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Urban forest restoration enhances soil microbial functional potential and functional insurance via shifts in β-diversity

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Abstract

Background Forest restoration has primarily been evaluated through changes in aboveground communities, while belowground microbial communities—critical drivers of ecosystem functions—remain less understood. Moreover, studies of soil microbes have focused largely on community structure, which does not necessarily reflect the recovery of functional capacity and stability.

Methods To determine how forest restoration affects microbial community structure and function and how microbial diversity relates to ecosystem multifunctional potential and stability, we analysed soil microbial communities from 79 urban forest restoration sites across New Zealand, spanning 0–63 years since initial plantings. Shotgun metagenomic sequencing was used to characterize taxonomic composition and functional potential, with diversity quantified using alpha and beta metrics. To evaluate links between diversity and ecosystem function, we assessed ecosystem multifunctional potential (EMF) which describes the ecosystem's capacity to simultaneously provide multiple functions, and we developed a novel functional insurance (*FI*) index grounded in ecological theory as an indicator of functional stability and resilience. To calculate *FI* in microbial systems from sequencing data, we quantified functional overlap by estimating over 250 million species-function correlations per sample.

Results Contrary to our expectations, only beta diversity, not alpha diversity, was positively associated with EMF and *FI*, indicating that community composition and dissimilarity rather than species richness underpins microbial functional capacity and stability. EMF and *FI* were positively correlated, showing that high functional diversity and functional overlap can co-occur in microbial systems. In addition, archaeal turnover increased with closing forest canopies, contributing to higher EMF and *FI*, while bacterial turnover was only weakly associated with restoration parameters. Notably, restoration time did not play a role in shaping microbial diversity, EMF and *FI*.

Conclusions Our findings demonstrate that microbial compositional turnover, rather than increases in species richness, are critical for restoring soil ecosystem functions. Incorporating microbial functional metrics like the *FI* index into restoration frameworks that recognise both above and belowground dynamics could promote resilient and multifunctional urban forests.

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Introduction

In the Anthropocene, urbanization, globalization, and climate change are reshaping ecosystems in profound ways. Yet the impacts on soil microbial biodiversity—a key driver of nutrient cycling, carbon sequestration, plant growth promotion, and disease control—are often overlooked [15, 46, 73]. Soil microbiomes influenced by urbanization have an increased potential for pathogens, loss of plant–microbe mutualism, and global homogenization rather than local adaptation [15, 64, 75]. It is therefore imperative to not only avoid further habitat loss but to actively restore biodiversity in degraded soils. Urban ecosystems are subjected to particularly severe disturbance and ecosystem degradation [20, 72]. Therefore, urban landscapes could especially benefit from ecosystem restoration efforts that rebuild microbial diversity and function, facilitating crucial ecosystem services in the process.

While increasing biodiversity is often linked to improved ecosystem function across virtually all biological domains of life [48], Shiping [13, 14, 28], the relationship is complex and often non-linear, especially in microbial systems [15], (Nannipieri et al. 2017; Maron et al. 2018). Inconsistent evidence between studies in controlled versus natural environments highlights the strong environmental context-dependence of microbial diversity-function relationships [7]. This context-dependence arises in part from unique microbial traits, including rapid species turnover, metabolic plasticity, and buffering effects of functionally overlapping species [34]. Key measures of alpha diversity (richness and/or evenness of taxa/functions found per sampling unit) and beta diversity (turnover or dissimilarity of taxonomic/functional assemblages between sampling units) also depend on scale and habitat heterogeneity. As a result, predicting how changes in microbial diversity will influence key ecosystem processes remains a challenge. To better understand these dynamics, additional research is needed on the role of 'functional insurance' in mediating the relationship between microbial diversity and ecosystem stability.

Functional insurance (also commonly referred to as functional redundancy; but see [19]) is described as the coexistence of distinct taxa with overlapping traits and functional roles within a community and promotes the stability of biodiversity and ecosystem functioning [39]. Functionally overlapping species with different ecological strategies also contribute to more efficient resource usage and multifunctionality (the simultaneous provisioning of multiple ecosystem functions), increasing ecosystem stability, adaptability, and productivity [8, 33, 36]. For example, Yi Li et al. [35, 37] found that increased plant diversity in grassland and forest experiments bolstered multifunctionality indirectly by elevating the diversity of other trophic groups such as herbivores, predators, and decomposers. More generally, both alpha and beta diversity have been found to promote multifunctionality to varying degrees depending on the ecosystem and study organism in question [25, 51, 65]. To date, our understanding of functional diversity and insurance has mostly been derived from studies of macro-organisms like plants and animals [38], which typically interact at much larger temporal and spatial scales than microbes. As studies on macro-organisms cannot confidently be extrapolated to microbial systems, targeted research into microbial structure and function is needed to understand the impacts of disturbance and restoration on belowground functional insurance [16, 59].

Drawing on the concepts of multifunctionality and functional insurance, effective ecosystem restoration should aim to rebuild the structure and function of biodiversity, both above- and belowground. Vegetation planting to recreate a specific species assemblage has traditionally been the focus of terrestrial restoration (here meaning forest reconstruction; [23, 79]), but this has resulted in the foundational role of soil microbiomes often being overlooked [29, 52, 63]. To secure long-term functional stability and multifunctionality, restoration goals should extend beyond establishing forest plant species richness and include strategies that foster the recovery of soil microbiomes [52, 53], with microbiomes being defined as a microbial community occupying a defined habitat with distinct physicochemical properties, as well as their activity [82]. Although integrating microbial ecology into restoration planning could enhance critical ecosystem processes, evaluations of microbial multifunctionality in applied settings remain scarce [24, 68, 78]. Additionally, microbial functional insurance in a restoration context has received little attention, likely due to methodological constraints and fundamental knowledge gaps. As a result, we still lack answers to key questions: How do forest restoration plantings shape soil microbial diversity, and to what extent does this diversity confer functional insurance across multiple ecosystem processes?

Here, we use a metagenomics approach to investigate soil prokaryotic community structure and functional potential spanning a restoration planting sequence of 79 urban forest sites. We assess how microbial alpha and beta diversity, as well as the functional capacity for major

microbial functions such as carbohydrate and nitrogen metabolism, are influenced by forest restoration plantings. Additionally, we develop a novel functional insurance index to explore how restoration and microbial diversity shape ecosystem multifunctional potential and functional insurance in urban forest soils, and to provide a method to quantify soil microbial functional insurance and ecosystem resilience using metagenomic sequencing data. We hypothesized that an increase in alpha and beta diversity compared to unrestored sites leads to (1) an increase in multifunctional potential and (2) an increase in functional insurance. A greater local variety of taxa (i.e., alpha diversity) could increase the chances of including both functionally complementary and overlapping species. This could expand the range of available functional traits while also providing a buffer against species loss, contributing to increased ecosystem stability. Further, greater microbial community dissimilarity (i.e., beta diversity) from unrestored sites may signal restoration progress and a transition toward more mature, stable forest ecosystems, where microbial assemblages underpin a broader and potentially more resilient range of ecosystem functions.

Methods

Study sites

We surveyed 79 sites across nine cities in Aotearoa New Zealand, comprising 74 urban forest restoration sites with no trees present before planting and five unrestored sites. These sites spanned a wide latitudinal range (– 46.45137°S to – 37.67842°S) and encompassed most of the country's climatic variation (Figure S1, Table S1). Restoration sites ranged from 9 to 63 years since initial planting of juvenile native plant species (referred to as 'restoration time'). The sites ranged from 0.1 to 148 hectares (mean = 9.7 ha), with patch size estimates incorporating adjacent remnant forest. Each restoration site included a single permanent sampling plot established through the People, Cities & Nature program (PCaN; https://www.peoplecitiesnature.co.nz/), a research initiative aimed at providing a scientific foundation for restoring nature in urban environments. Sampling plots each measured 10 × 20 m and were positioned > 1 m from the forest edge. All plots excluded streams, lakes, or coastal access. Within each plot, three 1 × 1 m subplots were established—one at the centre and two in opposite corners along the diagonal. The five unrestored sites with an effective restoration time of 0 years were situated near restoration sites in Hamilton, New Plymouth, Tauranga, Wellington, and Invercargill, spanning the entire longitudinal range of the sampling network, and consisted of either mown or unmanaged grassland that represented the state of restoration sites prior to initial planting. Since the focus of the study was on the trajectory and changes along the restoration gradient, we focused on having a large sample size of restoration sites rather than a matching unrestored site for each restoration site.

Sample collection

Fieldwork took place between February and May 2023 (spanning summer and autumn). Data collection at all 79 sites included: (1) soil sampling for DNA extraction, soil moisture and physicochemical testing, enzyme activity, and microbial respiration, (2) measurements of canopy openness and leaf litter depth, and (3) assessment of ground cover. Vegetation survey data from 2018 was available for all previously established plots, while the newly established unrestored plots were surveyed at the time of sampling.

Using decontaminated and/or UV-sterilized tools, four soil cores (10 cm depth, 5 cm diameter) were collected from each 1 × 1 m subplot. Cores from the three subplots within each plot were sieved through an 8 mm mesh, combined, and homogenized. The soil was kept on ice throughout the sampling day. Half of the homogenized sample was then sieved through a 2 mm mesh and transferred into two 15 ml falcon tubes (30 ml total) for metagenomic analysis; these were stored at – 8 °C during the fieldwork campaign and at – 20 °C upon return to the University of Waikato. From the remaining soil, 250 g was bagged for soil moisture and physicochemical testing, enzyme and respiration assays (see Supplementary methods) and refrigerated at + 4 °C. Soil moisture (wet basis) was calculated per sample as 1 – (dry weight / wet weight) after oven drying at 35–40 °C overnight. Physicochemical properties were analysed by Hill Labs (Hamilton NZ) and included pH, carbon, nitrogen, phosphorus, and organic matter content.

Vegetation data collection and calculations

Forest canopy openness—a proxy for understorey light availability—was measured at the centre and each corner of the plot (= five locations per plot) using a spherical crown densiometer (convex model A). Leaf litter depth was assessed to the nearest 0.5 cm three times at each of these five locations (15 measurements per plot) using a ruler to measure down to the humic layer; these values were then averaged to produce a single mean litter depth estimate per plot. Ground cover was visually estimated across the entire plot, categorised into five types: herbaceous vegetation, mulch, leaf litter, bare soil, and moss. Data on woody vegetation were available from a 2018 survey of all established plots. This included adult trees, saplings, and tree ferns with a diameter at breast height (DBH, measured 135 cm above ground) greater than 2.5 cm, for which both species identity and DBH were recorded. Seedlings were counted by species and

summed per plot. Tree evenness was calculated as Shannon diversity / log(richness) [56].

To quantify landscape context, a forest connectivity index was calculated for each site following Matesanz et al. [43]. This index considered the number of native forest fragments within a 10 km radius of each site, weighted by their distance from the focal plot, using data from the Land Cover Database [3].

Soil DNA extraction, sequencing, and bioinformatic data processing

We extracted soil DNA from all 79 samples using 0.25 g soil per sample with the Qiagen DNeasy PowerLyzer PowerSoil Kit (Qiagen, Venlo, Netherlands), following the manufacturer's protocol. DNA concentrations were measured fluorometrically using the Qubit 1X dsDNA High Sensitivity (HS) Assay Kit (Invitrogen, Waltham, MA, USA). Three negative extraction controls using lab-grade water were included and processed identically to the soil samples. Both DNA extracts and negative controls were sent to Auckland Genomics (University of Auckland, Auckland, NZ) for library preparation and sequencing. Libraries were prepared using the Illumina DNA Prep (M) Tagmentation kit (Illumina, San Diego, CA, USA), with 100 ng of input DNA per sample. Library quality was assessed using the Agilent Bioanalyzer High Sensitivity DNA Kit (Agilent Technologies, Santa Clara, CA, USA) and quantified again with the Qubit HS Assay. Libraries were then normalized and pooled in equimolar concentrations and sequenced in triplicate on the Illumina NovaSeq X Plus platform using 300-cycle kits, generating 2×150 bp paired-end reads. The run yielded 8.3 billion raw reads, an average sequencing depth of 90.2 ± 20.8 million raw reads per sample after merging technical triplicates and excluding controls.

Sequencing data were processed on the New Zealand eScience Infrastructure (NeSI) high-performance computing platform to assign taxonomic and functional classifications to the metagenomes. Raw sequence reads (FASTQ files) underwent quality assessment using FastQC [1] and were trimmed for quality and adapter removal with Fastp (Shifu [13, 14]). Cleaned triplicate reads were then merged. Taxonomic assignment was performed using Kraken2 [83] with the PlusPFP database (Standard plus RefSeq protozoa, fungi, and plant; database dated 10/9/2023: https://benlangmead.github.io/aws-indexes/k2). We used Bracken [40] to estimate relative abundances of taxa and generated an abundance table with KrakenTools [41]. In parallel, functional potential profiles were generated from high-quality sequences using the SUPER-FOCUS annotation tool [69]. This workflow employed the DIAMOND aligner (v0.9.19 [10],) and used the 100% identity-clustered version 2 reference database (100_v2; https://github.com/metagenni/SUPER-FOCUS/issues/66). SUPER-FOCUS assigns metagenomic sequences to the SEED subsystem functional annotations [50] and provides relative abundance estimates following the methodology outlined in Silva et al. [69].

All subsequent analyses were conducted in R Statistical Software v4.4.2 [58], using the *vegan* [49] and *phyloseq* [44] packages. To prepare the taxonomic data for modelling microbial community variation across the urban forest restoration sequence, taxa not assigned at the phylum level or occurring in only one sample (i.e. singletons) were removed. This standard quality filtering step helps eliminate low-confidence taxa, which may result from sequencing artefacts or incomplete database matches. Taxa classified as viruses, plants, or animals were also excluded to focus the analysis on target microbial taxa, including bacteria, archaea, protists, and fungi. To account for differences in sequencing depth and ensure sample comparability, taxon abundances were normalized using the *transform_sample_counts* function in *phyloseq*. The functional dataset was normalized, as well, and any functional annotations that included the term "in plants" were removed because they represented non-target functions.

Calculating functional insurance

The following functional insurance (*FI*) calculations were adapted from Ricotta et al. [61] who proposed an *FI* index for biological communities (termed functional redundancy there):

$$FI = S - Q \tag{1}$$

where S is Gini-Simpson diversity [70] and Q is Rao's quadratic entropy [60]. Rao's Q is a measure of diversity that accounts for both species abundances and the difference in functional traits between species, with the latter typically expressed as a trait-species matrix or dissimilarity matrix. For macro-organisms such as plants or animals, trait differences between species can be identified through observation and direct measurement. This is not the case for soil microbiomes due to the scale and high complexity of the system in terms of heterogeneous soil microhabitats, high taxonomic and functional diversity, and horizontal gene transfer, so trait-based dissimilarities between microbial species need to be inferred in a different way. Using metagenomic sequencing data, the following options were considered:

1. Calculating phylogenetic similarity using phylogenetic distance between species (as opposed to trait-based dissimilarity);

2. Assembling MAGs (metagenome-assembled genomes) to link functional genes to individual species;
3. Estimating trait–species correlations based on their co-occurrence in samples.

Here, we chose the third option for the following reasons: Phylogenetic similarity is not equivalent to functional insurance and thus should not be interpreted as such in the context of ecosystem functioning. That is because a phylogenetic approach cannot account for convergent evolution of traits nor for horizontal gene transfer [31, 35, 37]. Further, even though the assembly of MAGs is more reliable than correlation tests in linking species and functional traits, a considerable portion of sequence data typically cannot be successfully assembled into MAGs, resulting in information loss [45, 77].

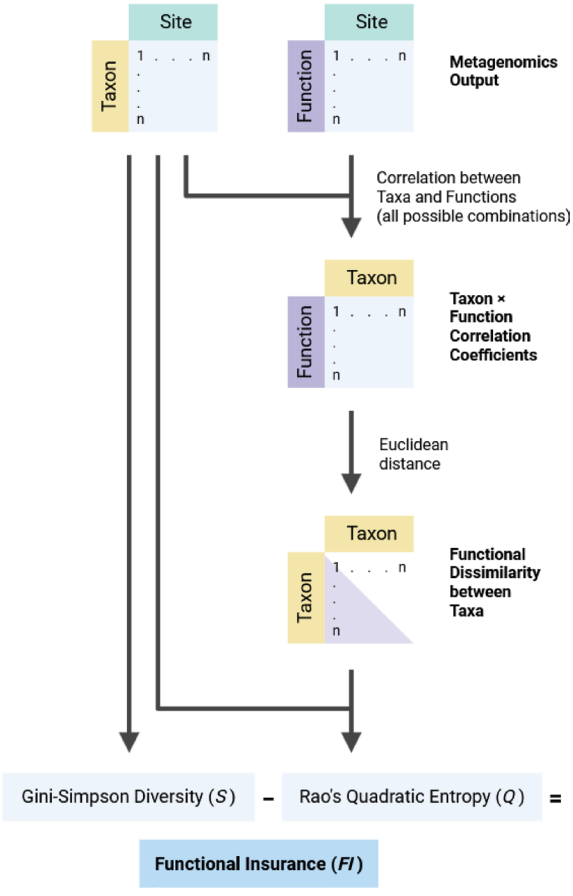

Fig. 1 Conceptual workflow for calculating Functional Insurance (*FI*) from taxonomic and functional metagenomics data, using relative abundance tables. Here, "function" refers to protein-coding gene sequences. *FI* is defined as Gini-Simpson Diversity (*S*)—Rao's Quadratic Entropy (*Q*), with *S* directly calculated from taxonomic abundance data. Calculation of *Q* requires both taxonomic abundance data and a measure of functional dissimilarity between taxa. Functional dissimilarity is derived by calculating correlation coefficients between all taxon–function pairs, retaining only significant positive correlations, and then calculating Euclidean distances between taxa based on their functional correlation coefficients

Functional insurance was calculated based on a subset of the full data due to technical limitations of computing capacity. Taxa with a mean abundance of > 0.001% and functions with a mean abundance of > 0.00001% were included, retaining 56.6% of taxa and 61.5% of functions. To assess how our subsetting approach to analyses may affect the true representativeness of our results, we also tested an alternative subset of functions by applying a threshold of functions that appeared in at least 50% of sites. This yielded a 98% identical functional subset compared to the abundance-based subset described above. Due to the similarity in outputs, hereafter we only use the former abundance-based subsetting approach. To identify significant trait–species correlations, we calculated the correlation estimate and *p*-value for each combination of individual taxa and functions (> 250 million combinations) using the *cor.test* function (method = "kendall") from the *stats* package. Only positive, significant ($p < 0.05$) correlations were retained. Negative and/or non-significant correlations were interpreted as 0. The resulting co-occurrence matrix between taxa and functions returned an estimate of which functions likely co-occurred with individual taxa. Any functions that showed no significant correlations with any taxa were removed from the matrix. This estimation does not aim to assign specific functions to taxa or make claims about their functional repertoire. Instead, the co-occurrence matrix is used solely to produce a measure of functional dissimilarity between taxa. For that purpose, we derived a taxon-specific Euclidean distance matrix from the co-occurrence matrix and used it to calculate *Q* with the *divc* function from the *ade4* package [18]. Finally, *FI* was calculated as $S - Q$ as described above (Fig. 1), using the *estimate_richness* function in *phyloseq* to calculate *S*. Compared to Shannon diversity, which weighs rare taxa more strongly and therefore tends to vary more in highly diverse soil communities, Simpson diversity (*S*) plateaus more quickly. Hence, variation in *FI* is largely dependent on *Q*. In a taxonomically highly diverse community such as soil, high *FI* values indicate a large functional overlap between taxa, while low *FI* values indicate low functional overlap. In a community with low taxonomic diversity, *FI* would always be in a low range because $S \geq Q$ [61]. In summary, *FI* indicates a community's potential to retain ecosystem functions throughout disturbance or species loss, with a high *FI* indicating a higher likelihood.

Statistical analysis

To test the relationship between environmental parameters and restoration time, we z-scaled all environmental parameters and fitted Generalized Linear Mixed Models (GLMMs) using the *glmmTMB* function with a Gaussian

distribution, city as a random effect, and restoration time as the predictor. To test for relationships between microbial taxonomic alpha diversity and restoration parameters (Table 1), we first calculated the Shannon–Wiener alpha diversity (α, [67]) per site with the *estimate_richness* function in *phyloseq*, and then calculated the log response ratio of alpha diversity (LRR_α) compared to unrestored sites:

$$LRR_\alpha = log\left(\alpha_{restored}/\overline{\alpha_{unrestored}}\right) \quad (2)$$

We fitted GLMMs with a lognormal distribution using $LRR_\alpha + 1$ as a response variable and city as a random effect. The log response ratio serves as a benchmarking metric to express restored sites relative to a baseline of unrestored sites, influencing only the model intercept without altering the underlying statistical relationships or slopes among predictors. Since the unrestored sites span the entire climatic and environmental range of the sampling network but are represented by a small sample size only, heterogeneity among unrestored sites may increase uncertainty in the unrestored baseline. Consequently, log response ratios are interpreted as indicators of the direction of restoration trajectories over time, rather than estimating recovery relative to a fixed or absolute baseline. We further conducted model selection based on AIC scores to perform model ranking using the *dredge* function from *MuMIn* [4]. Predictor coefficients were z-standardized for all statistical models. We repeated this analysis for bacterial and archaeal alpha diversity separately.

Similarly, we tested for correlations of restoration parameters with microbial taxonomic beta diversity, with the mean Bray–Curtis dissimilarity [9] of each restored site to all unrestored sites as an index of beta diversity (β). Bray–Curtis dissimilarity between sites was calculated using the *distance* function from *phyloseq*. Since Bray–Curtis dissimilarity is bounded between 0 and 1, we fitted GLMMs with a beta distribution using city as a random effect and conducted model selection based on AIC scores using *dredge* as described above, then repeated the same approach for bacteria and archaea separately.

Additionally, we tested for correlations of restoration parameters as well as alpha and beta diversity with different metabolic groups, including carbohydrate metabolism, nitrogen metabolism, phosphorus metabolism, and microbial respiration. The change in sequence counts for each metabolic group was calculated as the log-response ratio compared to unrestored sites. We then fitted GLMMs with a Gaussian distribution using city as a random effect and conducted model selection based on AIC scores as above.

Ecosystem multifunctional potential (EMF) was calculated using the threshold approach [12] with the *getFuncsMaxed* function from the *multifunc* package [11]. This approach integrates multiple ecosystem functions into a single metric by assessing how many functions simultaneously perform at high levels. Using functional relative abundance data, each function is standardized relative to its observed maximum in the dataset, and EMF is calculated as the proportion of functions in a given sample that exceed specified performance thresholds. We used thresholds of 0.4, 0.6, and 0.8, corresponding to functions achieving at least 40%, 60%, or 80% of their maximum observed value. We tested the correlation of LRR_α and beta diversity with EMF for all three thresholds. Since EMF represents count data (i.e., the number of functions exceeding a threshold), we applied GLMs (Generalized Linear Models) with a negative binomial distribution. We then tested the correlation of LRR_α, beta diversity, and EMF with functional insurance (*FI*) using beta regression models with the *betareg* package [86] because *FI* is bounded between 0 and 1. Additionally, we tested the effect of restoration time on alpha and beta diversity as well as on EMF and *FI*, with city as a random effect. For alpha diversity, we used GLMMs with a Gaussian distribution; PERMANOVA was used to assess the relationship with beta diversity; for EMF and *FI* we used GLMMs with a negative binomial distribution and a beta distribution, respectively.

Table 1 List of tested restoration parameters

Variable	Range	Unit
Basal area	0–18,456	cm^2
Canopy openness	1.0–99.8	%
Connectivity	– 2.7–1.3	–
Diameter at breast height	0–113.2	cm
Ground cover—bare ground	0–18	%
Ground cover—herbs	2–100	%
Ground cover—leaf litter	0–97	%
Ground cover—moss	0–4	%
Litter depth	0.6–6.2	cm
Patch size	0.03–148.00	ha
Restoration time	0–63	y
Sapling number	0–250	No. of individuals
Sapling richness	0–19	No. of species
Seedling number	0–13,685	No. of individuals
Seedling richness	0–40	No. of species
Tree evenness	0–0.96	Pielou's J
Tree number	0–174	No. of individuals
Tree richness	0–18	No. of species

Results

Vegetation richness shapes bacterial but not archaeal alpha diversity

The assessment of vegetation parameters in relation to restoration time revealed significant ($p < 0.05$) increases of sapling, seedling, and tree numbers, seedling and tree

richness as well as tree evenness, tree basal area and mossy ground cover over time. The canopy openness significantly decreased ($p < 0.05$), and so did herbaceous ground cover. There were no significant relationships with time detected for any of the tested physicochemical properties (Figure S2).

Sequencing and quality control of the taxonomic dataset produced 538 million high-quality reads and 10,756 unique taxa. 94.4% of those taxa were identified as bacteria, 4.3% as archaea, and 1.3% as eukaryotes. Shannon alpha diversity ranged from 6.86 to 8.08 with a mean of 7.86. No significant relationship with restoration time was detected (Figure S3A, Table S6). The mean LRR_{α} of restored sites approached zero, indicating that alpha diversity was of a similar magnitude regardless of restoration status (restored or unrestored), except for a few sites with lower alpha diversity compared to unrestored sites. A negative LRR_{α} indicates lower alpha diversity compared to unrestored sites, and a positive value indicates higher alpha diversity, respectively. We found a minor but significant decline in microbial alpha diversity with increasing tree richness relative to unrestored sites ($p < 0.05$). Sapling richness and seedling richness were positively associated with overall and bacterial alpha diversity relative to unrestored sites, respectively (Fig. 2, Table S2; $p < 0.05$). Archaeal alpha diversity in restored sites was significantly lower relative to unrestored sites with increasing forest connectivity (Fig. 2, Table S2; $p < 0.05$).

The mean Bray–Curtis dissimilarity of microbial community composition from unrestored sites (i.e., beta diversity) ranged from 0.11 to 0.24 with a mean of 0.15, and no significant relationship with restoration time was detected (Figure S3B, Table S6). While the overall microbial community response was largely driven by the dominant domain of bacteria, bacterial and archaeal communities both showed distinct responses to forest restoration. Specifically, an increase in overall and bacterial beta diversity was positively associated with seedling number ($p > 0.05$), while archaeal beta diversity was associated with canopy openness ($p < 0.05$) and moss cover ($p > 0.05$) (Fig. 2, Table S2). Further, alpha and beta diversity patterns appeared completely decoupled from each other, with no consistent correlations with forest restoration parameters observed between these two facets of diversity.

Functional gene abundances vary dynamically with restoration

Sequencing and quality control of the functional dataset produced over 500 million high-quality reads (6.2 ± 1.4 million per sample) and identified 38,035 different functions (25,774 ± 1011 functions per sample). 14.2% of those were associated with Carbohydrates, 1.1% were

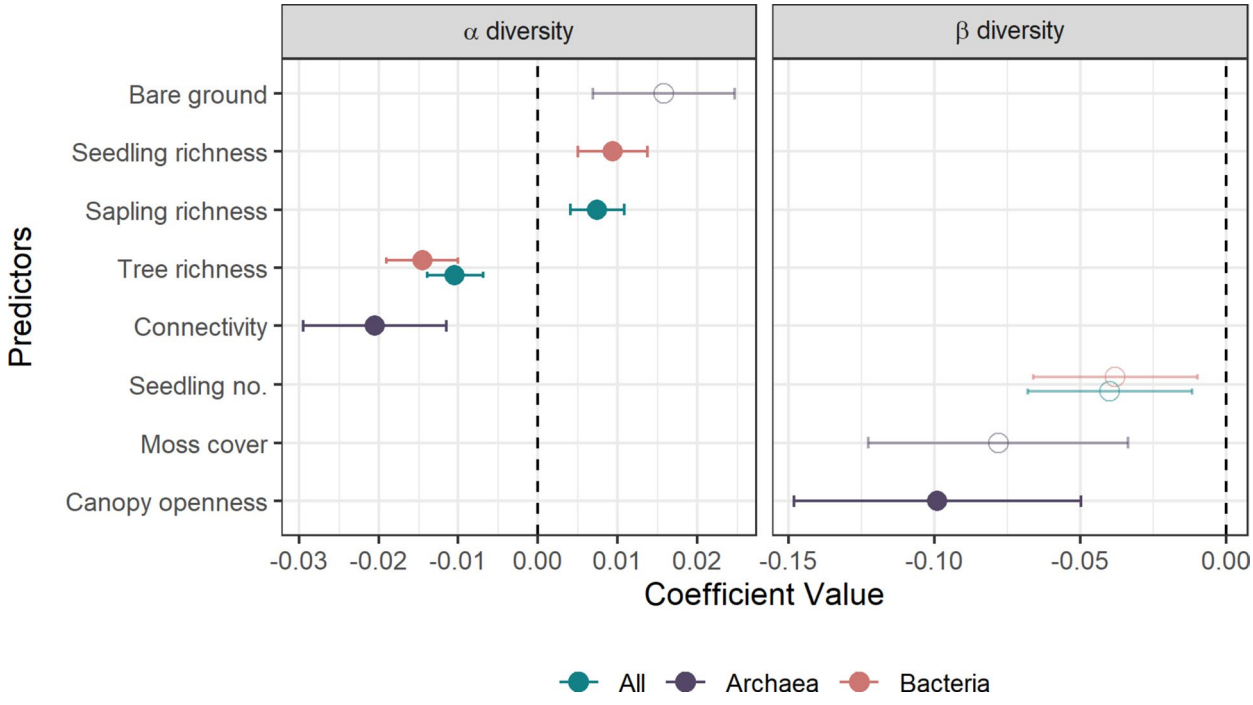

Fig. 2 Relationship of forest restoration parameters and soil microbial diversity. Alpha diversity refers to the log response ratio (LRR) of alpha diversity in restored sites compared to unrestored sites and beta diversity refers to the mean Bray–Curtis dissimilarity to unrestored sites. Effect sizes are depicted as z-scaled coefficients. A positive coefficient represents **a** a higher alpha diversity compared to unrestored sites, and **b** a higher dissimilarity to unrestored sites (i.e., a shift away from unrestored sites), respectively. In total, 18 predictors were tested but only those that were retained in the minimal models are depicted. Closed and open symbols denote statistically significant ($p < 0.05$) and non-significant ($p > 0.05$) relationships, respectively

associated with Nitrogen metabolism, 0.7% with Phosphorus metabolism, and 5.1% with respiration. Since this DNA-based dataset reflects functional capacity underpinned by genes, rather than expressed functional activity, we refer to multifunctional potential rather than multifunctionality when discussing the following results.

We found that numerous forest restoration parameters were significantly associated with changes in relative abundances of all four tested metabolic groups (i.e., potential metabolism of carbohydrates, nitrogen, phosphorus, and respiration), with no consistent trends observed across those groups. Notably, phosphorus metabolism and respiration showed opposite trends in all relevant predictors (Fig. 3, Table S5). We also tested the relationship between restoration parameters and enzymatic activity. The paired models (functional potential based on the proportion of sequences, and enzymatic activity) showed very little overlap. The proportion of sequences and enzymatic activity related to respiration increased with seedling number ($p < 0.05$) but varied in response to other restoration parameters (Figure S4). The remaining metabolic groups showed no congruence in observed relationships between restoration parameters and functional potential or enzymatic activity (Figure S4, Table S5).

Multifunctional potential changes with beta diversity rather than alpha diversity

We calculated ecosystem multifunctional potential (EMF) for three thresholds (0.4, 0.6, and 0.8), with the highest threshold including dominant functions only and the lower thresholds including dominant as well as increasingly rare functions. We did not find significant relationships between LRR_{α} and EMF for any threshold (Fig. 4A, Table S3). In contrast, EMF increased significantly at thresholds 0.6 and 0.8 ($p < 0.05$) as dissimilarity between restored and unrestored sites increased, but not at the lowest threshold of 0.4 (Fig. 4B, Table S3). This suggests that the number of available functions was not affected by microbial diversity when including both rare and dominant functions. Even sites that were compositionally similar (i.e., with low community dissimilarity) to unrestored sites showed high functional diversity. When focussing on common functions exclusively (higher EMF thresholds), beta diversity became a significant predictor of multifunctional potential ($p < 0.05$), but with the addition of rarer functions we observed a plateau effect where further increases in beta diversity no longer translated to higher multifunctional potential (Table S3). This likely meant as assemblages increasingly differed in composition from unrestored sites, the evenness of their dominant functions also increased, such that more functions were retained in EMF counts above the higher threshold

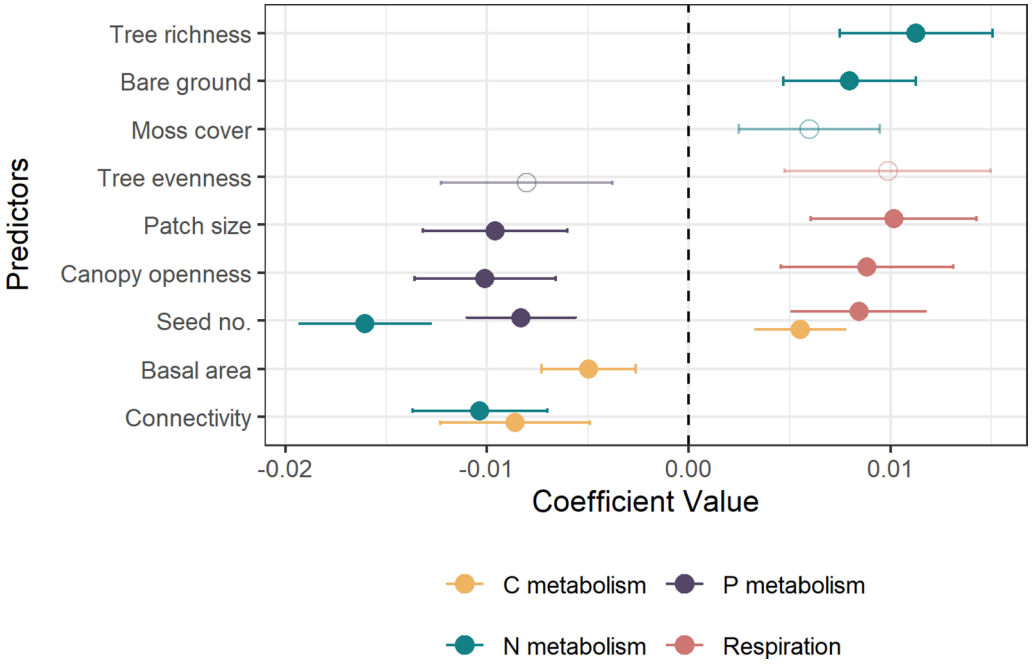

Fig. 3 Relationship between forest restoration parameters and soil microbial metabolic groups. Each metabolic group is calculated as the LRR of its respective normalized sequence counts compared to unrestored sites. Effect sizes are depicted as z-scaled coefficients. A positive coefficient represents an increase in sequence counts compared to unrestored sites. Closed and open symbols denote statistically significant ($p < 0.05$) and non-significant ($p > 0.05$) relationships, respectively

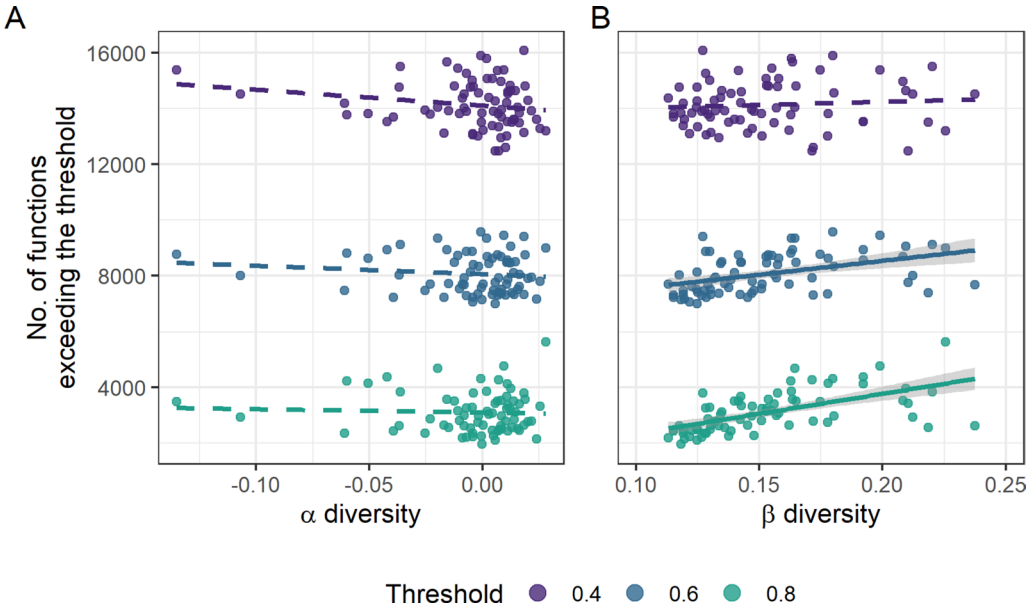

Fig. 4 Relationships between alpha as well as beta diversity and ecosystem multifunctional potential (EMF). The EMF was calculated for three different thresholds, with higher thresholds including only common/dominant functions and lower thresholds including both common and rarer functions. Alpha diversity refers to the LRR of alpha diversity compared to unrestored sites, and beta diversity refers to the mean Bray–Curtis dissimilarity to unrestored sites. Lines with grey confidence intervals indicate significant ($p < 0.05$) relationships while dashed lines indicate non-significant ($p > 0.05$) relationships

values. Restoration time was not a significant predictor of EMF at any threshold (Figure S3C, Table S6; $p > 0.05$).

Functional insurance is driven by community composition rather than alpha diversity

The functional insurance index (*FI*) for the 79 microbial communities ranged from 0.69 to 0.73 with a mean of 0.71. As *FI* is calculated as *S*–*Q* (Simpson alpha diversity—Rao's quadratic entropy) and *S* is high in all sites (mean = 0.99), the differences in *FI* are mostly dependent on *Q*—in other words, the functional dissimilarity of communities.

While we did not observe any significant changes in *FI* with Shannon alpha diversity (Fig. 5A, Table S4), we found a significant positive relationship between beta diversity and *FI* (Fig. 5B, Table S4). As the community dissimilarity to unrestored sites increased, the variation in *FI* extended in a fan shape. The higher the taxonomic dissimilarity, the higher the *FI* and its variation, indicating that community compositional differences rather than species richness cause shifts in functional insurance. We also observed a significant positive correlation between *FI* and EMF at lower thresholds (i.e., thresholds that include rare functions, Fig. 5C, Table S4; $p < 0.05$). Restoration time was not significantly associated with *FI* (Figure S3D, Table S6; $p > 0.05$).

Discussion

We investigated how forest restoration affects soil microbial communities across a sequence of 79 urban planted forests in New Zealand. We characterized microbial communities in restored versus unrestored sites using alpha and beta diversity metrics, evaluated ecosystem multifunctional potential (EMF)—the capacity to provide multiple functions simultaneously –, and developed a functional insurance (*FI*) index which quantifies the coexistence of distinct taxa with functional overlap as an indicator of functional stability and resilience. We hypothesized that both alpha and beta diversity would positively correlate with EMF, but our findings only supported an association of beta diversity with increased EMF. Similarly, we expected both diversity metrics to have positive effects on *FI*, yet only beta diversity showed a positive effect, suggesting that community composition, not species richness, drives soil microbial functional capacity and stability. Further, EMF and *FI* were positively correlated, indicating that high functional diversity and functional overlap are not mutually exclusive in microbial systems. These findings underscore that to promote the recovery of soil ecosystems and restore vital ecosystem functions, a shift in community composition rather than an increase in species richness per se is needed.

With less than 2% of all sequences pertaining to eukaryota, this domain was underrepresented in the metagenomic dataset. Even though eukaryota were retained in the dataset, taxonomic and functional

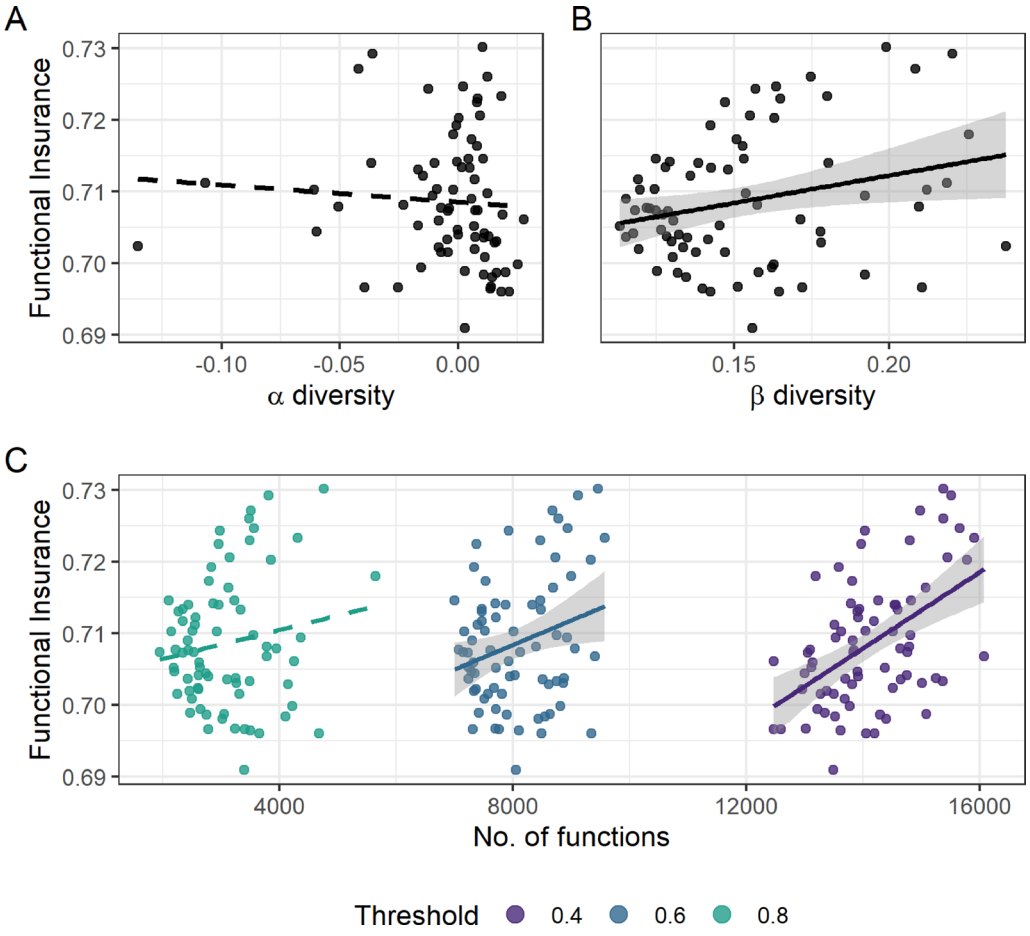

Fig. 5 Relationships between functional insurance and **A** alpha diversity, **B** beta diversity, and **C** multifunctional potential. Alpha diversity refers to the log response ratio of alpha diversity in restored sites compared to unrestored sites, and beta diversity refers to the mean Bray–Curtis dissimilarity to unrestored sites. Lines with grey confidence intervals indicate significant ($p < 0.05$) relationships while dotted lines indicate non-significant ($p > 0.05$) relationships

inferences primarily reflect prokaryotic potential. Changes in relative abundances pertaining to prokaryota could therefore either reflect a true change in prokaryotic abundances or a shift in eukaryotic abundances that were not captured sufficiently. Similarly, an observed loss of functions could either reflect a true loss or a functional compensation via eukaryotic taxa. With that in mind, metagenomic sequencing data should be interpreted with caution.

We found alpha diversity to be consistently high regardless of whether an area had restoration plantings or not, with a slight decrease in restored forests compared to unrestored sites. This aligns with prior studies indicating that soil microbial alpha diversity often remains stable during restoration, while beta diversity shifts during succession [81] and with plant richness [57]. For example, Smenderovac et al. [71] observed no change in microbial alpha diversity but a decline in metabolic activity during deforestation. Furthermore, Zhou et al. [89] noted higher microbial alpha diversity in agricultural monocultures than in diverse natural ecosystems, emphasizing that soil functionality depends on community composition more than alpha diversity. A decline in alpha diversity at certain restoration sites may also reflect microbial adaptation to more mature plant communities, where complex substrates and root exudates favour specialist taxa over the broader pool of generalists [87]. Our results provide further evidence that higher alpha diversity does not necessarily indicate improved ecosystem recovery and functioning [21, 27, 66].

While changes in alpha diversity represent species loss or gain, changes in beta diversity also capture compositional turnover, and environmental patterns of these diversity metrics can be largely independent [30]. Accordingly, alpha and beta diversity were affected by restoration parameters in unique ways. Vegetation richness primarily affected alpha diversity, whereas canopy openness was linked to archaeal beta diversity. Observed effects of vegetation richness may stem from deterministic filtering by root exudates and litter quality, among others [85], while changes in canopy openness affect the forest microclimate and with it soil temperature and

moisture. Likewise, the positive correlation between seedling/sapling richness and alpha diversity coupled with the negative correlation of tree richness and alpha diversity suggests higher alpha diversity in early stages of restoration where mature trees are still scarce. The overall lack of observed associations between restoration parameters and beta diversity suggests a higher dependency on soil properties (e.g., pH) rather than vegetation [32, 76].

Archaea are less studied than bacteria and fungi [78], not least due to limited genetic databases, and we currently lack the knowledge needed to fully understand their contribution and reassembly during ecosystem recovery. Due to their distinct environmental niches [42], they likely respond distinctly to forest restoration. Unlike bacteria, which were mainly associated with vegetation, archaea were correlated with canopy openness and forest connectivity, suggesting strong ties to microclimate, soil nutrient levels, and dispersal limitations. Archaeal diversity and abundance have been shown to vary with soil C:N ratios and pH [5, 84] as well as climate and vegetation cover [2].

While many microbial studies have used taxonomy to infer function [17], this approach can be misleading for multiple reasons. External stressors can induce non-lethal physiological effects and dormancy in microbes, altering function without affecting taxonomy, and structurally distinct communities may perform similar roles due to functional overlap [22]. In this study, we examined the functional potential of soil microbiomes using DNA sequence abundances, reflecting the genetic repertoire of microbial communities rather than currently active gene expression. However, to also estimate functional activity alongside functional potential, we used enzyme assays targeting one indicator enzyme per metabolic group. Since functional capacity and activity diverge from each other depending on biotic and abiotic factors, there was little overlap in results between the two methods. The activity of different metabolic groups might depend more or less strongly on environmental factors. For instance, nitrogen cycling rates depend on external cofactors and redox conditions, causing a decoupling between genetic potential and active gene expression [54, 55]. These external dependencies also explain in part why metabolic groups responded differently to restoration parameters. For example, some functions rely on vegetation type for specific carbon sources or root exudates, while others are mainly regulated by soil pH or moisture [88].

Our first hypothesis predicted a positive correlation of both alpha and beta diversity with ecosystem multifunctional potential (EMF), but we only found support for a relationship with beta diversity. While alpha diversity reflects the species richness of a sample but not the functional overlap of taxa within that sample or with other samples, beta diversity reflects compositional turnover and, by extension, functional changes in response to environmental drivers, promoting niche complementarity and therefore a higher multifunctional potential [47]. Changes in alpha diversity, on the other hand, could result in little variation in EMF depending on the degree of functional overlap between taxa of a given sampling site. Our observation aligns with previous studies identifying beta diversity more than alpha diversity as a driver of EMF in soil microbial communities [65, 74, 89], though results vary across landscape types, methodological approaches, and taxonomic groups [26, 51, 68]. Wan et al. [80] also found that rare bacterial species contribute more strongly to EMF than abundant species, suggesting a greater functional overlap among abundant taxa. In practical terms, management approaches solely aiming for a species richness increase may not ensure a functionally diverse ecosystem.

The positive relationship between beta diversity and EMF weakened with decreasing EMF thresholds. While high thresholds capture common functions only, low thresholds also capture rare functions, resulting in a larger subset. Consequently, EMF appears to plateau at low thresholds (e.g., 0.4), where the functional space is saturated. This suggests that with increasing dissimilarity from unrestored sites, the number of very frequent functions (i.e., high gene frequencies) simultaneously present increases, but the number of all functions simultaneously present, regardless of their frequency, is consistent. That is likely because even unrestored sites, or restoration sites similar to unrestored sites, are highly functionally diverse. The number of functions present was not affected by time since initial restoration.

We further hypothesised that both alpha and beta diversity would positively correlate with functional insurance (*FI*), but like EMF, we only found evidence for a relationship with beta diversity. As soil microbial communities become increasingly dissimilar to unrestored sites, the compositional turnover from an unrestored state towards a more mature forest soil ecosystem causes an increase in *FI* and its variability between sites. This suggests a shift towards more functionally stable, resilient soil communities that are more strongly adapted to local conditions. Notably, we did not see a reduction in the number of distinct functions. Instead, EMF and *FI* were positively correlated, suggesting that functional stability supports multifunctional potential by allowing soil communities to provide multiple processes simultaneously rather than mainly reacting to environmental fluctuations [89]. More generally, our results suggest that to enhance multifunctional potential and functional insurance of soil microbial communities in urban restored forests, an increase in microbial species richness alone is not sufficient. Changes in beta diversity seem to play a vital role, with archaeal compositional turnover being

facilitated by closed forest canopies. We also demonstrate that our proposed functional insurance metric, *FI*, is a viable alternative to currently available methods. Future research can build on this foundation by integrating metatranscriptomics data to directly test whether genetic functional overlap translates into realized ecosystem processes. Refining and expanding on this metric will strengthen its ecological relevance, extend its utility in restoration frameworks, and advance our understanding of how microbial diversity supports ecosystem resilience and multifunctionality.

Our study of soil microbial communities in urban forest restoration sites in New Zealand reveals that beta diversity, rather than alpha diversity, drives ecosystem multifunctional potential (EMF) and functional insurance (*FI*). Compositional dissimilarity, influenced by environmental factors like forest canopy openness, enhances functional stability and multifunctional potential, while an increase in alpha diversity does not necessarily improve soil ecosystem recovery. These findings emphasize that restoration efforts should prioritize shifts in microbial community composition over species richness to promote resilient, multifunctional soil ecosystems, but further research is needed to identify potential levers of bacterial turnover in the context of forest restoration.

Supplementary Information

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Supplementary Material 1.

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Author contributions
SB, KK, CL, KW, MB, and AB contributed to conceptualization and methodology. SB and KK collected the data. SB, KK, and CL curated the data. SB conducted the formal analysis, visualized the results, and wrote the original draft. AB and NE validated the results. AB, KW, MB, and KK contributed to supervision. All authors contributed to the manuscript review and editing process. MB, AB, and KW contributed to funding acquisition and project administration.

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Data availability
Raw sequencing data is available at the AGDR [Project NZ-00045; https://doi.org/10.57748/8s3r-w363] upon request to protect indigenous data sovereignty. Processed data and code are provided here: https://figshare.com/s/f815661e4581e9ce3e10.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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