Soil Physiochemistry and Microbiology Assessment of Aotearoa Native Species Nursery

Durham University Expeditions Grant

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1. Abstract

Soil quality underpins terrestrial ecosystems and critically affects the success of restoring landscapes and biodiversity levels. The report discusses soil properties through microbial communities and physiochemical tests (on active C, pH, bulk density, soil moisture, and gravimetric water content).

Using broad genetic and physiochemical characterisation, the study (1) investigated differential growth success across kānuka and mixed(-species) nursery treatments and (2) correlated physiochemical variables and microbial community compositions to nursery treatments and relative growth rate, and (3) evaluated those physiochemical variables and genomic analyses as low-cost proxies of soil microbial biodiversity.

Molecular analyses concluded that nursery treatment more strongly influenced bacterial and fungal DNA abundance compared to above-ground observations of improved growth rates. Relative abundance, however, is insufficient to determine which nursery treatment fosters greater microbial biodiversity.

Non-significant differences between each assessed physiochemical variable and nursery treatments also indicated that, despite different species compositions, native nursery flora supports similar soil structures. Of the measured physiochemical properties, SM shows the greatest potential use in assessing soil health, reflected in above-ground growth.

2. Introduction

Climate change as a result of human activity poses an existential threat to ecosystems. The UN estimates a peak global temperature increase of 2.1-2.7 °C under current policies[1], in which

scenario the International Panel on Climate Change warns of a high to very high risk of global biodiversity loss[2].

Wildwood restoration or creation of nascent forests is a strategy to reduce drivers of climate change by sequestering carbon emissions. In Aotearoa (New Zealand), the New Zealand Emissions Trading Scheme incentivises reforestation through carbon credits and permit trading. However, there are concerns[3] that this incentivises the more economical planting of alien, fast-growing tree species over the restoration of native terrestrial biodiversity.

The Auckland University of Technology (AUT) Living Laboratories Project seeks to fill the knowledge gap around restoration in Aotearoa, and divert strategies from monoculture, non-native *Pinus radiata* forestry systems[3]. From its inception in 2019 , the Living Laboratories Project aimed to provide scientific knowledge—regarding restoration site preparation and species planting—to restore ecosystems in alignment with mātauranga Māori (indigenous knowledge)[4]. As part of this effort, AUT and the Ngāti Whātua Ōrākei have collaborated at the Pourewa Creek Recreation Reserve under the Living Laboratories project umbrella.

At this site, the efficacy of native nursery treatments are under investigation to determine how to make replanting native forest more efficacious and economical. The nursery treatments studied at the site are a ‘mixed’ treatment—of māhoe (*Melicytus ramiflorus*, whiteywood), ngaio (*Myoporum laetum*, mousehole), tarata (*Pittosporum eugenioides*, lemonwood), karamū (*Coprosma robusta*)—and a ‘kānuka’ treatment solely comprised of kānuka (*Kunzea ericoides*, white tea-tree). It was noted before sampling that some areas of the site displayed better relative growth rates than others, raising the question of how these above-ground differences relate to underground physiochemical and microbial properties.

Physiochemical and microbial assessments are fundamental to characterising soil health and recognising bacterial and fungal contributions to underground and above-ground geochemical processes and ecosystem services. This study investigates the correlation of rudimentary physiochemical measures—in addition to microbial abundance levels—to above-ground floral growth and designed nursery treatment. Abiotic conditions drive biological and greater ecological processes and produce disparate microhabitats. Therefore, physiochemical properties were expected to vary across nursery treatments and align with above-ground growth observation on the restored sampling plot.

Our project investigated low-cost measures of commonly investigated soil metrics: active carbon (C), pH, bulk density (BD), gravimetric water content (GWC), and soil moisture (SM). pH is a fundamental soil property that broadly underpins organic nutrient content and biological activity, and active C directly influences underground and above-ground communities[5]. Active C is the small, labile fraction of soil carbon that is an energy source for biological activity[6]. Changes in these properties may be early indicators of change in soil quality[6], which shapes microbial processes and activity levels. Water content, assessed through BD, GWC, and SM measurements, offers distinct interpretations of the levels of soil compaction, structure, and water saturation.

These physiochemical aspects were investigated in conjunction with microbial community composition through RT-qPCR amplification of fungal and bacterial DNA. Since bacterial community composition within soil can predict soil quality and physiochemistry[7], we posited the ratios of fungal to bacterial DNA would be similarly predictive of above-ground growth levels.

Subsequently, we aim to conclude whether nursey treatment and relative growth rate—as observed by above-ground growth—is correlated to (1) relative bacterial and fungal DNA abundances and (2) several physiochemical variables. These physiochemistry measurement tests are then (3) evaluated as a low-cost proxy of soil health.

Figure 1. Map of Tāmaki Makaurau (Auckland) with marked sampling locations of Pourewa (blue) and Kepa Bush (green) reserve. Pourewa contains the Living Laboratories planting sites and sample transect.

3. Methods

3.1 Field Sampling

Soil sampling took place in the Kepa Bush reserve (-36.8631894, 174.8277260) as an undisturbed control measure and in 10 quadrats located along a 100 m transect within the 2.2 ha Living Laboratories experimental site at the Pourewa Creek Recreation Reserve. Kepa Bush sampling occurred within a 5 m^2 area at points of limited ground cover and exposed soil areas sufficiently exposed to insert soil corers.

The plot of land alternated between nurseries with ‘mixed’ and ‘kānuka’ treatments (Figure 2, Nursery Treatment). Greater and lower relative growth rates are reported as “good” and “bad” respectively.

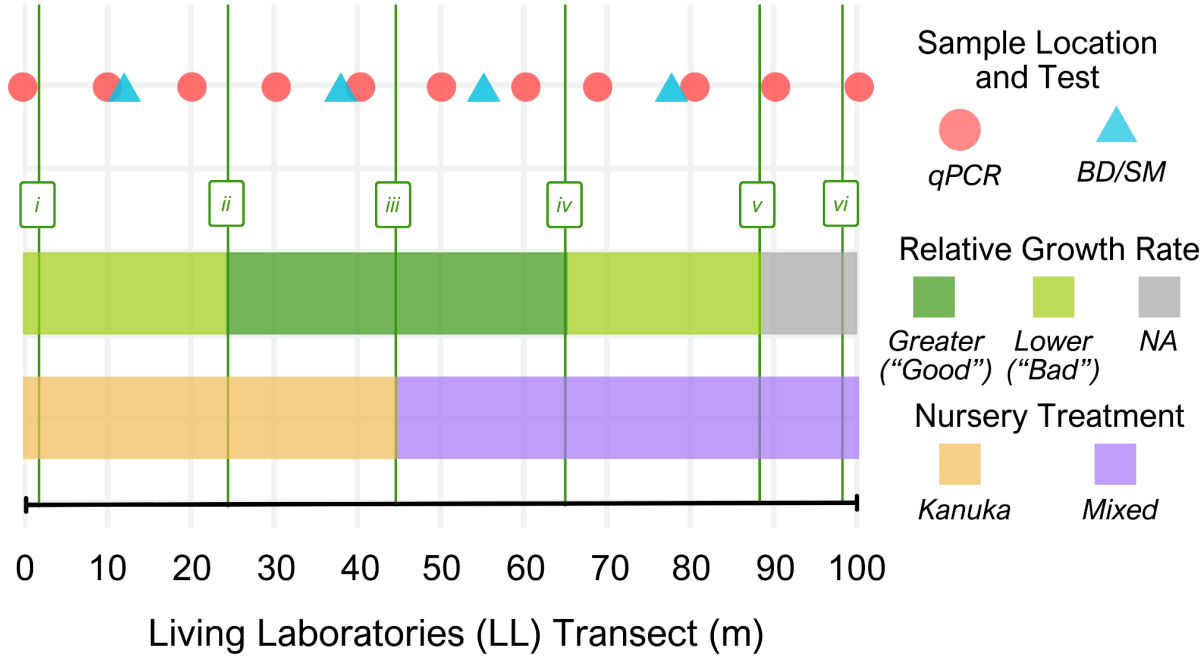

Figure 2. Samples were collected along a 100 m transect through the Living Laboratories nursery sites (LL). 11 soil samples were taken for qPCR analysis from regions spanning both nursery treatments (Nursery Treatment). Four additional samples were taken for bulk density (BD), and soil moisture (SM) assessments. SM samples were also used in Gravimetric Water Content testing. Transect regions, denoted by Roman numerals, are employed in multiple figures.

Kepa Bush samples employed for qPCR analysis used homogenised soil samples from two 10 cm soil cores. Four additional samples were cored at 10 cm depths[8] from Kepa Bush for bulk density measurement.

Soil samples were frozen or refrigerated as necessary.

3.2 Physiochemical Analysis

3.2.1 Active C Potassium-oxidisable carbon (POXC) in mg per kg of soil was determined to measure active C. Samples (2.50 ± 0.05 g) were homogenised, oven-dried (60 °C), and sieved (2 mm). Potassium permanganate digestion solution (20 mL, 0.015 M) was added before the sample was shaken (2 min) and left to stand (10 min). The solution was diluted (1:60) and the absorbance measured (λ = 530 nm).

3.2.2 pH Soil samples (2 g) were combined with deionised water (2 g). They were shaken (30 min) and left to settle (8 hrs); this cleaning process was repeated before soil pH was assessed using pH test strips. Where colouration was unclear due to debris, strips were lightly wiped to reveal colour change and measure alkalinity/acidity relative to the manufacturer's colour chart.

3.2.3 Bulk Density (BD) and Gravimetric Water Content (GWC) BD samples were taken with cylindrical soil corers (depth = 10 cm, volume = 502.4 cm^3) and oven dried (60 hrs, 60°C). Initial and dry masses were recorded to the nearest gram. BD measurements were calculated by dividing dried sample mass by core volume and GWC by dividing the (lost) water mass over the dry sample mass.

3.2.4 Soil Moisture (SM) SM was measured on site using the Campbell Scientific Hydrosense II. SM estimates water content by directly measuring soil resistivity and concluding moisture levels. Soil composition and compaction also influences SM measurements, as resistivity is influenced by salinity and temperature.

3.3 Genomic Analyses

3.3.1 DNA Extraction PowerSoil Pro DNA Extraction Kit was used per manufacturer instructions to process soil samples (250 mg) with the following modifications to manufacturer instructions: Fastprep-24 vortexing at 5.5 ms^{-1} for 60 seconds replaced initial horizontal Vortex Adapter vortex; EA Buffer was allowed to evaporate for 2 min prior to centrifugation; lastly, samples incubated in the C6 Elution Buffer for 2 min before centrifugation.

3.3.2 DNA Quantification Invitrogen Life Technologies (ThermoScientific) Qubit protocol was followed for 1 µl extracted DNA from each sample.

3.3.3 qPCR Analysis qPCR analysis followed Applied Biosystems protocol for real-time PCR analysis of bacterial and fungal relative presence through PowerTrack™ SYBR™ Green dye fluorescence. Bacterial and fungal DNA were assessed using the broad-range primers EUB338/EUB518 and 5.8s/ITS1f, respectively. Technical triplicates of all samples for bacterial and fungal analyses were located on a single 96-well plate. Each well contained 1µl DNA (1:10 dilution). qPCR cycling conditions are provided in Table 1.

Cycle stage	Temperature (°C)	Time (seconds)	Number of Cycles
Polymerase Activation	95	120	1
Denaturation	95	5	40
Annealing (Bacterial)	53	30	40
Annealing (Fungal)	53	30	40
Elongation	72	60	40

Table 1. qPCR cycle conditions for bacterial and fungal analysis, using broad-range bacterial primers, EUB338/EUB518, and broad-range fungal primers, 5.8s/ITS1f, and the PowerTrack SYBR Green Master Mix—which include an antibody-mediated hot-start DNA polymerase—with protocol-specified polymerase activation temperature.

3.4 Statistical Analyses

Mann-Whitney U, Kruskal-Wallis, Spearman correlation coefficient, and one-way ANOVA were conducted ($\alpha = 0.05$) using R Studio software[9] and the *dplyr*[10] and *nortest*[11] packages. Figures 3-6 and Tables 2-3 were constructed using R Studio software[9].

4. Results

4.1 Bacterial and Fungal DNA

To grossly estimate the microbial abundance within the Living Laboratories soil, we investigated the relative DNA abundance for a broad range of bacterial and fungal species. RT-qPCR analyses revealed a general increase in DNA abundance with increasing transect distance (Figure 3), determined by its inverse relationship with Ct value, in both bacterial and fungal species (Figure 3). Additionally, the microbial abundance at all transect sites—except for at 0 m—was greater than the Kepa Bush site, for bacterial (Ct = 16.58) and fungal (Ct = 20.55) species.

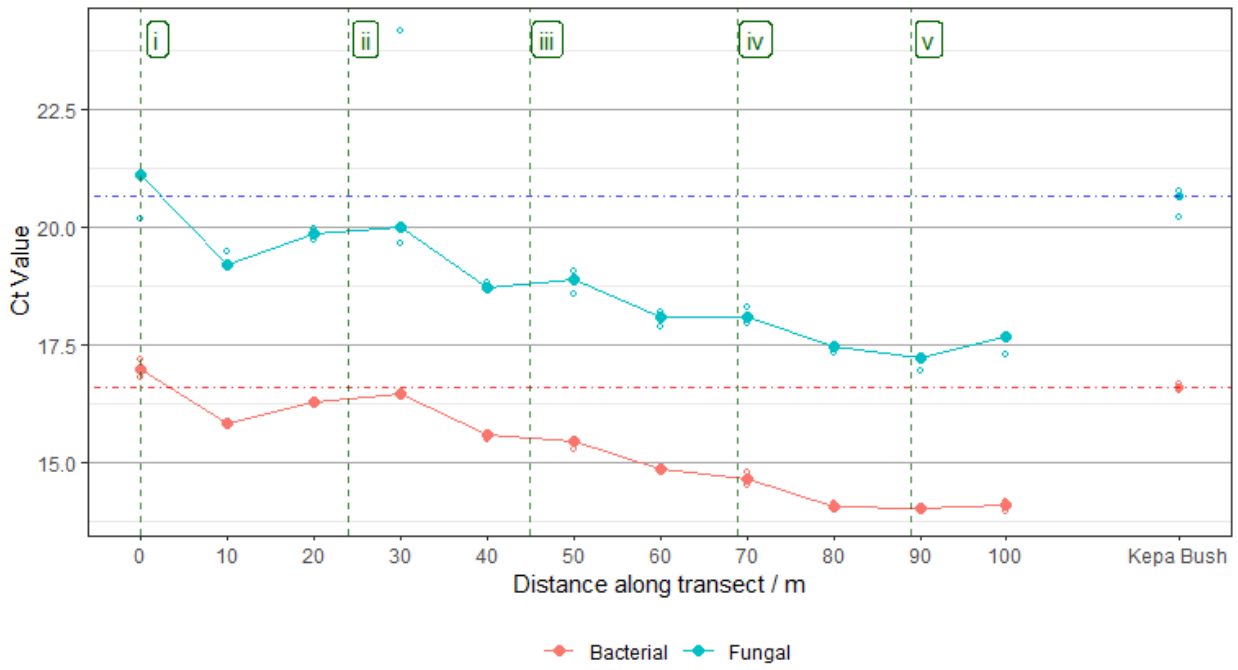

Figure 3. Bacterial and Fungal DNA abundances, as Ct values, along the Living Labs transect and Kepa Bush site. The Roman numerals correspond to the regeneration technique and its perceived relative success, as per Figure 2. Horizontal dashed lines represent the Ct values for the Kepa Bush site.

A Mann-Whitney U (MWU) test ($\alpha = 0.05$) was performed to determine if there were any significant differences in bacterial and fungal abundances amongst the transect and the Kepa Bush sampling site. This analysis of bacterial and fungal Ct values (Figure 2) determined non-significant differences in DNA abundance between sample sites (Table 2).

Transect points were categorised based on visible differences in floral growth levels; these sectors were categorised as “good” and “bad” to indicate their relatively greater or lower growth across both nursery treatment types. The distribution of these sectors has been visualised in Figure 2 by Roman numerals and Figure 1. MWU analysis of Ct values (Table 3), based on “good” and “bad” growth rates, as well as treatment type, found that above-ground observations had limited correlation to soil microbial communities. Discussion of qPCR results relative to above-ground growth omits data from regions without determinable relatively greater or lower growth rate (at 90 m and 100 m).

Table 2. P-values generated via MWU analysis comparing Ct values from every point along the LL transect to that of the Kepa Bush site.

	0m	10m	20m	30m	40m	50m	60m	70m	80m	90m	100m
Bacteria	0.081	0.081	0.077	0.081	0.081	0.081	0.149	0.149	0.081	0.081	0.081
Fungal	0.663	0.081	0.081	0.663	0.081	0.081	0.081	0.081	0.081	0.077	0.081

Table 3. MWU test p-values compare the Ct values of points along the LL transect relative to the Kepa Bush Ct values (not recorded). p-values are grouped by nursery treatment type and relative growth success (“Good” and “Bad).

	Bad.Kanuka	Good.Kanuka	Good.Mixed	Bad.Mixed
Bacterial	0.459	0.028	0.037	0.037
Fungal	0.196	0.156	0.028	0.028

The Spearman’s rank correlation coefficient between bacterial and fungal Ct values (ρ = 0.96) and similar Ct ratios (Figure 3) showed near-parallel DNA abundance patterns across sampled sites. Therefore, the bacterial and fungal DNA abundance is discussed concurrently.

4.2 Active Carbon (C)

A Kruskal-Wallis (KW) analysis (α = 0.05) of active C abundance amongst transect samples determined non-significant differences in active C levels (chi-squared (□²) = 15.813, df = 11, p-value = 0.1482), with no observable trends amongst points statistically supported by the large □² value. MWU (α = 0.05) of mg active C per kg soil grouped by relative growth rate (p = 0.9245) or nursery treatment (p=0.9273) was also non-significant. There was no correlation between active C and bacterial (ρ = -0.04) or fungal (ρ = 0.13) Ct.

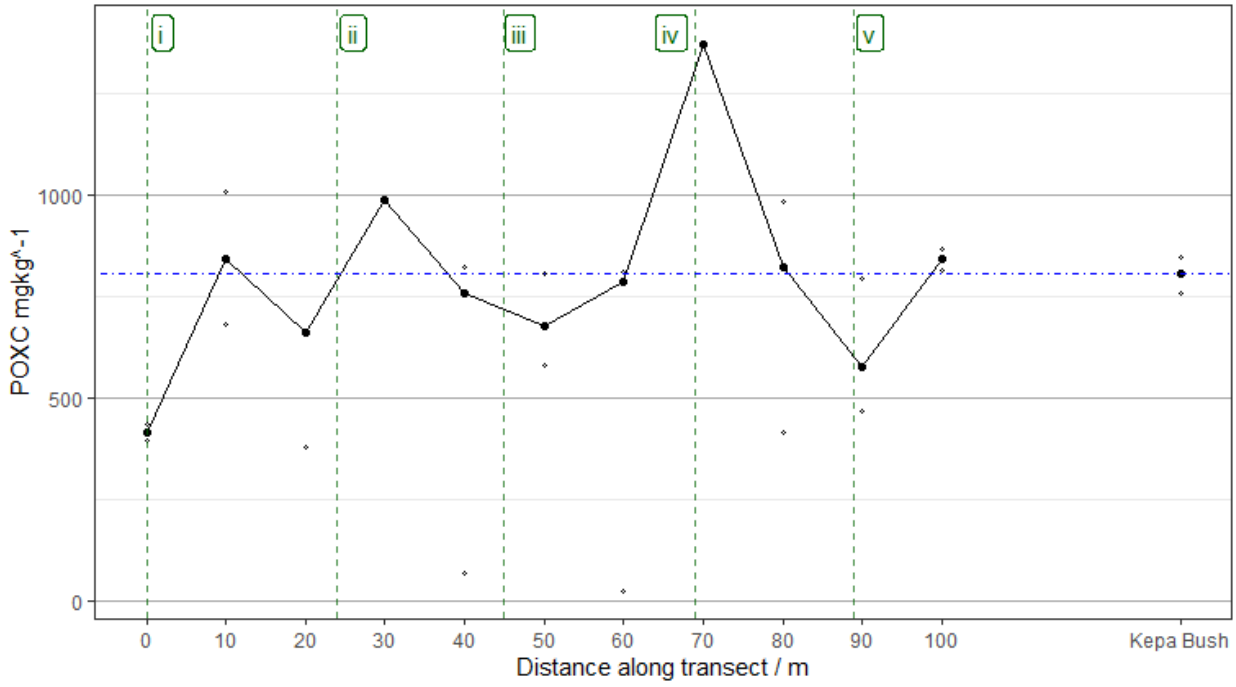

Figure 4. Active C quantities along the LL transect and Kepa Bush site. Roman numerals correspond to

the regeneration technique and its perceived relative success, as per Figure 2. The horizontal dashed line represents the POXC value of the Kepa Bush site.

4.3 pH

Including the abnormal acidic sample (90 m), a KW test (α = 0.05) found no significant pH differences (chi-squared = 5.0449, df = 5, p-value = 0.4104) along the transect. Analysis following the omission of the outlier at 90 m still found no significant differences (chi-squared = 10, df = 10, p-value = 0.4405).

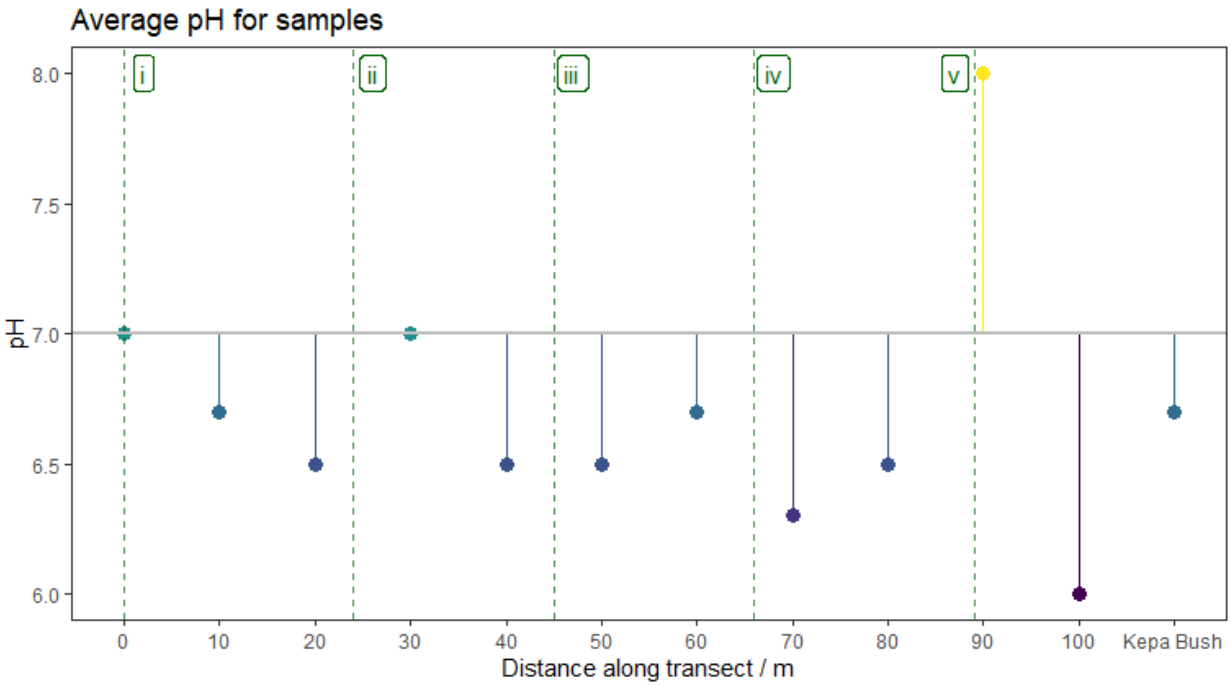

Figure 5. pH values of the points along the LL transect and Kepa Bush site, where darker colours represent greater acidity. Roman numerals correspond to the regeneration technique and its perceived relative growth success.

4.4 Bulk Density, Soil Moisture, and Gravimetric Water Content

A one-way ANOVA test (α = 0.05) was performed to compare the effects of the region along the transect on each of the physical soil properties. ANOVA analysis concluded no significant differences between the transect and each of the measured properties. Soil moisture ($F(1) = 0.213$, p-value = 0.66), bulk density ($F(1) = 0.391$, p-value = 0.566), and gravimetric water content ($F(1) = 0.791$, p-value =0.424), all showed no statistically significant differences.

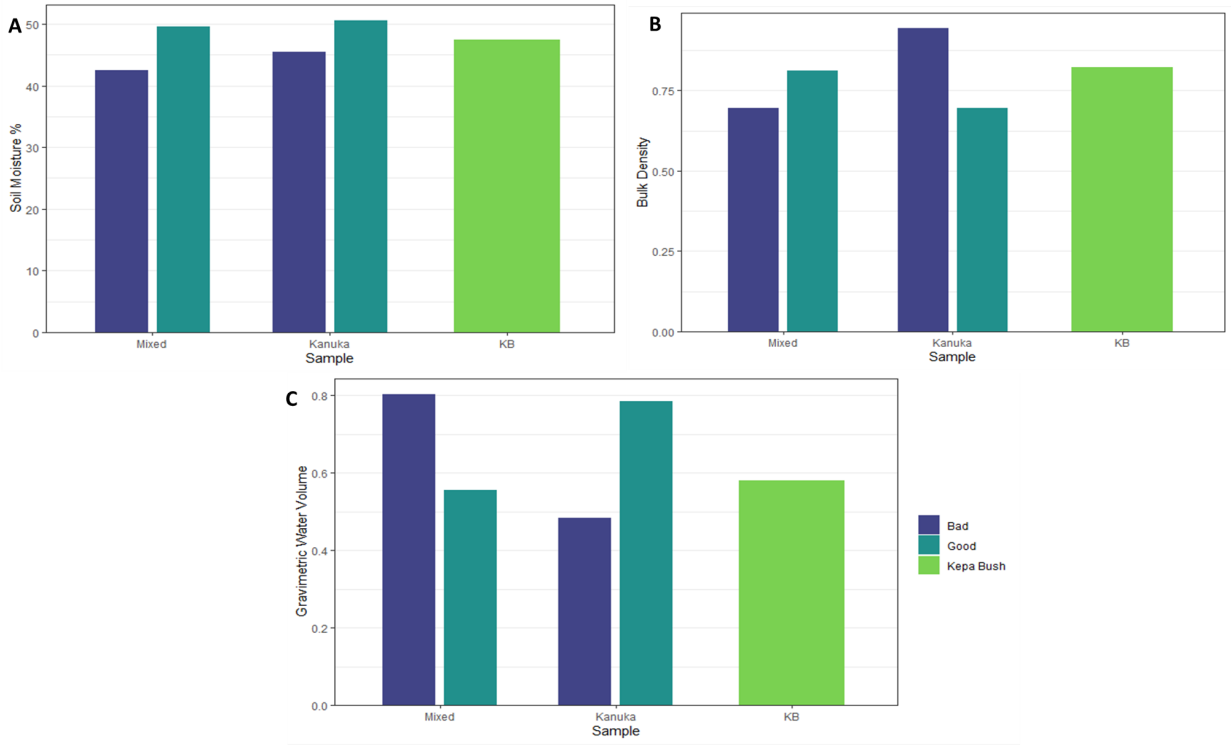

Figure 6. Soil Moisture (A), Bulk Density (B), and Gravimetric Water Volume for each region along the transect (as per Figure 2) and Kepa Bush site.

5. Discussion

5.1 Bacterial and fungal DNA abundance is more strongly correlated to nursery treatment than relative growth rates

Nursery treatments, kānuka or mixed, appear to more strongly influence the microbial abundance patterns than the visible ‘good’ or ‘bad’ growth rates. Restricting Ct values by nursery treatment produces narrower DNA abundance ranges than restriction by visible growth rates (Figure 3). The importance of nursery treatment suggests that floral species composition directs microbial community structure and warrants further investigation of the floral-microbial biodiversity connection[12].

Mixed nurseries demonstrate greater microbial abundance levels relative to the Kepa Bush and kānuka treatment sites, and a significant divergence (p-value ≤ 0.037, Table 3) from the unadulterated and innately biodiverse Kepa Bush habitat. However, there are limited conclusions about the relative soil health of these regions based on qPCR results.

Microbial abundance (Figure 3) does not equate to levels of genetic diversity within the detected bacterial and fungal species within the soil; high levels of microbial biodiversity may suggest good soil health, but high levels of microbial abundance cannot appropriately rule on the quality or health of the soil. The use of broad-range primers during qPCR analysis, thus, limits the commentary this study can make regarding the success of restoration efforts based on below-ground microbial communities.

Given the critical role of high microbial diversity in maintaining soil quality[13,14] and as a key indicator of soil health[15], the choice between monospecies (kānuka) or multispecies (mixed) restoration should be supported beyond relative assessments of bacterial and fungal abundances.

While floral diversity directly improves through mixed species nurseries, the non-significant microbial community difference between kānuka nurseries and native bush (Table 3) may support the use of kānuka-exclusive nurseries. These limited conclusions may be improved through further investigation of the phylogenetic diversity of soil microbial species.

To return to discussions of abundance patterns, there is a general increase in DNA abundance along the transect (Figure 3). Therefore, nursery species composition may not influence microbial communities. Rather, an unaccounted factor e.g. unequal nutrient deposition due to transect topography may have impacted DNA abundance within the nursery treatments. If this factor is transient and has a limited range of influence, a longer transect may resolve the potential for its confounding bias.

5.2 Limited ability of physiochemical metrics to estimate above-ground floral growth

5.2.1 Active C

The mass of active C per kg of soil from the LL samples showed no significant difference along the transect (Figure 4), even when analysed by relative growth rate nor nursery treatment. In addition, there was no correlation between active C and bacterial ($\rho = -0.04$) or fungal ($\rho = 0.13$) Ct. The main reason for this is likely technical error; literature[6] shows strong linear correlations between active C and measures of microbial activity so the lack of correlation is unexpected.

Although this methodology is financially accessible, it is complex, requiring precise timing at shaking and settling steps[16], and analysis must be conducted swiftly due to the degradation of

the potassium permanganate test solution. Based on this study's investigation, we do not recommend this low-cost POCX assessment of active C[16] based on its low reproducibility by non-scientist users (e.g. farmers, as suggested by Weil et al.[6]).

5.2.2 pH

No correlation was found between the above-ground observations in growth and pH levels across the transect and Kepa Bush (Figure 5). The nonsignificant pH difference between transect regions of greater and lower growth may be due to unusual climatic effects. The small coverage and sample size (n = 36) along the transect have minimised discernable pH differences; the coinciding periods of sampling and the Aotearoa rainy season (winter) likely also reduced inter-sample pH variation compared to the potential dry season (summer) measurements. Whangaparāoa (Auckland Peninsula) also witnessed drastic increases in rainfall in 2023[17], above the historical rainfall levels (>1000 mm[18]).

In contrast to well-characterised influence of pH in shaping microbial communities[19, 20, 21], we conclude that there is no pH-based relationship to microbial abundance across our sample site based on the non-significant pH trends across the Living Laboratories transect. Despite the readily-available commercial pH test strips, pH assessment does not appear to produce sufficiently useful data. Therefore, this study does not suggest pH to be a worthwhile measurement of soil physiochemical differences across small areas.

5.2.3 Bulk Density, Soil Moisture, Gravimetric Water Content

The composition of native species within the Living Laboratories, defined by the mixed and kānuka nursery treatments, did not produce significant soil SM, BD, or GWC differences (Figure 6). Rainfall patterns also likely influence the non-significant SM, BD, and GWC, for reasons discussed in *5.2.2 pH*. Future investigations should record physiochemical metrics over an extended, climatically-diverse period. However, there is SM, BD, and GWC variation between the regions of greater and lesser floral growth.

Although the measured physiochemical variables are not significantly related to growth rates, SM displays the strongest positive correlation. Relative growth rates on both nursery treatments (Figure 5a) suggest that SM is the most promising diagnostic method to estimate soil quality within the Living Laboratory project. SM is not the most accessible method studied for measuring soil quality, as it requires specialised technology (Campbell Scientific), but SM does estimate soil water content with less processing time than BD and GWC measurements.

Neither BD nor GWC showed significant differences within the transect or when compared to Kepa Bush, likely because of the similar soil texture, moisture and organic matter content across the 100 m transect, which are determinants of soil compaction[22]. The authors propose that while BD and GWC serve as useful metrics of soil physiochemistry across wider regions, BD and GWC assessments will be uninformative, and should be ignored in plots, similar to the Living Laboratories, that cover small areas and have limited differences in soil identity or usage.

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7. References

[1] UNFCCC. *Nationally determined contributions under the Paris Agreement* (UNFCCC, 2022).

[2] IPCC. *Climate Change 2022: Impacts, Adaptation and Vulnerability* (IPCC, 2022).

[3] Concannon, C. *The Living Laboratories project*. RNZ News (2022). Available from https://www.rnz.co.nz/national/programmes/ourchangingworld/audio/2018851659/the-living-laboratories-project

[4] Auckland University of Technology (AUT). *AUT Living Laboratories*. (AUT, 2019). Available from: https://wedocs.unep.org/handle/20.500.11822/28861

[5] Tang, C., Yang, F., & Antonietti, M. Carbon Materials Advancing Microorganisms in Driving Soil Organic Carbon Regulation. *Research* (2022.

[6] Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B. & Samson-Liebig, S.E. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *American Journal of Alternative Agriculture* **18**, 3-17 (2003).

[7] Hermans, S.M., Buckley, H.L., Case, B.S., Curran-Cournane, F., Taylor, M., & Lear, G. Using soil bacterial communities to predict physico-chemical variables and soil quality. *Microbiome* **8**, 1-13 (2020).

[8] Al-Shammary, A. A. G., Kouzani, A. Z., Kaynak, A., Khoo, S. Y., Norton, M., & Gates, W. Soil bulk density estimation methods: A review. *Pedosphere* **28**, 581-596 (2018).

[9] Posit team. *RStudio: Integrated Development Environment for R*. Posit Software, PBC, Boston, MA (2023).

[10] Wickham H., François R., Henry L., Müller K., & Vaughan D. *dplyr: A Grammar of Data Manipulation*. R package version 1.1.4 (2023)

[11] Gross J. and Ligges, U. *nortest: Tests for Normality*. R package version 1.0-4 (2015)

[12] Thakur M.P., Phillips H.R., Brose U., De Vries F.T., Lavelle P., Loreau M., Mathieu J., Mulder C., Van der Putten W.H., Rillig M.C., & Wardle D.A. Towards an integrative understanding of soil biodiversity. *Biological Reviews* **95** 350-64 (2020).

[13] Rao, D.L.N. Microbial diversity, soil health and sustainability. *Journal-Indian Society of Soil Science* **55**, 392 (2007).

[14] Sharma, S.K. et al. Microbial Community Structure and Diversity as Indicators for Evaluating Soil Quality. *Biodiversity, Biofuels, Agroforestry and Conservation Agriculture* **5** 317-58 (2010).

[15] Chourasiya, D., Sharma, M.P., Maheshwari, H.S., Ramesh, A., Sharma, S.K. & Adhya, T.K. Microbial diversity and soil health in tropical agroecosystems. *Advances in Soil Microbiology: Recent Trends and Future Prospects* **2** 19-35 (2017).

[16] Vanek, S., Fonte, S., & Magonziwa, B. *Soil Health Evaluation Manual Version 6.2* (Soils Cross Cutting Project, McKnight Foundation, 2018). Available from https://smallholdersha.files.wordpress.com/2018/07/soiltoolkitmanual_sv6-2.pdf

[17] Morton J. *Weather: New Zealand's dramatically wet-and-dry 2023 - in 10 charts*. Available from https://www.nzherald.co.nz/nz/new-zealands-dramatically-wet-and-dry-2023-in-10-charts/7BPCBRLSWZE25BQ24MGEENLN5E/ (NZ Herald, 2023)

[18] Stats NZ. *Rainfall | Stats NZ*. Available from https://www.stats.govt.nz/indicators/rainfall (updated 2023)

[19] Hermans, S.M., Buckley, H.L., Case, B.S. et al. Using soil bacterial communities to predict physico-chemical variables and soil quality. *Microbiome* **8**, 79 (2020).

[20] Li, K., Hu, J., Li, T., Liu, F., Tao, J., Liu, J., Zhang, Z., Luo, X., Li, L., Deng, Y., & Che, R. Microbial abundance and diversity investigations along rivers: Current knowledge and future directions. *Wiley Interdisciplinary Reviews: Water* **8**, e1547 (2021)

[21] Zhalnina, K., Dias, R., de Quadros, P.D. et al. Soil pH Determines Microbial Diversity and Composition in the Park Grass Experiment. *Microb Ecol* **69**, 395–406 (2015)

[22] Carter, M.R. & Gregorich, E.G. *Soil Sampling and Methods of Analysis.* 2nd ed. 771 - 772 (2008)