Ecological Engineering 183 (2022) 106741

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Interactions of treated municipal wastewater with native plant species

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ARTICLE INFO

Keywords:
Treated municipal wastewater
Land treatment
Native ecosystems
Soil-plant interactions
Ecological restoration

ABSTRACT

Potentially, the restoration of native ecosystems could be combined with the land application of treated municipal wastewater (TMW), reducing TMW discharge into waterbodies. High levels of nutrients, pathogens, and other contaminants from TMW can degrade water quality. The land application of TMW onto native vegetation reduces the nutrient load in water bodies and may create zones of ecological value. However, establishing native plants may be challenging if the species are not adapted to highly fertile environments, such as those resulting from TMW irrigation. There is a critical knowledge gap about the response of native plant species to irrigation with TMW. We aimed to determine the distribution and speciation of nutrients in the soil-plant system following application of TMW onto 11 species of native plants in a long-term field trial on Banks Peninsula, New Zealand (NZ). TMW was irrigated at a rate of 1000 mm per annum, equivalent to N, and P loading rates of 194 and 110 kg ha yr^{-1}, respectively. We determined physicochemical properties from soil profiles (0–65 cm) under selected species as well as the growth and chemical composition of the plants. Despite the site receiving 950 kg ha^{-1} yr^{-1} of Na, there was no evidence of impaired soil structure following TMW irrigation. Nitrogen did not accumulate in the soil, and it is likely to have been taken up by plants or lost through denitrification and nitrate leaching. The accumulation rate of P indicated that soil P concentrations will remain within the range found in NZ agricultural soils for at least 50 years. TMW irrigation increased plant height by 10% compared to the control after 3.5 years of growth. Plant species significantly affected the concentrations of total C, total N, nitrate (NO_3^-), and Na in the soil. TMW application had negligible effects on the elemental composition of plant foliage. NZ native vegetation can facilitate the land application of TMW. Future work should elucidate the maximum rates that can be applied as well as the effect of TMW on the soil microbiota.

1. Introduction

United Nations Sustainable Development Goals (SDGs), Target 6.3, calls for increased wastewater recycling and reuse to improve the water quality in aquatic ecosystems (WWAP, 2017). When discharged into waterways, wastewater can exacerbate eutrophication (Carey and Migliaccio, 2009). Its origin and level of treatment determine the concentrations of sodium, nutrients, pathogens, and trace elements in wastewater (Norton-Brandão et al., 2013). Applying Treated Municipal Wastewater (TMW) to land may reduce waterway degradation and mitigate the public health risks (Hamilton et al., 2007). This practice is particularly valuable in arid and semiarid areas where the pressure on available water is high due to the irrigation of agricultural land (Pedrero et al., 2010). The nutrients in TMW reduce the need for synthetic fertilisers (Cirelli et al., 2012). However, the application of wastewater onto productive agricultural land is not appropriate where insufficient treatment poses a public health risk (Sato et al., 2013). Additionally, public and industry perceptions can hinder the use of TMW for agricultural production (Lowe, 2009; Simcock et al., 2019). Many indigenous peoples, including Māori in New Zealand (NZ), do not allow the combination of waste from human origin with food production chains (Pauling and Ataria, 2010).

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https://doi.org/10.1016/j.ecoleng.2022.106741
Received 5 April 2022; Received in revised form 4 July 2022; Accepted 19 July 2022
Available online 5 August 2022

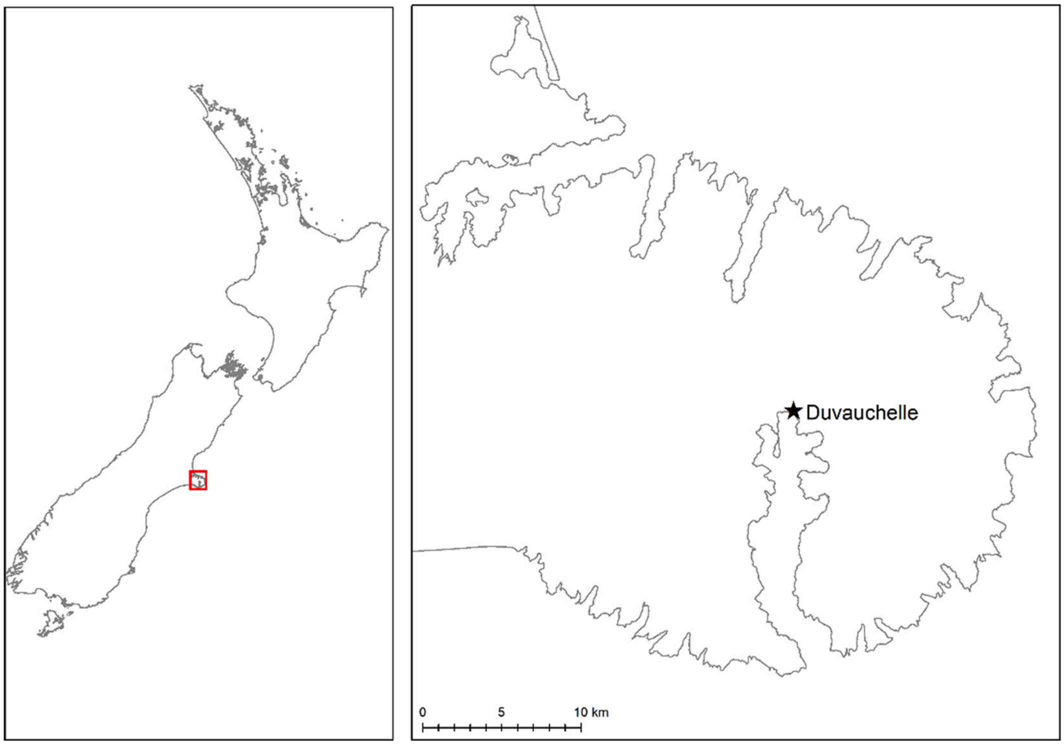

Fig. 1. Location of the field site in Duvauchelle, Banks Peninsula, on the east coast of the South Island of New Zealand.

Non-agricultural practices for the land application of TMW include the creation of artificial wetlands (Kivaisi, 2001), the production of energy crops, such as *Salix* spp. (Gonzalez-Garcia et al., 2012), and the irrigation of public and private green spaces, including golf courses (Qian and Mecham, 2005). Many of these systems consist of monocultures and plants that are exotic to the local area. Alternatively, native plants could be used in wastewater land treatment systems to mitigate contaminants. Native hyperaccumulators are used for the phytoremediation of land contaminated with trace elements from irrigation with municipal and industrial wastewater (Hasan et al., 2021; Irshad et al., 2015; Sharma et al., 2021). In artificial wetlands, native species are used to support local biodiversity and to prevent the naturalisation and spread of exotic species (Maschinski et al., 1999; Quadros et al., 2017). Combining the land application of TMW with restoration projects may not only provide an opportunity to increase native biodiversity (Cunningham and Gharipour, 2018), but may also accelerate public support for both concepts (Van Diggelen et al., 2001).

Table 1
Characteristics of the treated municipal wastewater (TMW) used at the field site in Duvauchelle and application rate in the TMW irrigated treatment, receiving 1000 mm yr^{-1}. Adapted from Gutierrez-Gines et al. (2020).

Variable	Concentration[a]	Application rate (kg ha^{-1} yr^{-1})
pH	7.5	–
EC (μS cm^{-1})	423 (40)	–
Total suspended solids	32	320
NH_4^+-N	0.49 (0.15–0.80)*	4.9
NO_3^--N	18 (7.5)	180
NO_2^--N	0.86 (0.09)	8.6
Al	0.43 (0.11–1.7)*	4.3
B	0.10 (0.04)	1
Ca	59 (12)	590
Cd	<0.001	–
Cu	0.04 (0.03)	0.4
Fe	0.96 (0.25–3.6)*	9.6
K	22 (5.0)	220
Mg	19 (5.5)	190
Mn	0.06 (0.03)	0.6
Na	95 (21)	950
P	11 (5.0)	110
S	25 (11)	250
Zn	0.17 (0.11)	1.7
Sodium Adsorption Ratio (SAR)[b]	2.75	–

Values are means and standard deviations of the mean (* geometric mean and standard deviation range), $n = 54$, except for trace elements $n = 14$.
[a] Values are in mg L^{-1} unless otherwise indicated.
[b] Relative concentration of Na to Ca and Mg, calculated according to Ayers and Westcot (1985)

The value of native ecosystems is increasingly recognised worldwide, and the interest in rehabilitation and restoration of native forests and degraded lands is growing (Thomas et al., 2014). In New Zealand (NZ), native land cover has decreased by 72% since the arrival of humans and eventual agricultural intensification (Ewers et al., 2006). Within intensive agricultural landscapes, native vegetation now covers as little as 0.5% of the land (Franklin et al., 2015). Native vegetation increases local biodiversity by providing habitats for indigenous birds, arthropods, mammals, and other successional plant species, and helps to reduce greenhouse gas emission through carbon sequestration (Dymond et al., 2013). Many native ecosystems are adapted low fertility soils (Wardle, 1985). Therefore, it may be challenging to establish native species in environments receiving high-nutrient TMW irrigation. Mohammadi et al. (2021) found that a desert plant native to Iran (*Niteria schoberi*) responded positively to TMW application. TMW irrigation could, therefore, accelerate the revegetation of native plants with beneficial effects on local economies and environments (Abdullah et al., 2022). Franklin et al. (2015) reported that some NZ native species tolerated high application rates of N but did not show an increase in plant biomass. However, Reis et al. (2017) and Gutierrez-Gines et al. (2019) measured large increases of *L. scoparium* biomass following the application of biosolids. A positive growth response to biosolids application was described in various, but not all, native species by Gutierrez-Gines et al. (2017). Therefore, it is unknown how the addition of water and nutrients from TMW irrigation would affect the growth of different

Table 2
Eleven perennial New Zealand native plant species were planted at the field trial, divided into three vegetation types. Māori names and scientific names are shown. Scientific names were reviewed on 30 June 2022 according to https://nzflora.landcareresearch.co.nz/.

Vegetation type	Species	Common name
1	*Leptospermum scoparium* J.R.Forst. & G.Forst.	Mānuka[a]
	Kunzea robusta de Lange & Toelken	Kānuka[a]
2	*Olearia paniculata* (J.R.Forst. & G.Forst.) Druce	Akiraho[a]
	Pseudopanax arboreus (L.f.) K.Koch	Puahou[a]
	Coprosma robusta Raoul	Karamu[a]
	Podocarpus laetus Hooibr. ex Endl.	Hall's tōtara[a]
3	*Griselinia littoralis* (Raoul) Raoul	Kapuka[a]
	Pittosporum eugenioides A.Cunn.	Tarata[a]
	Cordyline australis (G.Forst.) Endl.	Tī kōuka[b]
	Phormium tenax J.R.Forst. & G.Forst.	Harakeke[c]
	Phormium cookianum Le Jol.	Wharariki[c]

[a] Dicotyledonous trees and shrubs.
[b] Monocotyledonous tree.
[c] Monocotyledonous herbs

species. Nevertheless, if biomass is not harvested for native products, there is no removal of nutrients from a TMW land application site. This might result in nutrient imbalances in the soil or increased leaching and runoff. The application of TMW to land is largely limited by N, P, sodicity and heavy metals (Barton et al., 2005; Houlbrooke et al., 2011; Tzanakakis et al., 2009), and the fluxes of the fate of these compounds when TMW is applied to native vegetation require investigation to determine the sustainability of such TM land application systems.

We hypothesised that the land application of TMW combined with the re-establishment of native ecosystems on marginal land would increase native biodiversity, without unacceptable accumulation or loss of inorganic contaminants. This research aimed to quantify responses of NZ native vegetation to irrigation with TMW. Specifically, we sought to determine (i) changes in the distribution and speciation of nutrients and inorganic contaminants in the soil-plant system, and (ii) the establishment and growth of NZ native species under TMW irrigation.

2. Materials and methods

2.1. Field site

A field trial was set up in July 2015 in Duvauchelle (43°45′9"S, 172°56′36″E), Banks Peninsula, on the east coast of the South Island of NZ (Fig. 1). The soil (a Pawson silt loam, 46% sand, 29% silt, 25% clay) has a short period of summer moisture deficit (Molloy, 1988). The average annual rainfall in the nearby town of Akaroa is 969 mm (Macara, 2016). The trial consisted of 24 blocks of 5 m × 5 m with a total of 1350 plants (Fig. S-1). Half of the blocks were irrigated with TMW from the local wastewater treatment plant (WWTP) at a rate of 1000 mm yr^{-1} through surface drip irrigation, which started in January 2016. Table 1 shows the properties of the TMW and annual application rates of the components. The other half were control blocks that did not receive any form of irrigation. The trial included eleven perennial NZ native species divided into three different vegetation types (Table 2), which resulted in four replicates per vegetation type and treatment. Vegetation type 1 included the most common early successional species in NZ (Sullivan et al., 2007), type 2 contained species that are well adapted to wet environments (Czernin and Phillips, 2005), and type 3 contained species that are naturally regenerating on Banks Peninsula (Wilson, 1994). The species used were of local provenance (Department of Conservation, 2021). Seedlings were 2 years old when they were planted into established *Dactylis glomerata* (cocksfoot) at the site, which was used for extensive sheep grazing prior to the experiment. Tree guards were used to protect the plants from weed competition. A high density of 2 plants m^{-2} was chosen to achieve fast canopy closure and data collection.

2.2. Plant monitoring

The number of dead plants was recorded in October 2015, November 2015, December 2015, and June 2019 to calculate survival rates. The height of each plant was measured in June 2019, using a measuring tape.

2.3. Soil and plant sample collection

Soil samples were collected in October–November 2018, from underneath five species: *L. scoparium*, *K. robusta*, *Coprosma robusta*, *Cordyline australis* and *Phormium tenax* (Table 1). These species were chosen because they are commonly used in restoration plantings throughout NZ and are adapted to wet environments that can result from TMW irrigation (Franklin et al., 2019). We dug a total of 24 soil pits, four per vegetation type and treatment combination. The pits in vegetation types 1 and 3 were dug in a place that allowed to sample both targeted species on either side of each pit. Soil pits were dug to a depth of 65 cm and had a width of 60 cm × 60 cm. The locations of the pits within each plot were chosen randomly. A hand trowel was used to access densely rooted soil directly underneath the plant, where soil samples were taken at five different depths: 0–5 cm, 10–20 cm, 25–35 cm, 40–45 cm, and 55–65 cm. This ultimately resulted in a total of 200 soil samples that were transported to the laboratory on ice, where they were kept at 4 °C overnight until further analysis.

In June 2019 the foliage of the 40 plants where soil samples were taken from was sampled. For each plant, 10 branches or leaves of different age and aspect were cut by secateurs and combined to generate a representative sample. For *P. tenax* and *C. australis*, the entire leave was cut at its base. For *C. robusta*, *K. robusta*, and *L. scoparium* branches with a stem diameter < 10 mm were selected and cut off at the stem.

2.4. Soil and plant analysis

Soil exchangeable NO_3^- and NH_4^+ were extracted from 4 g of fresh soil with 2 M KCl (Blakemore et al., 1987). Colorimetric methods were used to determine NO_3^- (Miranda et al., 2001) and NH_4^+ concentrations (Mulvaney, 1996) in the extract, using a Cary 100 Bio UV–visible spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

Soil moisture content was determined by drying a subsample of fresh soil at 105 °C for 24 h (Blakemore et al., 1987). The rest of the soil was dried at 40 °C for 4 days and sieved to <2 mm. Plant samples were washed with deionised water before being dried at 60 °C for 4 days. Leaves of *C. robusta*, *K. robusta*, and *L. scoparium* were then separated from the stems. Dried plant leaves and soils were ground with a Rocklabs Bench Top Ring Mill (Scott, Dunedin, New Zealand).

A Vario-Max CN Elemental Analyser (Elementar, Langenselbold, Germany) was used to determine total C and N in the ground soil samples. A CN828 Carbon/Nitrogen analyser (LECO, St. Joseph, MI, USA) was used to determine total C and N in the ground plant samples. Soil pH was determined in deionised water using a 1: 2.5 soil: water ratio with a HQ 440d Multi-Parameter Meter with pH probe PHC735 (HACH, Loveland, CO, USA). In all, 0.2 g of ground soil and plant samples were digested in 5 mL 69% HNO_3. Samples were left to pre-digest overnight prior to digestion in a ultraWAVE microwave digester (Milestone Srl, Sorisole, Italy) at 220 °C and 110 bar. Elemental concentrations in the soil digests were analysed by ICP-OES (Varian 720-ES, Agilent Technologies, Santa Clara, CA, USA). Elemental concentrations in the plant digests were analysed by ICP-MS (7500cx, Agilent Technologies, Santa Clara, CA, USA). To determine phytoavailable concentrations of elements, 5 g of soils were extracted with 0.05 M $Ca(NO_3)_2$ (Gray et al., 1999). Elemental concentrations in the extracts were analysed by ICP-MS (Agilent 7500cx). Plant-available phosphorus (Olsen P) was determined in a 0.5 M $NaHCO_3$ extract, using a Cary 100 Bio UV–visible spectrophotometer for colorimetric analysis (Olsen et al., 1954). Certified reference materials were included for soil and plant digestions (SRM

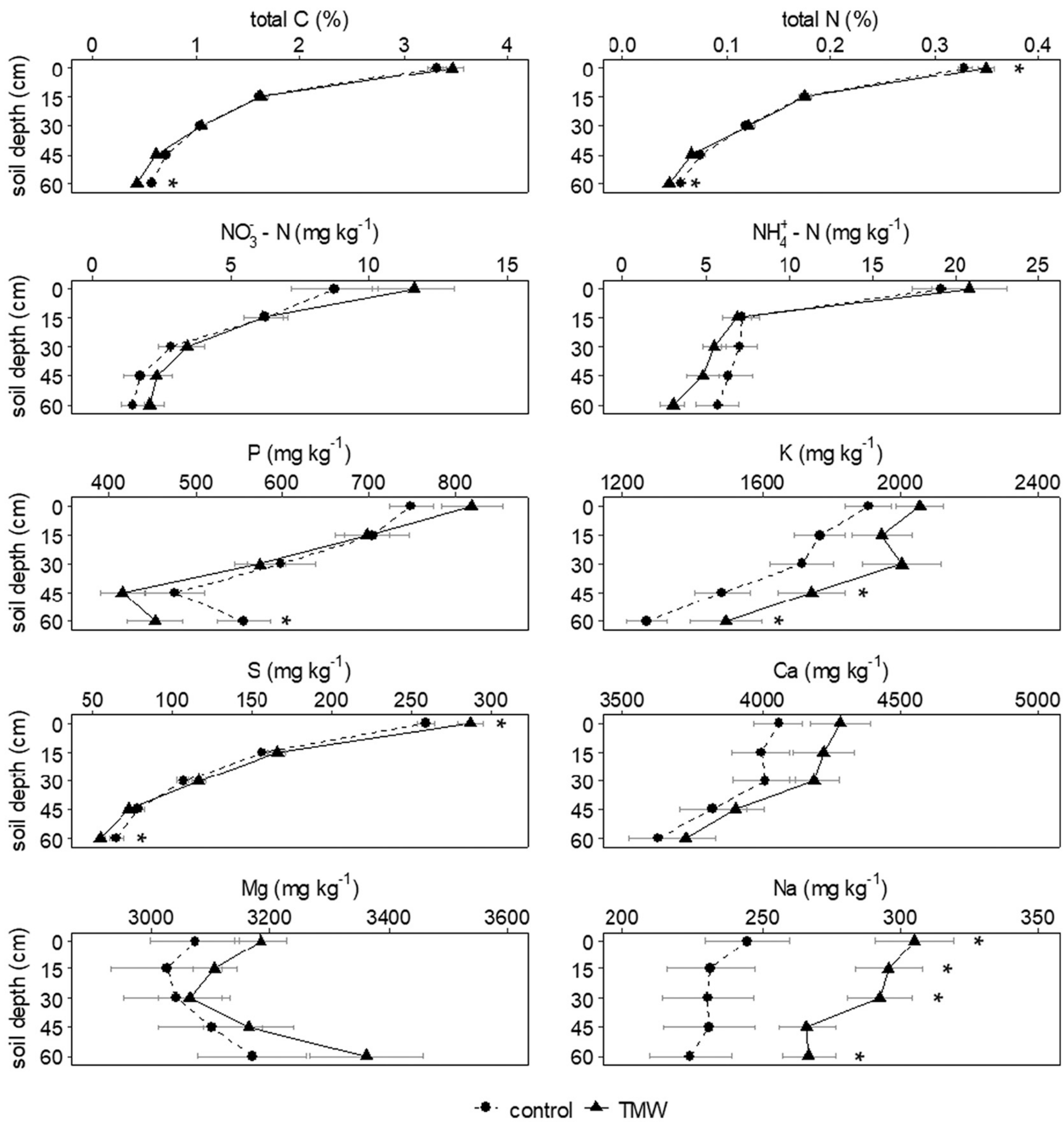

Fig. 2. Concentrations of elements in the soil profile (0–60 cm) at the site in November 2018, comparing TMW irrigated plots and non-irrigated control (all plants combined). Values show means and associated standard errors ($n = 20$). Asterisks (*) indicate significant differences between treatments at $p < 0.05$ according to two-tailed unpaired *t*-test.

2710a Montana I Soil and SRM1573a Tomato Leaves, National Institute of Standards and Technology, U.S. Department of Commerce). Recoveries ranged from 9 (for Na) to 110% for soil digests and from 92 to 125% for plant digests. While recoveries were low for soil digests, they agreed with results reported by other laboratories that used USEPA SW-846 Method 3050B (NIST, 2018). Analysis of a subset of samples for Na by an accredited laboratory correlated with our results, with y = 1.0× at $r = 0.95$ and $p < 0.001$ (Fig. S-2).

2.5. Statistical analysis

Data were analysed with R (R Core Team, 2021). Three-way analysis of variance (ANOVA) was carried out for soil parameters. Depth, species, and irrigation were used as independent variables with interactions. The residuals were plotted to test the assumptions of normality and homoscedasticity. Data were $\log_{10}$ or square-root transformed where the assumptions were not met. Tukey's honestly significant difference (HSD) post-hoc test was used where significant effects were found, using the package *agricolae* (de Mendiburu, 2021). A two-tailed unpaired *t*-test was used to compare element concentration at each depth individually after combining results from all plant species. Two-way ANOVA was carried out for plant parameters, using species and irrigation as independent variables with interaction effects. The package *multcomp* (Hothorn et al., 2021) was used for Tukey's HSD post-hoc test where significant effects were found. Assumptions for ANOVA were tested as described for soil parameters. Plant heights in the TMW and control blocks were compared for each species individually by two-tailed unpaired t-test. The significance level for all results was at $p \leq 0.05$. The package *factoextra* (Kassambara and Mundt, 2016) was used to perform a principal component analysis (PCA) of plant variables.

3. Results and discussion

3.1. Elemental concentrations in soils

The Sodium Adsorption Ratio (SAR) of the TMW was 2.75 (Table 2), which was within the limit of 6 that is used as a guideline for effluent irrigation in Australia (Hanjra et al., 2012). However, irrigation of TMW with this SAR and an electric conductivity (EC) of 423 µS cm^{-1} (Table 2) can lead to the degradation of soil structure through clay dispersion (ANZECC, 2000; Mojid and Wyseure, 2013). There were no visual signs of ponding or runoff following the irrigation of TMW at the site that would have indicated that infiltration was impaired. This is consistent with findings by McIntyre (2018), whereby infiltration rates of local soils were not impaired by TMW irrigation with similar Na loading rates. Na significantly increased throughout the soil profile, except at 40–50 cm depth (Fig. 2). Nevertheless, only 735 kg Na ha^{-1} was recovered in the soil following TMW irrigation equivalent to 2700 kg Na ha^{-1} over the experimental period. This reflects the mobility of Na in the soil and indicates that the majority of applied Na leached through the soil profile. Our results were consistent with those of Gutierrez-Gines et al. (2020), who reported that Na accumulation in TMW irrigated silt loams from the same region was not proportional to Na application. While the accumulation of Na can increase soil pH (Blume et al., 2016), we did not find significant differences between treatments. The average pH in the topsoil at 0–5 and 10–20 cm was 5.7 and 5.6, respectively. This is within the optimal range for plant growth (Neina, 2019).

High application rates of Na compared to Ca and Mg can lead to increased leaching of these cations and K^+ from the soil (Chahal et al., 2011). This was not observed at our field site, with a trend of all these elements to increase with TMW irrigation (Fig. 2). K was significantly higher in the subsoil (40–50 and 55–65 cm) of TMW irrigated plots. K can be preferentially adsorbing in the soil and therefore result in a reduction of Mg (Liang et al., 2021). However, Ca and Mg showed an overall (0–65 cm) increase of 5.5% and 4.6% respectively following TMW irrigation. This was likely a result of their high application rate and low SAR of the TMW (Table 2). Ca and Mg can offset possible negative effects of Na and K on the soil structure (Gutierrez-Gines et al., 2020). Our results indicated that TMW had no negative effects on the equilibrium of cations in the soil and that there was a low risk of Na impairing soil structure and plant growth.

While total N significantly increased in the topsoil (0–5 cm) by 6%, it significantly decreased by 21% in the subsoil (55–65 cm) (Fig. 2). The application rate of N at the site was 194 kg N ha^{-1} yr^{-1}, but total N throughout the soil profile only increased by 5.6 mg kg^{-1} over the experimental period, equalling to an increase of 17 kg N ha^{-1} yr^{-1}. Possible pathways of N losses are through plant uptake, NO_3^- leaching, or emissions of N_2 or N_2O following denitrification. The latter can be elevated with increased soil moisture and elevated pH in the TMW irrigated plots (Clough et al., 2004; Šimek and Cooper, 2002). Barton et al. (1999) reported that just 1% of N applied with TMW is emitted as N_2O. Assuming such losses we could expect 2 kg N_2O-N ha^{-1} yr^{-1} to be emitted from the irrigated plots at our site. Despite N_2O having a global warming potential 300 times higher than that of CO_2 (Griffis et al., 2017) this emission rate is low compared to normal soil respiration in NZ native shrubland of ca. 10 t CO_2-C ha^{-1} yr^{-1} (Hedley et al., 2013). Leaching could account for a larger proportion of N losses, as Sparling et al. (2006) reported that up to 22% of applied TMW-N leached from soil after 4 years of irrigation. However, Gutierrez-Gines et al. (2020) found that NO_3^- leaching from a local Silt Loam irrigated with TMW from the same WWTP at double N loading rates equalled to just 2 kg N ha^{-1} over 17 months. Soil NO_3^- concentrations were unaffected by TMW irrigation. This is consistent with results of Sparling et al. (2006) who found no increased NO_3^- in the soil after 4 years of TMW irrigation onto four different NZ soils. Given the high mobility of NO_3^- in soil (Cameron et al., 2013), it is likely that NO_3^- that was not taken up by plants was leached from the soil. Soil NH_4^+ was not significantly different in the TMW irrigated plots but tended to be reduced under TMW irrigation, where NO_3^- was increased. This could indicate accelerated nitrification with TMW irrigation and increased NH_4^+ supply (Robertson and Groffman, 2015).

The lowest concentration of NO_3^- was measured under *C. robusta* (3.1 mg kg^{-1}, Table S-1) and it was significantly higher under *K. robusta* (6.2 mg kg^{-1}). The results are consistent with those of Franklin et al. (2015), who reported that the selection of plant species affects N fluxes in the soil profile. However, unlike reported by Esperschuetz et al. (2017) and Halford et al. (2021), NO_3^- concentrations in the soil under the myrtaceous species *L. scoparium* and *K. robusta* were not lower than under other species. Low NO_3^- concentrations under *C. robusta* were not reported previously, and underlying mechanisms are unknown. NZ native plant species have diverse root morphologies (Franklin et al., 2019), which can affect fluxes of waters through impacts on preferential flow and hydraulic conductivity (Clothier et al., 2007). In addition, distinct exudation from plant roots can affect the mobility of elements and the rates of biogeochemical nutrient cycling, as is the case for N (Carlton et al., 2019). Due to the high mobility of NO_3^- (Di and Cameron, 2002), lower concentrations indicate reduced likelihood of NO_3^- leaching and therefore ground- and surface water contamination and associated public health risk (McDowell et al., 2009).

As with total N, total C was significantly lower in the subsoil (55–65 cm) following TMW irrigation, showing a 25% decrease. It is possible that the high input of N and C with TMW irrigation lead to increased microbial activity, referred to as the priming effect (Kuzyakov et al., 2000). Jueschke et al. (2008) reported that while total soil organic C decreased in the subsoil under long-term TMW irrigation, total C in the topsoil significantly increased. At our site total C concentrations did not change at any other soil depths.

Phosphorus was not significantly affected by TMW irrigation, except for an 18% decrease in the subsoil (55–65 cm). P is generally immobile in soils and vertical movement in the soil profile occurs through macropores and preferential flow (Gupta et al., 1999). However, due to its immobility, P is mainly lost through surface runoff from a TMW irrigated area, and there were no signs of erosion and runoff at the site. The diverse morphology of native plant roots can stabilise the soil and reduce the risk of erosion (Franklin et al., 2019). Therefore, expected P losses from the site via runoff and erosion will be small. Gutierrez-Gines et al. (2020) reported that TMW irrigation onto a local silt loam at a rate of 75 kg P ha^{-1} yr^{-1} would not result in soil P exceeding 1750 mg kg^{-1}

Table 3
Results of three-way ANOVA for soil parameters with significant depth, interaction, species, or interaction effects (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). Variables with significant depth effects only were excluded.

Soil variable & data transformation	Depth	Irrigation	Species	Depth × Irrigation	Depth × Species	Irrigation × Species	Depth × Irrigation × Species
$Log_{10}(C)$	***	**	**	**			
$Log_{10}(N)$	***	**	**	**			
$Log_{10}(NO_3^-\text{-N})$	***		**		*		
$Log_{10}(NH_4^+\text{-N})$	***			*		***	
P	***			*			
K	***	***				*	
$Log_{10}(S)$	***		*	***			
Ca	***	*					
Mg	***	*	*				
$Na^{1/2}$	*	***	**			***	
$Log_{10}(As)$	***	***				*	
$Log_{10}(Cr)$		**				***	
$Log_{10}(Li)$	***	***					
$Log_{10}(Pb)$	***	*		*	*		

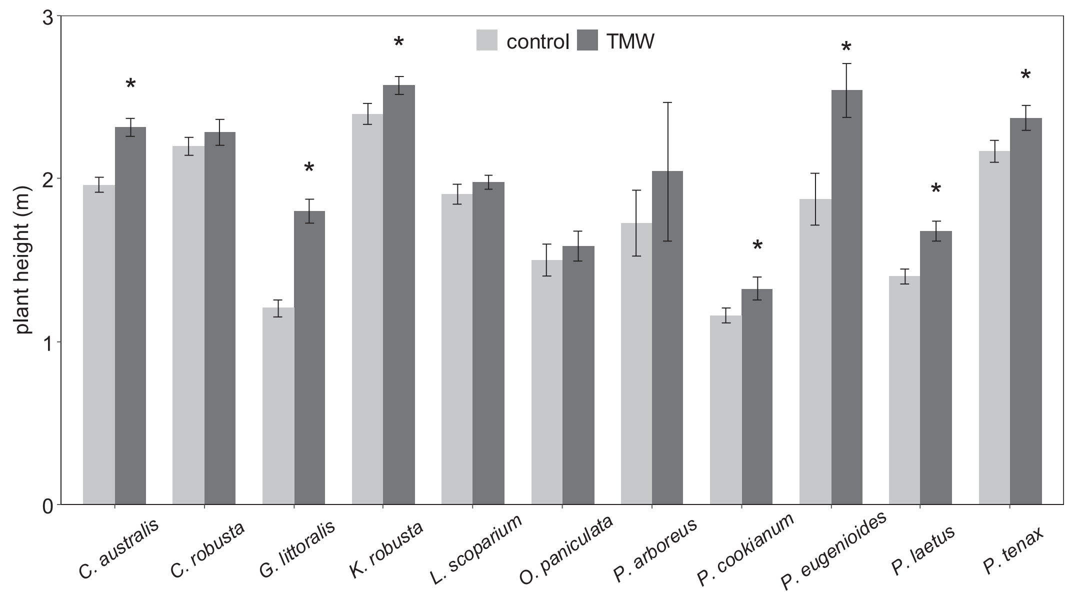

Fig. 3. Plant height at the site (4 years after establishment) by species, comparing plants growing in TMW irrigated plots and non-irrigated control plots. Bars show means and error bars show standard errors ($n = 7$–83). Asterisks (*) indicate significant differences ($p < 0.05$) between treatments according to *t*-test.

for at least 50 years. Similarly, based on the simulation of Gutierrez-Gines et al. (2020) with adjusted starting parameters (Table S-2) and no P removal, TMW irrigated soils at the site are not expected to exceed P concentration ranges in NZ productive soils (360–2640 mg kg^{-1}, Reiser et al., 2014) for at least 50 years. In our experiment, Olsen P in the TMW irrigated soils was not significantly increased. The Olsen P in the control and TMW irrigated plots was 14 and 17 mg kg^{-1} respectively, which is below recommended values for productive soils and indicates that native plants have low P requirements (Gutierrez-Gines et al., 2020).

Sulphur significantly increased with TMW irrigation in the topsoil (0–5 cm) and decreased in the subsoil (55–65 cm). While 250 kg S ha^{-1} yr^{-1} were applied with TMW, we observed no overall increase in the soil profile. This is consistent with the high mobility of S in soil and typical leaching losses of 20–120 kg S ha^{-1} yr^{-1} even from non-irrigated soil (Blume et al., 2016). However, while S can lead to acidification of soils, it is not associated with eutrophication (Posch et al., 2015).

None of the soil trace element concentrations were significantly affected by plant species, but the concentration of As, Cr, Li, and Pb was significantly increased by 6–14% following TMW irrigation (Table S-3). However, trace element concentrations did not exceed background concentrations in local soils (Percival et al., 1996). The application of trace elements with TMW was low, and results by Smith et al. (1996) suggested that it will take 50 to 100 years for trace elements to reach values of environmental concern. However, in contrast to their results, we did not observe increases of extractable element concentrations with TMW irrigation (Table S-4), and found significantly decreased concentrations of extractable Al, Cd, and Co in the topsoil (0–5 cm). Our results indicate that the risk of trace element accumulation in soil is lower from TMW irrigation than the application of mineral fertilisers, which can contain trace element contamination (Alloway, 2013).

Depth had the strongest effect on soil parameters (Table 3), which was expected due to the physicochemical changes within the soil profile (Blume et al., 2016). The plant species affected the soil concentration of total C, total N, NO_3^-, and Na, and to a lesser extent S and Mg. Irrigation affected the concentration of Na and K the most, and to a lesser extent total C, total N, Ca, and Mg. Interaction effects of species and irrigation were strong for NH_4^+ and Na concentrations.

The results demonstrate that the selection of plant species influences the fate of TMW applied nutrients and contaminants. Species differ in their biomass production and nutrient concentration, affecting the amount of nutrients taken up (Tzanakakis et al., 2009). Plant roots further influence the properties of the rhizosphere (Neumann et al., 2009): distinct root morphologies and rhizosphere processes affect the fate of nutrients and contaminants in the soil (Franklin et al., 2019). These properties need to be considered when selecting plants for TMW land application schemes.

3.2. Plant response to TMW irrigation

Following the transplantation of seedlings in July 2015, overall plant survival decreased from 97% in October 2015 to 87% in December 2015 before irrigation began in January 2016. The initial decline in plant survival was likely due to the high temperature and low rainfall during that period. In June 2019, 3.5 years after onset of irrigation, there were a total of 815 surviving plants at the site, equalling to an overall survival rate of 68%. The total survival rate of TMW-irrigated plants did not differ from the control. However, plants in the TMW-irrigated plots had begun to self-thin, a phenomenon where increased biomass production results in higher mortality in even-aged high-density plantings (Westoby, 1984). Across all species, the average height of the native vegetation receiving TMW was 2.1 m, significantly higher than the unirrigated plants in the control treatment at 1.9 m. Fig. 3 shows the heights of the individual species. There was no significant decrease in height in any species. Seven species showed a significant increase in plant height when irrigated with TMW. The largest increase was observed in *Griselinia littoralis*, where the plant height was 42% higher with TMW irrigation.

Our findings are consistent with accelerated plant growth following the application of other biowastes onto NZ native vegetation such as biosolids (Gutierrez-Gines et al., 2017) and vermi-compost (Xue et al., 2016). Species that showed no growth response appeared to do so for different reasons. *Pseudopanax arboreus* was not well adapted to the local environment at the site and showed signs of stress in both treatments. Its survival rate dropped to 57% before irrigation started and was at 21% in June 2019. Sooty mould (*Capnodium walteri*) was observed on all *L. scoparium* plants, which is likely a result of honeydew production by *Acanthococcus campbelli* and *Acanthococcus laptospermi*, the two common scale insects associated with *L. scoparium* (Bohórquez et al., 2019). *Olearia paniculata* showed evidence of stress and chlorosis in its leaves, which can indicate a nutrient imbalance (Sharma et al., 2021). *C. robusta*

Table 4
Concentrations of elements in plant foliage at the site in June 2019 by species and treatment; Irrigated with treated municipal wastewater (TMW) and non-irrigated (control).

	C. australis		*C. robusta*		*K. robusta*		*L. scoparium*		*P. tenax*	
	control	TMW	control	TMW	control	TMW	control	TMW	control	TMW
N (%)	1.3 ± 0.02	1.5 ± 0.11	1.6 ± 0.05	1.6 ± 0.07	1.9 ± 0.09	1.8 ± 0.09	1.6 ± 0.07	1.6 ± 0.05	1.4 ± 0.05	1.5 ± 0.07
Na	696 ± 135	690 ± 97	671 ± 41	729 ± 116	2767 ± 179	2765 ± 251	2503 ± 136	2336 ± 241	2031 ± 224	1541 ± 188
Mg	3247 ± 242	3478 ± 194	4143 ± 620	4439 ± 486	2434 ± 286	1841 ± 253	2492 ± 343	2497 ± 67	1546 ± 70	1606 ± 277
P	1651 ± 73	1687 ± 142	2226 ± 54	2151 ± 211	1710 ± 279	1576 ± 176	1191 ± 98	1114 ± 64	1871 ± 24	2145 ± 241
K (%)	0.81 ± 0.08	0.72 ± 0.10	1.2 ± 0.10	1.2 ± 0.21	0.66 ± 0.04	0.66 ± 0.05	0.67 ± 0.07	0.51 ± 0.04	1.3 ± 0.03	1.6 ± 0.12
Ca (%)	1.8 ± 0.23	1.5 ± 0.12	2.1 ± 0.12	2.3 ± 0.09	0.59 ± 0.11	0.46 ± 0.04	0.81 ± 0.08	0.84 ± 0.06	0.39 ± 0.03	0.34 ± 0.05
Zn	121 ± 15	126 ± 121	51 ± 4.2	50 ± 6.4	36 ± 2.8	24 ± 4.7	11 ± 0.57	9.3 ± 0.28	**32 ± 1.5**	**25 ± 1.3***
Mn	794 ± 172	946 ± 196	149 ± 26	162 ± 36	**691 ± 71**	**368 ± 58***	289 ± 67	127 ± 24	135 ± 17	181 ± 55
Fe	52 ± 2.2	63 ± 6.0	141 ± 15	157 ± 42	252 ± 17	283 ± 59	296 ± 34	575 ± 136	54 ± 3.1	56 ± 4.6
Cu	5.6 ± 0.53	5.0 ± 0.82	6.5 ± 0.27	5.7 ± 0.45	4.2 ± 0.31	3.6 ± 0.94	3.7 ± 0.64	2.5 ± 0.39	4.3 ± 0.08	4.2 ± 0.27

Values show means ± standard errors ($n = 5$). Values are in mg kg^{-1} unless otherwise indicated. Significant differences between treatments at $p < 0.05$ according to Tukey's HSD post-hoc test are indicated in bold followed by asterisk (*).

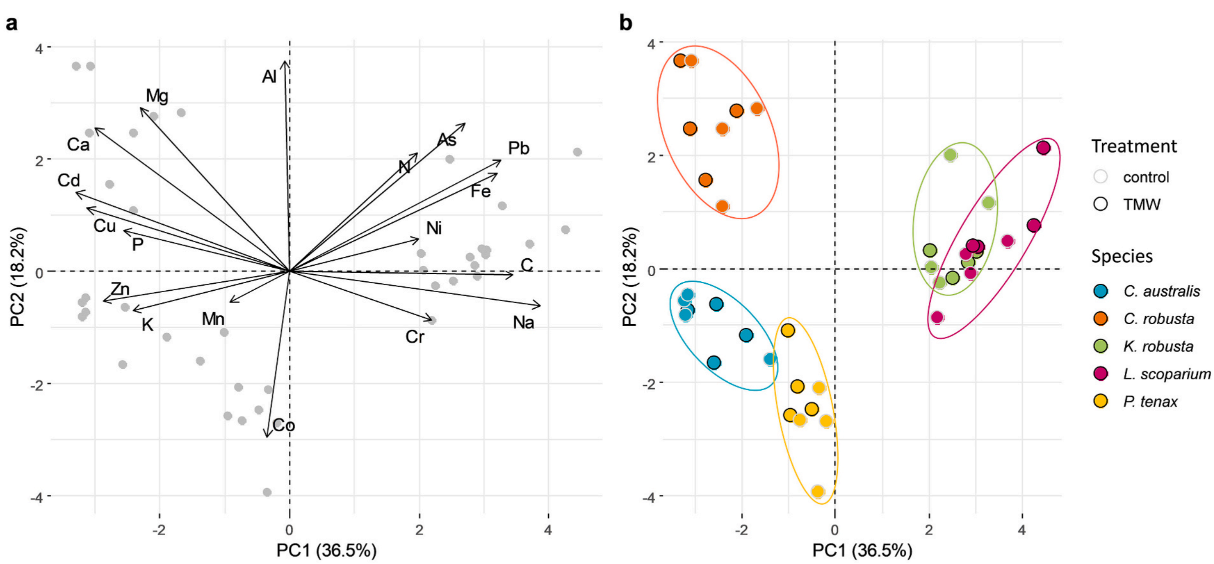

Fig. 4. Principal component analysis (PCA) of elemental plant composition at the Duvauchelle field site; (**a**) loading plot, and (b) score plot.

grew vigorously in both treatments, and there was no significant difference in plant height.

3.3. Elemental concentrations in plants

The concentrations of the essential elements in the plant foliage were not affected by TMW irrigation, except for Zn and Mn (Table 4). Following TMW irrigation, foliar concentrations of Mn in *K. robusta* and Zn in *P. tenax* decreased by 47% and 28%, respectively. This indicates dilution following increased biomass production with TMW irrigation (Jarrell and Beverly, 1981). Unlike previously reported with NZ native species (Franklin et al., 2015), there was no luxury uptake of elements with TMW irrigation. It appears that a combination of factors may have limited plant growth at the site (Marschner, 1995). However, other stressing factors such as light and temperature may have influenced the concentration of elements in the plant foliage (Güsewell and Koerselman, 2002). Not all species adapted to low-fertility conditions take up nutrients beyond their growth requirements when fertilised (Iversen et al., 2010). Concentrations of As, Cd, Cr, and Pb (Table S-5) were all within normal ranges for plant foliage (Chaney, 1989). Na, which was significantly increased in the soil with TMW irrigation, did not differ in the foliage between treatments. The small differences in elemental composition between treatments indicates that TMW did not impair the nutrition of NZ native plants.

PCA revealed that species were grouped based on their elemental composition (Fig. 4). PC1 (explaining 36.5% of variation) divided species into Myrtaceae (*L. scoparium* and *K. robusta*) and non-Myrtaceae species, while PC2 (explaining 18.2% of variation) divided species into mono- and dicotyledons. This is consistent with results of Hahner et al. (2014), who also found distinct elemental concentrations between native mono- and dicotyledons. Here, PC1 was mainly weighted by Na, C, Cd, and Pb, while PC2 was heavily weighted by Al, Co, and Mg.

The physiological traits of plants differ as a consequence of genetic influences, resulting in differential nutrient uptake among species (Dickinson et al., 2015). The selection of species with higher nutrient accumulation potential may mitigate elements associated with TMW (Tzanakakis et al., 2009). For example, we found that Na concentrations were four times higher in *K. robusta* than *C. australis*. However, the biomass of these species would need to be known to determine total plant uptake. Furthermore, elements that are taken up by plants will eventually return to the soil through litter, unless biomass is removed from the site. Possible uses for the biomass of NZ native plants at the field site include the production of essential oils from *L. scoparium* and *K. robusta* (Seyedalikhani et al., 2019), fibres from *P. tenax*, or timber from *Podocarpus laetus*. Additionally, some species could be used as a fodder supplement for livestock (Dickinson et al., 2015), particularly *G. littoralis* which was the most responsive species in the present study.

4. Conclusions

Application of TMW at 1000 mm yr^{-1} onto NZ native vegetation had negligible effects on the soil chemistry after nearly 3 years of irrigation. Soil Na concentrations were increased, but most of the applied Na was leached from the soil profile. The rate of P accumulation in the soil indicates that it will not accumulate beyond the range found in NZ agricultural soils for at least 50 years. Despite the application of 194 kg N ha^{-1} yr^{-1} with TMW irrigation, soil N concentrations were only significantly increased in the topsoil (0–5 cm). Plant species significantly affected the soil concentrations of C, N, NO_3^-, and Na. TMW irrigation did not impair the growth of NZ native plant species. The overall height of irrigated species was significantly increased, but the growth response differed between species. Overcoming challenges associated with establishing NZ native vegetation requires selection of species that are well adapted to the local environment. TMW irrigation did not increase concentrations of any elements to levels that may pose a risk to humans or ecosystems. Our results indicate that TMW irrigated at rates $\leq$1000 mm yr^{-1} could be used to support the establishment of native vegetation in NZ and elsewhere. Future research should investigate gaseous N losses and N leaching from TMW irrigated native vegetation to quantify the efficacy of the vegetation at protecting receiving waters.

CRediT authorship contribution statement

Alexandra Meister: Conceptualization, Formal analysis, Investigation, Writing – original draft, Data curation, Visualization, Funding acquisition. **Furong Li:** Investigation. **Maria Jesus Gutierrez-Gines:** Conceptualization, Formal analysis, Writing – review & editing, Supervision, Funding acquisition, Project administration. **Nicholas Dickinson:** Writing – review & editing, Supervision. **Sally Gaw:** Writing – review & editing, Supervision. **Mike Bourke:** Supervision, Resources, Project administration, Writing - review & editing. **Brett Robinson:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded by the Christchurch City Council and by the Centre for Integrated Biowaste Research and the Native Ecosystems projects under The Institute of Environmental Science Research Strategic Science Investment Fund from the Ministry of Business, Innovation and Employment, New Zealand [grant number C03X1701]. The lead author gratefully acknowledges the receipt of a University of Canterbury Doctoral Scholarship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2022.106741.

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