Diversity Patterns and Determinants of Soil Microorganisms and Nematodes along Elevation Gradients in a Temperate Forest in South Korea

Hae-In Lee[^1], Ke Dong[^4], Min-Ki Lee[^2,5], Yong-Ju Lee[^2,3,5], Hyung-Seok Sim[^2,5], Ari Bima Putra[^3,5] and Chang-Bae Lee[^1,2,3,5,*.]

[^1]: Biodiversity and Ecosystem Functioning Major, Department of Climate Technology Convergence, Kookmin University, Seoul 02707, Republic of Korea
[^2]: Creative Convergence Forest Science Specialist Training Center, Kookmin University, Seoul 02707, Republic of Korea
[^3]: Forest Carbon Graduate School, Kookmin University, Seoul 02707, Republic of Korea
[^4]: Department of Biological Sciences, Kyonggi University, Suwon 16227, Republic of Korea
[^5]: Department of Forest Resources, Kookmin University, Seoul 02707, Republic of Korea
[^*]: Correspondence: kecolee@kookmin.ac.kr; Tel.: +82-2-910-4812

Abstract: The elevational patterns of soil microbial and nematodes diversity (SMND) and the determinants remain controversial. Moreover, how the SMND are modified simultaneously with an elevational gradient has not yet been established. In this study, we investigated the elevational patterns of the SMND and the relative importance among/within tree factors (i.e., tree diversity, identity, and quantity) and environmental factors (i.e., climate and soil) on the SMND. For this purpose, we analyzed datasets from 27 plots across nine elevation bands in the temperate forests of Mt. Gariwang, South Korea. We performed multimodel inference tests and subsequently conducted a variance partitioning to determine the most prominent factors controlling each SMND and compare the relative contribution of the trees and environmental effects. Our results revealed that bacterial and fungal diversity decreased along the elevation gradient. However, nematode diversity did not change significantly, indicating that site-specific environmental conditions may be more influential than the elevation per se. Moreover, this indicates that bacterial diversity was affected by the pH and functional dispersion of the leaf size, and that fungal diversity was governed only by the pH. However, nematode diversity was driven by aboveground biomass, ammonium-nitrogen, and tree size diversity. In summary, the soil microbial diversity was more strongly controlled by the environmental factors, whereas the tree factors were more important for nematodes. Our results show that the elevational patterns and determinants of SMND differed among the taxonomic groups in the common micro-food web. These findings provide new insights into the factors controlling the SMND in a temperate forest and expand the local knowledge of soil biodiversity which is necessary for promoting its mainstreaming. Thus, our results contribute to establishing a basis for more targeted and effective biodiversity conservation and management practices in forest ecosystems.

Keywords: bacteria; elevation; environmental factor; fungi; nematode; soil; tree factor

1. Introduction

Ecologists have been studying elevational diversity patterns (EDPs) of various taxa, such as plants, animals, insects, and soil microorganisms, for over two centuries to establish and develop general notions of biodiversity [1–3]. These studies have provided valuable insights into ecosystem responses to global climate change and species distributions at various spatial scales, as well as advanced the current ecological theories [4–6]. The primary focus of EDP research has largely centered around macro-organisms, particularly plants and animals [1,3,7]. Recent advances in molecular biology and bioinformatics have enabled
the classification of soil microorganisms (i.e., bacteria and fungi) and microfauna (i.e., nematodes) in different environments, thereby improving the empirical understanding of soil microbial and nematode diversity (SMND) responses along elevational gradients [8–11]. Moreover, as the significance of soil biodiversity in regulating carbon and nutrient dynamics and supporting the ecosystem functions and services has been recognized [11–13], the EDP at this micro-scale has been extensively unveiled [8,10,14,15]. Within this framework, researchers have documented the inconsistent patterns of SMND, such as, the monotonic decline, hump-shape, and random patterns [2,6,8,9]. These patterns are likely due to complex and multifaceted effects of plant and environmental factors.

The patterns of SMND are likely regulated directly and indirectly by plant resource inputs and environmental factors across the elevational gradient. The diversification of resources, such as litter or root exudates, facilitated by greater plant diversity, increases SMND through the promotion of mutualisms [11,16]. Recently, soil microorganisms and nematodes have been observed to respond to plant trait identity, specifically the trait effect of the dominant species, rather than plant diversity, by influencing the quality and specific conditions of the resources used and preferred [16,17]. Furthermore, the tree effects can also include the aboveground biomass (AGB) [18], which serves as a proxy for the quantity of the plant-derived resources. An independent effect of the quantity per se and a dependent effect of tree diversity and/or identity contribute to the AGB effect [19,20]. Additional scientific evidence indicates that the SMND along the elevation gradient is governed by both climatic and edaphic factors as well as plant inputs [9,11,16]. Climatic factors, such as temperature and precipitation, have been shown to modulate the SMND through plant inputs and edaphic factors [8,11,21]. The edaphic factors, including pH and organic matter, have been demonstrated to affect the SMND both directly and indirectly through plant inputs and climatic factors [9,16,17,21]. However, the interrelationships among the environmental factors, the plant inputs, and the SMND remain controversial. This could be due to intricate mechanisms underlying the SMND pattern across various ecosystems.

To date, most studies of SMND have generally examined soil bacteria, fungi, and nematodes separately within a general microbial food web or compared diversity patterns among soil microbes and their predators including the nematodes [8,9,11,16,17,21]. This is partly due to the distinctive trophic levels and ecological roles of soil microorganisms (i.e., bacteria and fungi) and nematodes [22], which create different targets for analysis depending on the research interests. Beyond the single taxon level, advances in molecular biology technologies and increasing recognition of the interconnectedness of ecosystems have led to a shift to multi-taxon approach in order to comprehend ecological mechanisms including biological responses to environmental change [7,22]. The observation and comparison of EDPs across a wider range of organisms has also become a major issue for biodiversity conservation and management, as a larger number of taxa are considered for a holistic approach to biodiversity [7]. However, there have been few combined studies on how the diversity of soil bacteria, fungi, and nematodes changes simultaneously in conjunction with tree and environmental factors along an elevational gradient.

In this context, this paper aims to investigate the diversity patterns of soil bacteria, fungi, and nematodes along an elevational gradient ranging from 603 to 1493 m in Mt. Gariwang, a temperate mountain forest in South Korea, and to identify the primary factors controlling individual SMND. Furthermore, we confirm the relative importance of tree and environmental factors in determining SMND across soil biogroups and, in particular, examine the qualitative (i.e., diversity and trait identity) and quantitative (i.e., AGB) contributions of trees to SMND, as well as the contributions of climatic and edaphic factors. To this end, we compare the results of regression models and variance partitioning analyses to test the following four hypotheses: (i) the SMND exhibit distinct elevational patterns depending on taxon group; (ii) SMND are shaped by tree and/or environmental factors; (iii) SMND respond to the qualitative and quantitative effects of tree factors; and finally, (iv) SMND are affected differently by climatic factors and edaphic factors as environmental factors.
2. Materials and Methods

2.1. Study Area and Field Data Collection

The study site is located at Mt. Gariwang in South Korea (Figure 1), which has a cold temperate and inland alpine forest (1561 m a.s.l., 37°27.5′–37°30.5′ N, 128°30.5′–128°33.5′ E) and falls within a temperate deciduous and mixed coniferous forest biome and a mountain ecoregion [19]. The dominant tree species in this site are Acer pseudosieboldianum (Pax) Kom., Acer pictum Thunb. var. mono (Maxim.) Maxim. ex Franch., Lindera obtusiloba Blume, Quercus mongolica Fisch. ex Ledeb., and Fraxinus rhynchophylla Hance, covering more than half of the study site. The mean annual temperature (MAT) and annual precipitation (MAP) of Mt. Gariwang are 9.7 °C and 1761 mm, respectively [23]. The soil is classified as Inceptisols [24] with pH values 4.8–6.6 and an elevation from 603 to 1493 m a.s.l.

![Figure 1. Location of the study area and distribution of the 27 plots (400 m²) across the temperate forests of Mt. Gariwang, South Korea, and the soil sampling points at each plot.](image)

A total of 27 plots of 20 × 20 m² were established in nine elevation bands at 100 m intervals (i.e., three plots per elevation band) for soil sampling and tree inventory. Within the plot, we collected topsoil from 0–10 cm depth because plant roots are concentrated in this layer, which captures soil conditions affecting plant growth and nutrient availability, providing insight into overall plant-soil interactions [8,9]. We selected five soil sampling points, including a central point and four corners (Figure 1). The soil samples were extracted from these five points and subsequently mixed to create more representative and statistically reliable composite samples [9]. Therefore, a single mixed soil sample per plot was employed for soil chemical properties and SMND analysis.

To evaluate the effects of tree diversity, identity, and quantity (i.e., AGB), we identified the trees at the species level, and recorded all tree individuals with standing stems that had a stem diameter at breast height (DBH) of at least 2 cm in each 20 × 20 m² plot. Their DBH and height were measured using a diameter tape and a clinometer, respectively, based on a standardized protocol [19]. The measurements were conducted during the June 2021 growing season.

2.2. Quantification of Soil Microbial Diversity

The bacteria and fungi were extracted using the Maxwell® RSC PureFood GMO and Authentication Kit (Promega, Fitchburg, WI, USA) following the manufacturer’s instructions.

For the bacteria, fusion primers targeting the V3 to V4 regions of the 16S rRNA gene were used with the extracted DNA. For bacterial amplification, 341F fusion primers (5′-AATGATACGGGCGACCAGATCTACACXXXXXXXCTCGTCCGGACGCAGTCAGATGTTATAAGAGACAGCCTACGGGNGGCWGCAG-3′) were employed. The target region primer was identified by underlining and was paired with 805R (5′-CAAGCAGAAGACGGCATACGAGATXXXXXGTCTCTGTCGGCTCGAGATGTGTATAAGAGACAG-3′). The fusion primers were constructed in the following order:
the P5 (P7) binding site, the i5 (i7) index, the Nextera consensus, the sequencing adapter, and the sequence of the target region.

For fungi, DNA was extracted and PCR amplification performed using fusion primers targeting the ITS2 region of 16S ribosomal RNA gene. For fungal amplification, the corresponding primers are ITS3 (5'-AATGATACGGCGACCACGATCTACACXCCCCCCCGTCGGCAGCGTC-AGATGTGTATAAGAGACAGGCATCGATGAAGAACGCAGC-3'; the underlined sequence represents the target region primer) and ITS4 (5'-CAAGCAGAAGACGGCATACGAGATXXXXXXGTCTCGTGGGCTCGG-AGATGTGTATAAGAGACAGTCCTCCGTATATGGATATGC-3'). The fusion primers were constructed in the following order: P5 (P7) graft binding, i5 (i7) index, Nextera consensus, sequencing adapter, and the sequence of the target region.

The following conditions were used for the amplifications: initial denaturation at 95 °C for 3 min, followed by 25 cycles of denaturation at 95 °C for 30 s, primer annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final elongation at 72 °C for 5 min.

The PCR product was validated using 1% agarose gel electrophoresis and visualized using the Gel Doc system (BioRad, Hercules, CA, USA). The amplified products were purified using CleanPCR (CleanNA, Waddinxveen, The Netherlands) and pooled together in equal concentrations. Non-target products or short fragments were removed using CleanPCR (CleanNA). The DNA 7500 Chip was used to assess quality and determine product size using the Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA). Mixed amplicons combined and sequenced at Chunlab, Inc. (Seoul, Republic of Korea) using the MiSeq Sequencing system (Illumina, San Diego, CA, USA), based on the manufacturer’s instructions.

The nematode DNA was extracted from the centrifuged pellet using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) and stored at −80 °C until the PCR stage. The 18S rRNA genes were amplified using the NF1/18Sr2b primer sets (NF1, 5'-GGTGGTGCATGGCCGGCTTAGTT-3', 18Sr2b, 5'-TACAAAGGGCAGGGACGTAAT-3) [8,25]. The DNA library was prepared and sequenced on the Illumina MiSeq platform by Macrogen, Inc. in Seoul, Republic of Korea. The Mothur software version 1.48.0 was used to process sequence data in accordance with the MiSeq SOP. First, the make.contig command was used to merge the forward and backward files. Next, the screen.seqs command was employed to eliminate sequences with ambiguous bases and lengths outside the range of 300 to 450 bp. Finally, the de novo mode of the Chimeric Vsearch algorithm within Mothur was utilized to detect and remove chimeric sequences. Infrequent sequences with less than 10 reads were excluded to avoid inclusion of spurious reads generated by sequencing errors. High quality sequences were categorized into operational taxonomic units (OTUs) using a similarity level of ≥97% and were subsequently classified using the SILVA database (“http://www.arb-silva.de/” [accessed on 27 May 2022]) [9]. A summary statistic for SMND is illustrated in Table S1.

2.3. Quantification of Tree Diversity, Identity, and AGB

The effects of the trees were classified into three types: tree diversity, identity, and AGB. We chose four facets of tree diversity (taxonomic, phylogenetic, functional, and tree size diversity) as proxies for the effect of tree-derived resource range on soil microbes and nematodes, while trait identity was calculated by the community-weighted mean of trait values and served as a proxy for resource quality [16]. We also used AGB as a proxy to assess the effect of resource quantity.

Taxonomic diversity was measured using three metrics: species richness (SR), Shannon–Wiener species diversity (SD), and Pielou’s species evenness (SE). To evaluate phylogenetic diversity, a phylogenetic tree was initially created using the tree species identified in the study plots through a mega-tree method in the package “V.PhyloMaker” available in R studio version 3.6.2 [26]. This method is the largest dated phylogeny for vascular plant species, encompassing 74,533 plant species and all plant families. Next, we quantified four phylogenetic diversity metrics: phylogenetic species richness (PSR), phylogenetic species variability (PSV), phylogenetic species clustering (PSC), and phylogenetic species evenness (PSE) [27]. The phylogenetic tree used for this quantification is depicted in Figure S1.
We used four functional traits, namely maximum height (MH, m), wood density (WD, g cm\(^{-3}\)), and leaf size (LS, cm), which were quantified by the multiplication of the leaf length and width and the seed mass (SM, mg), to determine both functional trait diversity and community weighted mean (CWM) metrics. We sourced the functional traits documented for the tree species under observation from the published literature and open databases [19,28–31]. These key traits were identified as crucial for plant survival and growth as well as for various ecological functions [20,32]. To measure the functional diversity, functional dispersion (FD) was employed as a metric [33]. We calculated five functional dispersion indices: multi-trait functional dispersion utilizing all four traits, and four functional dispersions for each of the four traits (MH, WD, LS, and SM). The community-weighted means (CWM) of each trait were calculated by weighing the mean trait value in a plot by the relative basal area of each species.

To characterize the variation in individual tree size, we calculated the coefficient of variation in diameter at breast height (CV DBH) for each plot, which indicates size differences between/within tree species and stand structural feature [34,35].

To estimate the AGB of individual trees based on field data, we utilized allometric equations (Table S2) that were previously recommended for the tree species through stem DBH [36–42]. The summary statistics of tree diversity, identity, and AGB were provided in Table S1, and detailed information regarding the quantification of tree diversity and identity can be found in the Materials and Methods section of Supplementary Material S1.

2.4. Quantification of the Environmental Factors

To quantify the environmental factors, the chemical properties of each soil sample were analyzed at the National Instrumentation Center for Environmental Management at Seoul National University and the Korea Forestry Promotion Institute. The measured parameters included soil pH, total organic carbon (TOC), ammonium-nitrogen (NH\(_4^+\)-N), nitrate-nitrogen (NO\(_3^−\)-N), total phosphorus (TP), total nitrogen (TN), organic matter (OM), available phosphate (AP), and cation exchange capacity (CEC) following a standardized protocol for soil chemical properties [43,44]. The pH was analyzed using a pH meter with a 1:1 soil-to-water slurry. TN and inorganic nitrogen (NH\(_4^+\)-N and NO\(_3^−\)-N) were, respectively, measured using the Dumas and Kjeldahl methods. TP and AP were determined by the ICP (inductively coupled plasma) and the Lancaster methods. TOC and OM were evaluated using the Walkley-Black and dry combustion methods, respectively. CEC was analyzed by the 1N ammonium acetate leaching method.

The climatic factors including mean annual temperature (MAT) and mean annual precipitation (MAP) were obtained from a digital climatic map created by the National Institute of Forest Science using the ArcGIS 10.5 program [20]. The elevation was measured at a central point of each plot by using a handheld global positioning system. Summary statistics of the environmental factors are presented in Table S1.

2.5. Statistical Analyses

To assess the SMND along an elevational gradient, we utilized either linear or quadratic models based on the lower value of Akaike’s information criterion (AIC). To confirm whether spatial autocorrelation influenced the variables, we performed generalized least squares (GLS) models [45]. We then compared the spatial and nonspatial models representing the GLS models with the geographic coordinates of each plot as the spatial effect and the models without it, respectively. The spatial and nonspatial models’ suitability were assessed through AIC values. No significant effect of spatial correlation was found (Tables S3 and S4). Furthermore, Pearson’s correlational analysis was conducted on all predictors to avoid multicollinearity, which can decrease explanatory power due to strong correlations between the predictors, and highly correlated variables (|r| ≥ 0.7) were removed (Figures S2 and S3). We also selected MAP, which is relatively better at explaining other variables other than MAT, as a result of the climatic factor due to the strong correlation between MAP and MAT. Additionally, a variance inflation factor (VIF) analysis was con-
ducted to evaluate the impact of multicollinearity on the multiple regression models [46]. The variables used in this study had VIF values of <5, signifying that multicollinearity among the variables had a minimal effect on the results. Multimodel inference tests were conducted to identify the most significant variable for tree diversity and identity with environmental factors, as suggested by previous studies [19,32]. The variables considered were taxonomic, phylogenetic, and functional diversity, individual tree size diversity, and CWM values. AGB, the only metric representing resource quantity, was excluded from this selection stage within variable groups pertaining to tree diversity and identity. We performed three sets of multimodel inference tests for the bacterial, fungal, and nematode diversity, and evaluated all potential subsets of regression analyses. Employing a model averaging method, we identified the diversity and identity metrics that exhibited the highest standardized regression coefficient ($\beta$) and importance value from their subset models, respectively (Figure S4a). Similarly, a single variable was selected for the environmental factors (Figure S4b). We conducted additional multimodel inference tests to finally select the most important factor of the SMND from the tree and environmental factors, including MAP and AGB. Redundancy analysis (RDA) was used to quantify and test the effects of tree and environmental factors on variations in soil microbial diversity. We also performed variance partitioning analysis (VPA) to compare the relative importance of tree and environmental effects on SMND, both within and among them. To support the VPA results, we also examined bivariate relationships between SMND and each factor. All variables were standardized and log-transformed to enhance linearity and normality. R version 3.6.2 was used for RDA, multimodel inference, and VPA with the packages rda, MuMIn, and variancePartition [47].

3. Results

Soil bacterial and fungal diversity were significantly modified along with elevational gradient contrary to that for the nematodes (Figure 2). Microbial diversity tended to decrease with increasing elevation. On the other hand, soil nematode diversity had an unclear elevational pattern. For mean OTU richness along the elevational gradient, the bacteria, fungi, and nematodes were, respectively, 2276 (SE ± 296), 625 (SE ± 121), and 580 (SE ± 94) (Figure S1).

Subsequently, RDA was employed to identify the main tree and environmental factors explaining SMND (Figure 3). The tree and environmental factors, respectively, explained 28.81% (RDA 1: 15.36%, RDA 2: 8.64%) and 15.19% (RDA 1: 12.61%, RDA 2: 2.05%) of the SMND variation, indicating that the tree factors explained the SMND variation better than the environmental factors. The RDA also indicated that CWM LS, SR, and CWM WD strongly affected the SMND variation.

We used VPA to quantify the contribution of the tree and environmental factors to each SMND (Figure 4). The tree and environmental factors, respectively, explained 61%, 18%, and 57% of the bacterial, fungal, and nematode diversity variations. The tree effect, respectively, explained 27%, 5%, and 33%, while the environmental effect explained 61%, 22%, and 24% of the variations.

As a result of multimodel inference tests (Figure 5), the bacterial and fungal diversity were mainly driven by soil pH, and each of the important values was 1 and 0.94. On the other hand, nematode diversity was jointly regulated by NH$_4^+$ and AGB, and each of the important values was 0.91 and 0.88, respectively.

The bivariate relationship analysis supported these results (Figure 6), suggesting that bacterial diversity had significantly positive relationships with FDis LS and pH, while fungal diversity had a significantly positive relationship only with the pH. We also noted that nematode diversity had a significantly positive relationship with CV DBH, AGB, and NH$_4^+$.
The bivariate relationship analysis supported these results (Figure 6), suggesting that bacterial diversity had significantly positive relationships with FDis LS and pH, while fungal diversity had a significantly positive relationship only with the pH. We also noted that nematode diversity had a significantly positive relationship with CV DBH, AGB, and NH₄⁺.

Figure 2. The elevation patterns of soil microbial and nematode diversity and the related factors. Fitted regressions were significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Abbreviations: FDis, functional dispersion; SR, species richness; CV DBH, coefficient variation of DBH (diameter at breast height); CWM, community weighted mean; AGB, aboveground biomass; LS, leaf size; WD, wood density; MAP, mean annual precipitation; NH₄⁺, ammonium-nitrogen; $R^2_{adj}$, adjusted R-squared; n.s., nonsignificant.
Figure 3. Redundancy analysis (RDA) of soil microbial diversity representing the loadings of individual plots and the selected variables by each multimodel inference test for (a) tree and (b) environmental factors. Abbreviations: Bc, bacterial OTU richness; Fn, fungal OTU richness; Nm, nematode OTU richness. Abbreviations of variables are presented in Figure 2.

Figure 4. Variance partitioning analyses of tree and environmental effects on soil microbial and nematode diversity. Abbreviations of variables are presented in Figure 2.
Figure 5. Parameter estimates with 95% confidence intervals calculated by using the model averaging approaches of the multimodel inference tests for combined models with selected tree and environmental factors. Red asterisks indicate significant variables, and the significance levels are * $p < 0.05$ and *** $p < 0.001$. Abbreviations of all variables are presented in Figure 2.

Figure 6. Bivariate relationships between each soil microbial and nematode diversity and the relevant predictors. Fitted regressions were significant at $p < 0.05$. Abbreviations of variables are presented in Figure 2.
4. Discussion

4.1. Soil Microbial and Nematode Diversity Patterns along the Elevation Gradient

We found that soil bacterial and fungal diversity decreased along the elevational gradient, but that nematode diversity displayed a random pattern with elevation (Figure 2). This difference between soil microbial and nematode groups did not support our hypothesis.

The decreasing trend in soil microbial diversity with increasing elevation is consistent with that of previous research [48,49]. Theoretically, microbial organisms at higher elevations are more likely to have lower activity and diversity under harsh environmental conditions [50,51]. For instance, previous research has documented that such a decreasing pattern can be attributed to lower soil pH, temperature, moisture, nutrient availability, and so on [14,50–52]. In our study, microbial diversity patterns declined with decreasing pH along the elevation gradient, which may be attributed to the selection of specific microbial taxa that are adapted to acidic conditions or the limitation of certain microbial groups under low pH conditions [14,52,53].

In contrast, there was no significant specific pattern in nematode diversity along the elevational gradient. We confirmed that elevation is not the main determinant of nematode diversity, and that tree and environmental factors that have significant effects on nematode diversity do not also follow any particular patterns along the elevational gradient [17,54]. These results suggest that nematodes respond more sensitively to tree and environmental factors than elevation in the study area, which implies that nematodes have adapted to specific conditions related to the composition and structure of tree communities and environmental factors within their local habitat [10,15,55].

4.2. Trees and Environmental Effects on Soil Microbial and Nematode Diversity

Soil microbial diversity is mainly controlled by the combined effects of trees and environmental factors as well as the individual effect of environmental factors per se, whereas soil nematode diversity is affected by all combined and individual effects (Figure 4). Microbial diversity is strongly driven by the pH, whereas nematode diversity is mainly influenced by AGB, and NH$_4^+$ (Figure 5). Collectively, in terms of the environmental effects, the soil properties better explained the SMND than the climatic factors associated with elevation gradient at the local scale, which conforms to the results of previous research [11,15].

Our results showed that soil pH predominantly contributes to soil bacterial diversity (Figure 5), which is consistent with the general notion previously reported [56,57]. As the elevation increases, the pH tends to decrease due to factors such as reduced nutrient availability, increased leaching, and the accumulation of organic acids derived from decomposing vegetation [14,58]. Particularly, low pH conditions can select specific bacterial taxa that are adapted to acidic environments while inhibiting the growth and metabolic activity of others [52]. Thus, the decline in bacterial diversity can be partially attributed to the shift in the pH along the elevation gradient, which also implies that soil pH was close to neutral at the lower elevations, resulting in higher bacterial diversity [14] in our study area. In addition to the pH factor, the influence of FDis LS on bacterial diversity is an interesting finding (Figure 6), which may be attributed to factors such as increased resource availability or greater niche differentiation among plant species with different leaf sizes. The functional diversity of leaf size affects the litter quality, resource availability, and microhabitat heterogeneity, for example, water and temperature [16,21,59]. The different plant species with varying leaf sizes provide distinct types and amounts of OM inputs to the soil [16,59,60]. This variation in the litter quality and quantity influences the composition and activity of the bacterial community, affecting a change in bacterial diversity along the elevational gradient.

Similar to bacterial diversity, pH is also widely known to affect soil fungal diversity [2,53,58], which is consistent with the observations that fungi respond less sensitively to pH variation compared to bacteria [61]. However, these findings suggest that the tree effects on fungal diversity were negligible (Figures 3 and 4). In previous research, the controllable effects of tree diversity and identity have been reported, such as positive [62,63] and
minor or nonsignificant effects [64,65]. Such inconsistent results are likely to be attributed to the robust context-dependency of the tree effects on fungal diversity [66]. In addition, our result may not determine the significant effect because we synthetically assessed the diversity of the entire fungi community in contrast with previous research, which divided the fungal guilds and separately analyzed the diversity at the phylum level [61,66,67]. Furthermore, most forests were typically dominated by mycorrhizal fungi symbiotic with woody species [16,68], and the tree effects on fungal diversity might be seen if we focused on these fungi. Specifically, we could observe symbiotic woody species with endomycorrhizae, that is, *Acer pseudosieboldianum*, *Acer pictum* var. *mono*, *Lindera obtusiloba*, *Quercus mongolica*, and *Fraxinus rhynchophylla*, dominating in these study areas [69]. According to these observed facts, we suggest that forest soil fungal community-related research is necessary to focus on mycorrhizal fungi, particularly, endomycorrhizal fungi in our case.

Our results thus demonstrated that nematode diversity is driven by the tree factors rather than the environmental factors (Figures 3 and 4). In particular, AGB, NH$_4^+$, and CV DBH mainly regulate the diversity in this study area (Figure 6). Here, the role of AGB may be attributed to its potential effects on the soil microhabitats. AGB is a key determinant of aboveground productivity and belowground nutrient allocation [70], which regulates the quality and quantity of resources (e.g., leaf and root) available to soil nematode. For instance, the AGB could lead to LS or root length [70], thereby regulating the quantity of organic matter and nutrients or modifying soil physical spaces and structure, which may influence the distribution and diversity of the nematodes. We found that CV DBH, that is tree size diversity, drives nematode diversity, indicating that there are various intra- and interspecific niches among nematodes depending on tree sizes [35,71]. This means it may enable the complementary resource use of nematodes through niche partitioning and facilitation [34,71,72]. This result indicates that tree size facets play a key role in driving nematode diversity. Our findings also highlight the significant contribution of soil NH$_4^+$ to nematode diversity. Nitrogen is a key nutrient required for the growth and development of soil nematodes [15]. The higher nematode diversity was shaped in relatively nitrogen-rich soils [73]. In plant–nematode interactions, nematodes mineralize nutrients, and, notably, nitrogen is merely employed for assimilation [74], thus being egested in a mineral and/or easily mineralizable type such as NH$_4^+$-N in excess nutrition [74,75]. Collectively, this result may be partially elucidated through successive circular effects, implying that nematode diversity prompt in high-quality soils and the mineralization of numerous nematodes is simultaneously more active probably due to the abundance of NH$_4^+$-N.

Meanwhile, climate factors had a negligible effect on SMND, probably due to the low habitat heterogeneity at the local scale [15]. Namely, the influence of MAP could be observed on the regional scale rather than on the local scale.

5. Conclusions

We investigated the patterns and determinants of SMND along an elevational gradient and analyzed the relative importance of tree and environmental factors on SMND. These results indicated that soil bacterial and fungal diversity decreased along the elevational gradient, but nematode diversity did not change with the elevation. In contrast to microbial diversity, nematode diversity may be governed by site-specific conditions rather than the elevation per se. The key factors differed among the taxonomic groups. Soil pH played an important role in shaping the soil bacterial and fungal communities, probably by regulating the growth. The functional diversity of leaf size affected bacterial diversity, possibly through resource availability or niche differentiation. Moreover, nematode diversity was driven by AGB, NH$_4^+$-N, and tree size diversity, which likely had cascading effects on the quality and quantity of tree-derived resources. Collectively, microbial diversity responded more sensitively to environmental factors than to tree factors, while nematode diversity responded in the opposite trend. This research provides a context-dependent and taxon-integrated approach to soil biodiversity. It enhances the understanding of soil biodiversity at the local level, which is necessary for promoting its mainstreaming [76]. The
findings provide valuable information on the significant factors affecting soil microbial and nematode diversity across different elevations, thus contributing to establishing a basis for more targeted and effective biodiversity conservation and management practices in forest ecosystems.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/f14122428/s1; Table S1. Summary of the indices of tree diversity, trait identity and aboveground biomass (n = 27) in temperate forests of South Korea; Table S2. Allometric equations for the estimation of the aboveground biomass (AGB) of the woody plant species recorded in this study. Scientific names follow The Plant List “www.theplantlist.org” (accessed on 17 March 2022). AGB equations were obtained from the listed literature; Table S3. Summary of the generalized least squares (GLS) models indicating the effect of environmental factors on soil microbial and nematode diversity to confirm the influence of spatial autocorrelation; Table S4. Summary of the generalized least squares (GLS) models indicating the effect of environmental factors on soil microbial and nematode diversity in the variance partitioning plots to explain the influence of spatial autocorrelation; Table S5. Dataset used in this study; Figure S1. A phylogenetic tree used for the quantification of phylogenetic diversity indices; Figure S2. Pearson’s correlation coefficient among tree species and soil microbial and nematode diversity; Figure S3. Pearson’s correlation coefficient among environmental factors and soil microbial and nematode diversity; Figure S4. Standardized coefficient (β) with 95% confidence intervals and importance values of (a) tree and (b) environmental factors calculated using model averaging approach of a multimodel inference test. References [77–86] are cited in the Supplementary Materials.

**Author Contributions:** H.-I.L.: Formal analysis, Methodology, Writing—original draft, Writing—review, and editing. K.D.: Conceptualization, Investigation. H.-S.S., A.B.P., M.-K.L. and Y.-J.L.: Data curation, Investigation. C.-B.L.: Conceptualization, Methodology, Writing—review and editing, Project administration, Supervision. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Tree and environmental factors used in this study are included in Table S5 and further inquiries can be directed to the corresponding author.

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