemicals from manuka and kanuka, resulting in differences in their

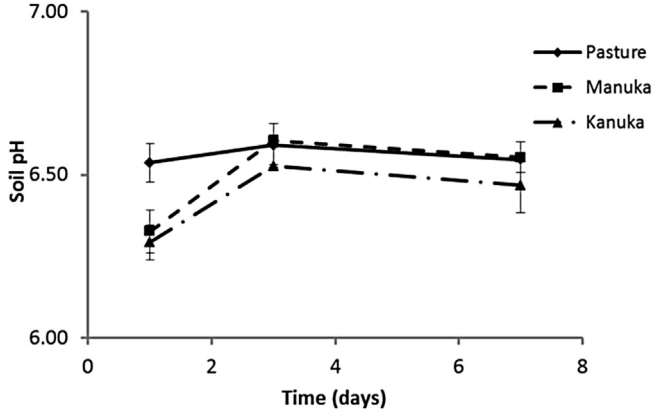

Fig. 3. Average soil pH from beneath pasture, manuka and kanuka plants grown in pots. Days represent time since *E. coli* application. Averages are that of three replicate plant pots, error bars represent standard error.

bioavailability to the pathogens. As noted previously, the chemical composition of these plants varies between regions due to both environmental and genetic factors (Porter et al., 1998; Porter and Wilkins, 1999).

The study indicates that antiseptic plant species may help reduce microbial contaminants in land applied organic wastes. Incorporating manuka and kanuka into shelter belts or riparian margins may help mitigate the risks of pathogen contamination of receiving waters.

References

Albihn, A., 2001. Recycling biowaste – human and animal health problems. Acta Veterinaria Scandinavica 95, 69–75.

Alexander, M., 1982. Most probable number method for microbial populations. In: second ed.Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties – Agronomy Monograph No. 9. ASA-SSSA, Madison, USA, pp. 815–820.

Bååth, E., 1996. Adaptation of soil bacterial communities to prevailing pH in different soils. FEMS Microbiology Ecology 19, 227–237.

Bergin, D.O., Kimberley, M.O., Marden, M., 1995. Protective value of regenerating tea tree stands on erosion-prone hill country, East Coast, North Island, New Zealand. New Zealand Journal of Forestry Science 25, 3–19.

Blakemore, L.C., Searle, P.L., Daly, B.K., 1987. Methods for Chemical Analysis of Soils. New Zealand Soil Bureau Scientific Report 80, p. 103.

Castaldi, P., Melis, P., Silvetti, M., Deiana, P., Garau, G., 2009. Influence of pea and wheat growth on Pb, Cd, and Zn mobility and soil biological status in a polluted amended soil. Geoderma 151, 241–248.

Cheng, Y., Howieson, J.G., O'Hara, G.W., Watkin, E.L.J., Souche, G., Jaillard, B., Hinsinger, P., 2004. Proton release by roots of medicago murex and medicago sativa growing in acidic conditions, and implications for rhizosphere pH changes and nodulation at low pH. Soil Biology & Biochemistry 36, 1357–1365.

Cornes, D., 2005. Callisto: a very successful maize herbicide inspired by allelochemistry. In: Proceedings of the Fourth World Congress on Allelopathy - Establishing the Scientific Base, Wagga Wagga, Australia.

Elving, J., Ottoson, J.R., Vinneras, B., Albihn, A., 2009. Growth potential of faecal bacteria in simulated psychrophilic/mesophilic zones during composting of organic waste. Journal of Applied Microbiology 108, 1974–1981.

Feng, P., Weagant, S.D., Grant, M.A., 2002. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Part 2. Chemical and Microbiological Properties – Agronomy Monograph no. 9, second ed. eds. ASA-SSSA, Madison, USA, pp. 815–820.

Horswell, J., Hewitt, J., Prosser, J., Van Schaik, A.P., Croucher, D., Macdonald, C., Burford, P., Susarla, P., Bickers, P., Speir, T., 2009. Mobility and survival of *Salmonella Typhimurium* and human adenovirus from spiked sewage sludge applied to soil columns. Journal of Applied Microbiology 108, 104–114.

Hutchison, M.L., Walters, L.D., Moore, A., Crookes, M., Avery, S.M., 2004. Effect of length of time before incorporation on survival of pathogenic bacteria present in livestock wastes applied to agricultural soil. Applied and Environmental Microbiology 70, 5111–5118.

Knowles, O.A., Robinson, B.H., Contangelo, A., Clucas, L., 2011. Biochar for the mitigation of nitrate leaching from soil amended with biosolids. Science of the Total Environment 409, 3206–3210.

Miaomiao, H., Wenhong, L., Xinqiang, L., Donglei, W., Guangming, T., 2009. Effects of composting process on phytotoxicity and speciation of copper, zinc and lead in sewage sludge and swine manure. Waste Management 29, 509–597.

Mohan, E., Mitchell, N., Lovell, P., 1984. Seasonal variation in seedfall and germination of *Leptospermum Scoparium* (Manuka). New Zealand Journal of Botany 22, 103–107.

Petersena, S.O., Sommerb, S.G., Bélinec, F., Burtonc, C., Dachd, J., Dourmade, J.Y., Leipf, A., Misselbrookg, T., Nicholsonh, F., Poulsena, H.D., Provoloi, G., Sørensena, P., Vinneråsj, B., Weiskek, A., Bernall, M.-P., Böhmm, R., Juhászn, C., Mihelico, R., 2007. Recycling of livestock manure in a whole-farm perspective. Livestock Science 112, 180–191.

Porter, N.G., Wilkins, A.L., 1999. Chemical, physical and antimicrobial properties of essential oils of Leptospermum scoparium and Kunzea ericoides. Phytochemistry 50, 407–415.

Porter, N.G., Smale, P.E., Nelson, M.A., Hay, A.J., Van Klink, J.W., Dean, C.M., 1998. Variability in essential oil chemistry and plant morphology within a *Leptospermum scoparium* population. New Zealand Journal of Botany 36, 125–133.

Prosser, J.A., Anderson, C.W.N., Horswell, J., Speir, T.W., 2014. Can manuka (*Leptospermum scoparium*) antimicrobial properties be utilised in the remediation of pathogen contaminated land? Soil Biology and Biochemistry 75, 167–174.

Quartacci, M.F., Irtelli, B., Gonnelli, C., Gabbrielli, R., Navari-Izzo, F., 2009. Naturally assisted metal phytoextraction by *Brassica carinata*: role of root exudates. Environmental Pollution 157, 2697–2703.

Sarmaha, A.K., Meyerb, M.T., Boxall, A.B., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65, 725–759.

Sidhu, J., Toze, S.G., 2009. Human pathogens and their indicators in biosolids: a literature review. Environment International 35, 187–201.

Stephens, J.M.C., Molan, P.C., Clarkson, B.D., 2005. A review of *Leptospermum scoparium* (Myrtaceae) in New Zealand. New Zealand Journal of Botany 43, 431–449.

Woomer, P.L., 1994. Most probable number counts. In: Weaver, R.W., Angle, S., Bottomly, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A. (Eds.), Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties, vol. 5. ASA-SSSA, Madison, USA, pp. 59–79.