Supplementary Material

Changes in soil chemistry and soil nutrient stocks after 30 years of treated municipal wastewater land disposal: a natural experiment

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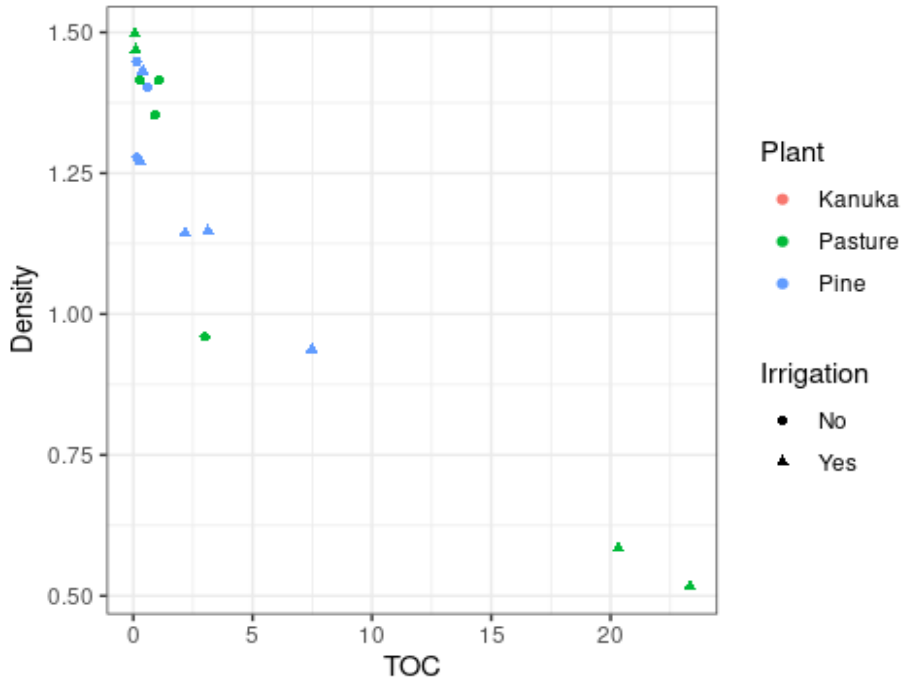
Interpolation of sample density based on soil C concentration

Figure S1. Relationship between soil C concentration and sample density

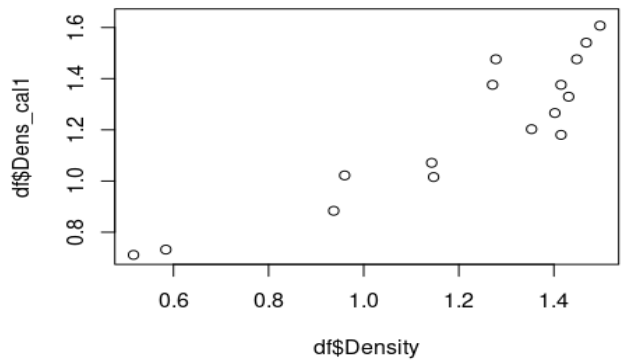



```
# Fitting an exponential model

model <- lm(Density ~ log(TOC), df)
summary (model)

##
## Call:
## lm(formula = Density ~ log(TOC), data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.197799 -0.106186  0.005635  0.108962  0.234662
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  1.18841    0.03449   34.46 6.15e-15 ***
## log(TOC)    -0.15149    0.01940   -7.81 1.81e-06 ***
## ---
```

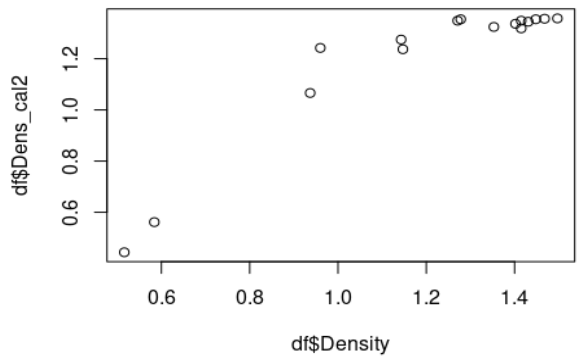
```
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1377 on 14 degrees of freedom
##   (323 observations deleted due to missingness)
## Multiple R-squared:  0.8133, Adjusted R-squared:    0.8
## F-statistic:    61 on 1 and 14 DF,  p-value: 1.81e-06

rm(model)

# Fitting a linear model

model2 <- lm(Density ~ TOC, df)
summary (model2)

##
## Call:
## lm(formula = Density ~ TOC, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.28194 -0.08053  0.04734  0.08850  0.13965
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)  1.359833   0.034861  39.007 1.10e-15 ***
## TOC         -0.039299   0.004326  -9.084 3.03e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1214 on 14 degrees of freedom
##   (323 observations deleted due to missingness)
## Multiple R-squared:  0.8549, Adjusted R-squared:  0.8446
## F-statistic: 82.51 on 1 and 14 DF,  p-value: 3.03e-07
```

```
rm(model2)
```

```
# Calculate densities with the different models

df <- df %>%
mutate (Dens_cal1 = 1.18841 - 0.15149*(log(TOC)),
        Dens_cal2 = 1.359833 - 0.03929 * TOC) %>%
  mutate(Dens_cal4 = (Dens_cal1 + Dens_cal2)/2)
plot(df$Density, df$Dens_cal1)
```

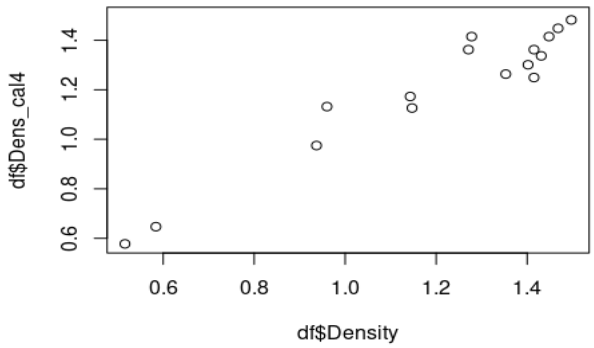


```
fit1 <- lm(Density ~ Dens_cal1, df)
summary(fit1)

##
## Call:
## lm(formula = Density ~ Dens_cal1, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.197799 -0.106186  0.005635  0.108962  0.234662
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept) 4.878e-06  1.580e-01    0.00        1
## Dens_cal1   1.000e+00  1.280e-01    7.81 1.81e-06 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1377 on 14 degrees of freedom
##   (323 observations deleted due to missingness)
## Multiple R-squared:  0.8133, Adjusted R-squared:    0.8
## F-statistic:    61 on 1 and 14 DF,  p-value: 1.81e-06

rm(fit1)

plot(df$Density, df$Dens_cal2)
```




```
fit2 <- lm(Density ~ Dens_cal2, df)
summary(fit2)

##
## Call:
## lm(formula = Density ~ Dens_cal2, data = df)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.28194 -0.08053  0.04734  0.08850  0.13965
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept) -0.0003142  0.1360165  -0.002    0.998
## Dens_cal2    1.0002312  0.1101129   9.084 3.03e-07 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Residual standard error: 0.1214 on 14 degrees of freedom
##   (323 observations deleted due to missingness)
## Multiple R-squared:  0.8549, Adjusted R-squared:  0.8446
## F-statistic: 82.51 on 1 and 14 DF,  p-value: 3.03e-07

rm(fit2)


plot(df$Density, df$Dens_cal4)
```




```
fit4 <- lm(Density ~ Dens_cal4, df)
summary(fit4)

##
## Call:
## lm(formula = Density ~ Dens_cal4, data = df)
##
## Residuals:
##       Min        1Q    Median        3Q       Max
## -0.164584 -0.017380 -0.001313  0.047632  0.161025
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  -0.1231     0.1075  -1.144    0.272
## Dens_cal4     1.1022     0.0873  12.625 4.86e-09 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.09058 on 14 degrees of freedom
##   (323 observations deleted due to missingness)
## Multiple R-squared:  0.9193, Adjusted R-squared:  0.9135
## F-statistic: 159.4 on 1 and 14 DF,  p-value: 4.86e-09
```

Table S1: Results of chemical analysis in the soil. Mean ± standard error. Different letters in each soil depth indicate significant differences between treatments (Tukey's test, $p<0.05$). When a transformation of the data was performed, it is represented over the results of ad-hoc analysis (ln = log transformation). When a non-parametric analysis of Kruskal-Wallis was performed, it is represented as KW.

Irrigation	Plant	Depth (cm)	NO3 – N (mg kg^{-1})		NH4 – N (mg kg^{-1})		TN (%)		TOC (%)		Olsen P (mg kg^{-1})		TP (mg kg^{-1})		P retention (%)		Mg (%)		Ca (%)		K (%)	
				KW		ln		ln		ln				ln		ln						
No	Kanuka	7.5	46 ± 4.2	a	15 ± 1	a	0.32 ± 0.02	a	4.8 ± 0.27	a	77 ± 9.5	a	532 ± 25	a			0.22 ± 0.003	b	0.67 ± 0.006	b	0.14 ± 0.003	a
No	Pasture	7.5	0.8 ± 0.14	c	7.4 ± 0.4	a	0.27 ± 0.01	a	3.1 ± 0.12	ab	40 ± 3	ab	548 ± 13	a	12 ± 0.4	b	0.26 ± 0.002	b	0.73 ± 0.005	ab	0.12 ± 0.004	a
Yes	Pasture	7.5	39 ± 4.1	a	20 ± 1.8	a	0.81 ± 0.1	a	7.1 ± 0.88	a	74 ± 6.3	a	680 ± 17	a	9.3 ± 0.2	b	0.24 ± 0.004	b	0.72 ± 0.007	ab	0.11 ± 0.002	a
No	Pine	7.5	0.04 ± 0.01	d	2.5 ± 0.2	b	0.08 ± 0	b	1.5 ± 0.06	b	18 ± 0.8	b	319 ± 6	b	11 ± 0.4	b	0.31 ± 0.001	a	0.79 ± 0.012	a	0.14 ± 0.006	a
Yes	Pine	7.5	5.3 ± 0.7	b	7.4 ± 0.5	a	0.22 ± 0.01	a	3.6 ± 0.17	ab	87 ± 3.1	a	566 ± 19	a	17 ± 0.8	a	0.25 ± 0.004	b	0.69 ± 0.012	b	0.11 ± 0.003	a
				KW		KW																
No	Kanuka	15	34 ± 3	a	10 ± 0.8	a																
No	Pasture	15	0.32 ± 0.02	d	3.8 ± 0.5	b																
Yes	Pasture	15	8.3 ± 0.3	b	3.9 ± 0.3	ab																
No	Pine	15	0.02 ± 0	e	0.52 ± 0.07	c																
Yes	Pine	15	2.3 ± 0.3	c	1.3 ± 0.1	c																
				KW		KW		ln		ln		KW		ln				KW		KW		
No	Kanuka	30	21 ± 2.2	a	3 ± 0.3	a	0.16 ± 0.01	a	2.5 ± 0.17	a	26 ± 6.7	bc	358 ± 12	bc			0.24 ± 0.003	c	0.78 ± 0.017	a	0.12 ± 0.004	a
No	Pasture	30	0.7 ± 0.1	c	1.2 ± 0.2	ab	0.10 ± 0.002	a	1.3 ± 0.03	a	33 ± 4.8	ab	470 ± 18	ab			0.27 ± 0.002	bc	0.74 ± 0.009	a	0.13 ± 0.005	a
Yes	Pasture	30	2.7 ± 0.1	b	1.1 ± 0.1	b	0.035 ± 0.001	b	0.31 ± 0.01	b	68 ± 1.7	a	621 ± 26	a			0.28 ± 0.002	ab	0.71 ± 0.006	a	0.11 ± 0.003	a
No	Pine	30	0.02 ± 0	d	0.26 ± 0.03	bc	0.031 ± 0.001	b	0.39 ± 0.01	b	5.1 ± 0.7	c	265 ± 4	c			0.29 ± 0.003	a	0.77 ± 0.014	a	0.13 ± 0.005	a
Yes	Pine	30	0.45 ± 0.03	c	0.16 ± 0.02	c	0.036 ± 0.001	b	0.45 ± 0.01	b	53 ± 7.5	ab	423 ± 21	b			0.27 ± 0.001	ab	0.71 ± 0.011	a	0.11 ± 0.004	a
				KW		KW																
No	Kanuka	45	22 ± 2.2	a	3 ± 0.4	a																
No	Pasture	45	0.7 ± 0.1	b	1.2 ± 0.1	ab																
Yes	Pasture	45	4.4 ± 0.2	a	0.9 ± 0.08	b																
No	Pine	45	0.03 ± 0	c	0.04 ± 0.01	c																
Yes	Pine	45	1.1 ± 0.1	b	0.02 ± 0	c																
				KW		KW		ln		ln		KW		KW				KW		KW		KW
No	Kanuka	60	15 ± 2.1	a	0.7 ± 0.15	a	0.061 ± 0.006	a	0.93 ± 0.11	a	3.4 ± 0.5	bc	264 ± 7	c			0.26 ± 0.001	b	0.73 ± 0.014	a	0.12 ± 0.004	a
No	Pasture	60	0.14 ± 0.01	c	0.3 ± 0.03	a	0.051 ± 0.002	a	0.62 ± 0.03	a	17 ± 4.1	ab	379 ± 19	ab	7.9 ± 1.2	a	0.26 ± 0.001	b	0.76 ± 0.006	a	0.12 ± 0.004	a
Yes	Pasture	60	2.5 ± 0.1	a	0.7 ± 0.13	ab	0.016 ± 0	c	0.12 ± 0	c	50 ± 4.1	a	540 ± 29	a	3.0 ± 0.4	a	0.29 ± 0.002	a	0.73 ± 0.007	a	0.11 ± 0.004	a
No	Pine	60	0.03 ± 0	d	0.08 ± 0.01	ab	0.019 ± 0.001	bc	0.16 ± 0.01	bc	0.8 ± 0.3	c	261 ± 6	c	4.1 ± 0.3	a	0.29 ± 0.002	a	0.74 ± 0.013	a	0.13 ± 0.005	a
Yes	Pine	60	1.0 ± 0.2	b	0 ± 0	c	0.031 ± 0.001	ab	0.32 ± 0.01	ab	38 ± 13.4	a	363 ± 30	bc	6.1 ± 0.8	a	0.3 ± 0.005	a	0.74 ± 0.011	a	0.12 ± 0.003	a
				KW		KW																
No	Kanuka	80	11 ± 1.1	a	0.12 ± 0.03	a																
No	Pasture	80	0.34 ± 0.02	c	0.02 ± 0	a																
Yes	Pasture	80	2.9 ± 0.1	a	0.08 ± 0.03	a																
No	Pine	80	0.09 ± 0.01	d	0.08 ± 0.03	a																
Yes	Pine	80	1.3 ± 0.2	b	0 ± 0	a																
				KW		KW		KW		KW		KW		ln				KW		ln		ln
No	Kanuka	100	12 ± 2.5	a	1.1 ± 0.11	a	0.019 ± 0.003	a	0.17 ± 0.024	a	6.7 ± 1.8	ab	250 ± 5	b			0.28 ± 0.003	a	0.78 ± 0.015	a	0.2 ± 0.003	a
No	Pasture	100	0.43 ± 0.1	b	0.2 ± 0.06	b	0.021 ± 0.006	a	0.28 ± 0.11	ab	3.8 ± 0.6	bc	275 ± 10	b	4.2 ± 0.6	a	0.27 ± 0.008	a	0.8 ± 0.029	a	0.18 ± 0.014	a
Yes	Pasture	100	3.1 ± 0.6	a	0.2 ± 0.07	ab	0.01 ± 0.001	ab	0.07 ± 0.004	c	20 ± 0.9	a	374 ± 3	a	1.6 ± 0.2	a	0.27 ± 0.005	a	0.81 ± 0.001	a	0.17 ± 0.003	a
No	Pine	100	0.05 ± 0.01	c	0.2 ± 0.08	ab	0.008 ± 0	b	0.07 ± 0.001	bc	1.3 ± 0.1	c	254 ± 3	b	2.1 ± 0.1	a	0.28 ± 0.001	a	0.75 ± 0.004	a	0.16 ± 0.004	a
Yes	Pine	100	0.25 ± 0.1	bc	0.98 ± 0.48	ab	0.012 ± 0	a	0.12 ± 0.006	a	4.4 ± 0.9	bc	264 ± 3	b	3.2 ± 0.2	a	0.28 ± 0	a	0.74 ± 0.007	a	0.15 ± 0.001	a
				KW		KW		KW		KW		KW		ln				KW				
No	Kanuka	200	8.6 ± 1.5	a	0.5 ± 0.12	a	0.011 ± 0.001	a	0.08 ± 0.008	a	5.7 ± 0.7	b	278 ± 2	b		ln	0.28 ± 0.001	b	0.81 ± 0.005	a	0.17 ± 0.004	ab
No	Pasture	200	0.8 ± 0.2	bc	0.1 ± 0.04	a	0.011 ± 0.001	a	0.1 ± 0.006	a	3.5 ± 0.7	b	279 ± 4	b	3.3 ± 0.3	a	0.27 ± 0.001	b	0.78 ± 0.007	ab	0.2 ± 0.001	a
Yes	Pasture	200	3.3 ± 1	ab	0.2 ± 0.09	a	0.011 ± 0.001	a	0.08 ± 0.003	a	15 ± 0.4	a	355 ± 5	a	1.6 ± 0.1	a	0.3 ± 0.004	a	0.82 ± 0.004	a	0.2 ± 0.009	a
No	Pine	200	0.01 ± 0.01	d	0.9 ± 0.36	a	0.009 ± 0.001	a	0.13 ± 0.046	a	1.1 ± 0.1	c	265 ± 2	b	2.0 ± 0.5	a	0.29 ± 0.003	a	0.78 ± 0.013	ab	0.17 ± 0.01	ab
Yes	Pine	200	0.96 ± 0.44	c	0.5 ± 0.25	a	0.013 ± 0.002	a	0.16 ± 0.035	a	4.5 ± 1.1	b	264 ± 1	b	2.3 ± 0.1	a	0.26 ± 0.003	c	0.73 ± 0.016	b	0.15 ± 0.002	b
				KW		KW		KW		KW		KW		KW				KW		ln		
No	Kanuka	300	8.5 ± 0.8	a	0.4 ± 0.05	a	0.016 ± 0.001	a	0.13 ± 0.012	a	15 ± 4.1	a	304 ± 17	a			0.27 ± 0.007	ab	0.81 ± 0.024	a	0.19 ± 0.005	a
No	Pasture	300	0.7 ± 0.1	b	0.3 ± 0.09	a	0.014 ± 0.001	a	0.12 ± 0.008	a	26 ± 1.4	a	347 ± 2	a			0.25 ± 0.002	b	0.74 ± 0.015	a	0.17 ± 0.004	a
Yes	Pasture	300	8.5 ± 1.6	a	0.4 ± 0.02	a	0.013 ± 0	a	0.09 ± 0.004	a	16 ± 1.3	a	340 ± 7	a			0.28 ± 0.007	a	0.81 ± 0.019	a	0.18 ± 0.008	a

Table S2: Results of chemical analysis in the soil. Mean ± standard error. Different letters in each soil depth indicate significant differences between treatments (Tukey's test, $p<0.05$). When a transformation of the data was performed, it is represented over the results of ad-hoc analysis (ln = log transformation). When a non-parametric analysis of Kruskal-Wallis was performed, it is represented as KW.

Irrigation	Plant	Depth	pH		EC (µS cm^{-1})		Na (mg kg^{-1})		Mn (mg kg^{-1})		Cu (mg kg^{-1})		Zn (mg kg^{-1})	
						ln		ln				KW		KW
No	Kanuka	7.5	4.2 ± 0.02	c	202 ± 12	a	469 ± 3	c	210 ± 3	b	6.9 ± 0.34	b	34 ± 0.5	b
No	Pasture	7.5	5.3 ± 0.04	a	62 ± 2.4	b	498 ± 5	bc	265 ± 6	a	7 ± 0.21	b	44 ± 0.5	a
Yes	Pasture	7.5	4.5 ± 0.03	bc	81 ± 5.9	b	599 ± 26	ab	224 ± 3	ab	9.9 ± 0.32	a	44 ± 0.6	a
No	Pine	7.5	5.2 ± 0.03	a	84 ± 3.9	b	677 ± 17	a	210 ± 5	b	3.3 ± 0.04	d	35 ± 0.5	b
Yes	Pine	7.5	4.7 ± 0.03	b	89 ± 3.8	b	491 ± 12	bc	195 ± 7	b	4.4 ± 0.07	c	33 ± 0.7	b
						KW								
No	Kanuka	15	4.4 ± 0.05	b	130 ± 7.3	a								
No	Pasture	15	5.4 ± 0.03	a	24 ± 0.6	d								
Yes	Pasture	15	4.4 ± 0.03	b	39 ± 0.9	c								
No	Pine	15	5.5 ± 0.03	a	50 ± 1	b								
Yes	Pine	15	5.1 ± 0.03	a	44 ± 2.3	c								
						ln		KW		KW		KW		ln
No	Kanuka	30	4.7 ± 0.07	c	97 ± 7.7	a	493 ± 5	b	251 ± 8	a	4.9 ± 0.16	a	32 ± 0.3	b
No	Pasture	30	5.4 ± 0.03	ab	21 ± 0.7	b	498 ± 8	b	235 ± 3	a	5.1 ± 0.22	a	36 ± 0.6	ab
Yes	Pasture	30	4.9 ± 0.06	bc	25 ± 0.6	b	561 ± 16	b	210 ± 4	ab	4.9 ± 0.16	a	37 ± 0.6	a
No	Pine	30	5.6 ± 0.02	a	48 ± 1.1	a	681 ± 5	a	208 ± 3	b	3.4 ± 0.04	b	32 ± 0.2	ab
Yes	Pine	30	5.4 ± 0.05	ab	21 ± 0.6	b	511 ± 24	b	208 ± 4	b	3.7 ± 0.07	b	32 ± 0.3	ab
						ln								
No	Kanuka	45	5 ± 0.08	bc	89 ± 6.9	a								
No	Pasture	45	5.6 ± 0.03	a	19 ± 0.5	c								
Yes	Pasture	45	4.5 ± 0.03	c	27 ± 0.6	bc								
No	Pine	45	6 ± 0.04	a	37 ± 1.2	b								
Yes	Pine	45	5.5 ± 0.04	ab	24 ± 0.9	bc								
						KW		KW		KW		KW		KW
No	Kanuka	60	5.1 ± 0.06	a	77 ± 9.4	a	530 ± 7	b	237 ± 8	a	4 ± 0.06	ab	32 ± 0.1	c
No	Pasture	60	5.9 ± 0.14	a	18 ± 1	b	521 ± 6	b	219 ± 3	a	4 ± 0.06	ab	35 ± 0.7	bc
Yes	Pasture	60	4.9 ± 0.04	a	21 ± 0.5	b	546 ± 15	b	222 ± 4	a	4.4 ± 0.09	a	37 ± 0.6	a
No	Pine	60	5.8 ± 0.04	a	36 ± 2.4	a	695 ± 13	a	207 ± 3	a	3.8 ± 0.06	b	33 ± 0.3	bc
Yes	Pine	60	5.7 ± 0.05	a	20 ± 0.6	b	528 ± 23	b	201 ± 5	a	3.6 ± 0.06	b	34 ± 0.6	ab
						ln								
No	Kanuka	80	5.8 ± 0.08	ab	47 ± 4.4	a								
No	Pasture	80	6.2 ± 0.11	a	10 ± 0.2	c								
Yes	Pasture	80	4.9 ± 0.04	b	19 ± 0.5	b								
No	Pine	80	6.4 ± 0.02	a	32 ± 2.1	ab								
Yes	Pine	80	6 ± 0.05	a	21 ± 1	b								
						ln		KW		KW		KW		KW
No	Kanuka	100	5.2 ± 0.04	b	63 ± 13	a	594 ± 13	abc	205 ± 3	a	4.9 ± 0.18	a	36 ± 1.4	a
No	Pasture	100	5.9 ± 0.12	a	11 ± 0.7	b	565 ± 25	bc	225 ± 8	a	5.1 ± 0.1	a	34 ± 1.3	abc
Yes	Pasture	100	5.6 ± 0.06	ab	21 ± 1.8	b	619 ± 26	ab	206 ± 3	a	4.5 ± 0.09	a	31 ± 0.3	bc
No	Pine	100	5.8 ± 0.06	a	82 ± 4.3	a	681 ± 18	a	174 ± 2	b	4.7 ± 0.07	a	31 ± 0.1	c
Yes	Pine	100	5.7 ± 0.02	ab	24 ± 2	b	504 ± 13	c	176 ± 2	b	4.6 ± 0.29	a	33 ± 0.2	ab
						ln		KW		KW		ln		ln
No	Kanuka	200	5.4 ± 0.02	a	43 ± 1.8	ab	569 ± 16	b	213 ± 2	a	7.8 ± 1.78	a	32 ± 0.1	a
No	Pasture	200	5.8 ± 0.03	a	10 ± 0.4	c	697 ± 3	a	204 ± 2	a	4.9 ± 0.05	a	32 ± 0.2	a
Yes	Pasture	200	5.5 ± 0.05	a	31 ± 3.5	b	608 ± 11	ab	212 ± 3	a	5 ± 0.23	a	32 ± 0.6	a
No	Pine	200	5.7 ± 0.13	a	88 ± 15	a	598 ± 11	ab	181 ± 2	b	4.6 ± 0.12	a	33 ± 0.3	a
Yes	Pine	200	5.7 ± 0.03	a	35 ± 2.2	b	571 ± 4	b	162 ± 3	b	4.2 ± 0.2	a	30 ± 0.1	a
						ln		KW		KW		KW		KW
No	Kanuka	300	5.3 ± 0.08	a	65 ± 11.2	a	637 ± 6	a	201 ± 7	a	5.4 ± 0.05	a	33 ± 0.5	a
No	Pasture	300	5.7 ± 0.07	a	11 ± 0.3	b	607 ± 4	a	195 ± 13	a	5.1 ± 0.16	a	30 ± 0.2	b
Yes	Pasture	300	5.4 ± 0.06	a	39 ± 5.9	a	674 ± 29	a	206 ± 6	a	5.1 ± 0.21	a	32 ± 0.6	a

Colour-blind friendly graphs equivalent to the ones in the main manuscript body:

Figure S2. Average and standard error of the soil parameters at the different treatments (irrigated vs non-irrigated, pine vs pasture vs kānuka) and at increasing soil depths.

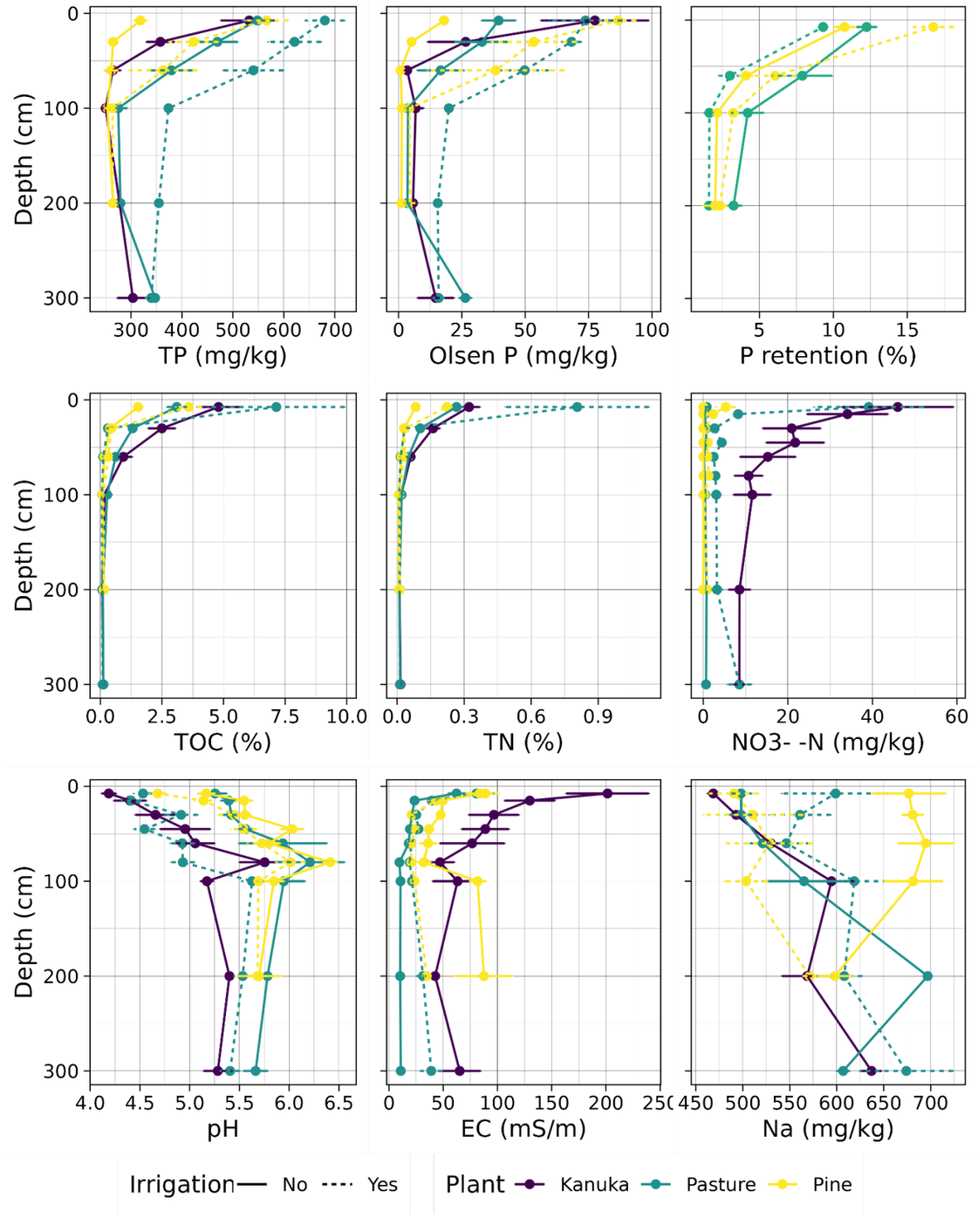


Figure S3. Principal component analysis of the chemical results in the topsoil (0 – 7.5 cm). A) biplot with the variable weighting and the scatterplot. B) Scatterplot with groups based on plant and irrigation. Ellipse shows confidence regions.

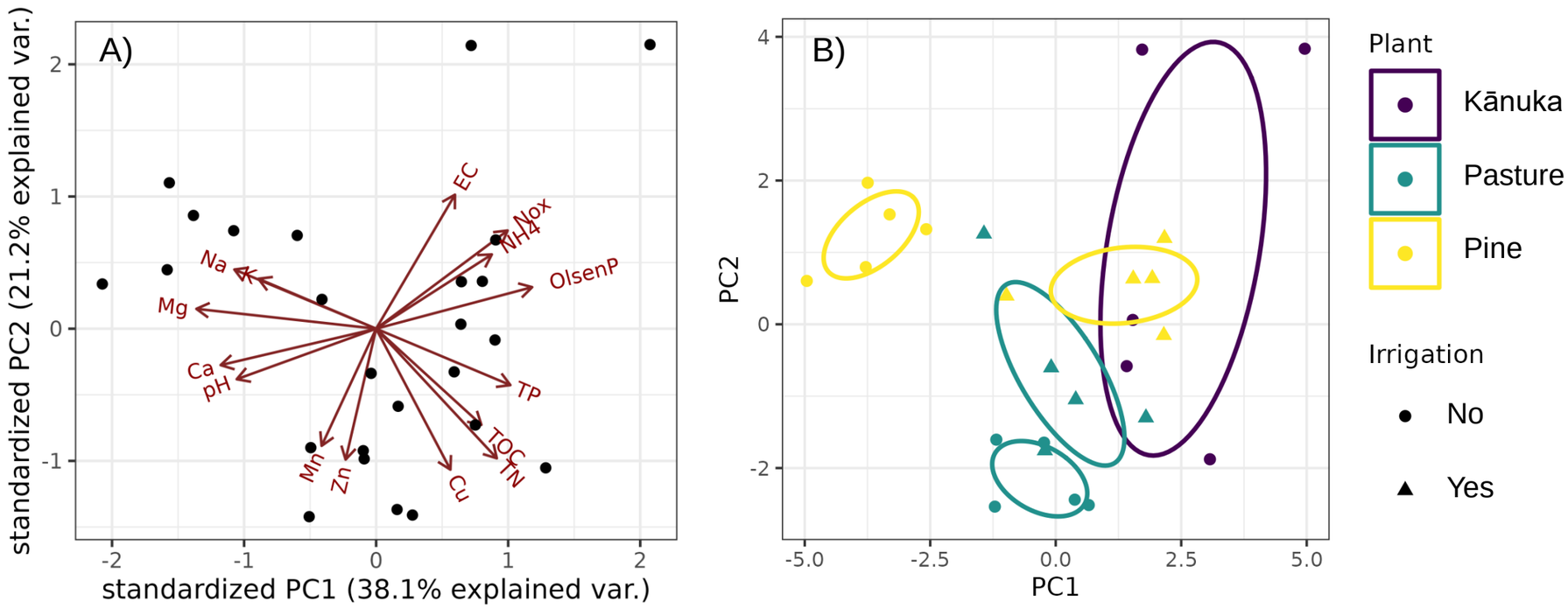


Figure S4. Boxplots of the element stocks in the soil (0 – 80 cm) calculated per hectare. Different letters in each graph indicate significant differences between groups (Tukey's test, $p < 0.05$).