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The Effect of the Conversion from Natural Broadleaved Forests into Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) Plantations on Soil Microbial Communities and Nitrogen Functional Genes

Jiahuan Guo [1,2], Huili Feng [1,2], Pierce McNie [2], Weifeng Wang [1], Changhui Peng [2,3], Lei Feng [4,5], Jiejie Sun [1,5], Chang Pan [1] and Yuanchun Yu [1,*]

1 Co-Innovation Center for Sustainable Forestry, College of Biology and the Environment, Nanjing Forestry University, Nanjing 210037, China; guojiahuan@njfu.edu.cn (J.G.); fenghuili@njfu.edu.cn (H.F.); wang.weifeng@njfu.edu.cn (W.W.); Chinasunjiejie@163.com (J.S.); panchang2020@163.com (C.P.)
2 Institute of Environmental Sciences, Department of Biological Sciences, University of Quebec at Montreal, Montreal, QC H3C 3P8, Canada; mcnie.pierce@courrier.uqam.ca (P.M.); peng.changhui@uqam.ca (C.P.)
3 School of Geographic Sciences, Hunan Normal University, Changsha 410081, China
4 College of Forestry, Nanjing Forestry University, Nanjing 210037, China; leifeng@njfu.edu.cn
5 Department of Forest and Conservation Sciences, Faculty of Forestry, University of British Columbia, Vancouver, BC V6T 1Z4, Canada
* Correspondence: ycyu@njfu.edu.cn; Tel.: +86-(25)-8542-8810

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Abstract: The conversion of forests could change soil characteristics and, in turn, impact the microbial community. However, the long-term effect of forest transformation on bacterial and archaeal composition and diversity, especially on nitrogen functional communities, is poorly understood. This study aimed to explore the response of soil bacterial and archaeal communities, as well as nitrogen functional groups, to the conversion from natural broadleaved forests to Chinese fir (*Cunninghamia lanceolate* (Lamb.) Hook.) plantations in subtropical China by 16S rRNA amplicon sequencing. Except for soil bulk density (BD) and ammonium nitrogen (NH_4^+–N) content, other soil properties all decreased with the conversion from natural forests to plantations. Alpha diversity of bacteria and archaea declined with the transformation from natural forests to plantations. The composition of bacteria and archaea was significantly different between natural forests and plantations, which could be mainly attributed to the change in the content of soil organic carbon (SOC), total nitrogen (TN), nitrate nitrogen (NO_3^-–N), and available phosphorus (AP). The conversion of natural forests to plantations decreased the gene copies of ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), and *nifH* (nitrogen fixation function) but increased denitrification gene copies (i.e., *nirS*, *nirK*, and *nosZ*). In summary, our study emphasizes the long-term negative effect of the conversion from natural broadleaved forests into Chinese fir plantations on the diversity and richness of soil microbial communities, thereby deeply impacting the cycling of soil nitrogen.

Keywords: Chinese fir (*Cunninghamia lanceolate* (Lamb.) Hook.); forest types conversion; microbial diversity; nitrogen cycle; 16S rRNA sequencing

1. Introduction

Forest ecosystems play an important role in regulating global biogeochemical cycles, maintaining species diversity, and resisting climate change [1], and are valued worldwide for the services they provide to society [2,3]. In forest ecosystems, tree species and composition have a great influence on soil biodiversity [4]. Soil biodiversity has as a critical role in determining the ecological and evolutionary response of terrestrial ecosystems to current and future environmental changes [5]. However, over the past 300 years, land-use activities, mainly for agricultural expansion and timber extraction, have resulted in a net

loss of about 7 to 11 million km^2 of the forest [6]. Land-use change has been identified as a major cause of soil carbon loss [7], and shows a significant contribution to greenhouse gas emissions [8]. Cultivation of natural land resources results in loss of organic matter, which directly affects the physical, chemical, and biological properties of soil [9]. As one of the most important parameters for the quality and sustainability of the ecosystem, soil organic carbon (SOC) is usually easily impacted by activities carried out for managing agricultural and forest ecosystems [7]. Highly managed forests, such as timber plantations in North America and oil-palm plantations in Southeast Asia, have also replaced many natural forests and currently cover 1.9 million km^2 worldwide [6]. These changes in forest cover will affect various important ecosystem service functions, including biodiversity maintenance, climate regulation, carbon storage, nitrogen cycles, etc. [10].

The process of forest ecosystems is driven by the interaction between aboveground and underground components, and its stability and sustainability depend on the conversion of nutrients [11]. The structure and function of forest ecosystems are deeply affected by the dominant tree species [12], as they determine the features of the soil and vegetation [13]. Different compositions of tree species could influence the physicochemical and biological characteristics of soils, as well as the cycling of nutrients [4,14], resulting in changes in the diversity and richness of resources available to soil microbial communities. The shift of soil properties (such as soil pH, soil texture, soil nutrient availability), soil enzyme activity, topography, and management activities may lead to a change in soil microbial communities [15–17]. Loss of microbial diversity may negatively impact climate regulation, soil fertility, and terrestrial ecosystem productivity [18]. Soil microbial communities serve as a link between soils, plants, and atmospheres by engaging in activities of decomposition and biogeochemical cycles [19,20], and their composition and diversity are usually treated as crucial indicators that reflect the function and stability of the ecosystem [21]. Therefore, it is critical to understand the structure and function of soil microbial communities in reflecting and predicting the response of forest ecosystems to environmental condition changes.

Subtropical forests have experienced extensive forestry land use, in which forests are generally regarded as crops, while there are relatively few long-lived natural forests [10]. In the subtropical region, many countries (e.g., Australia, central Chile, southeastern Brazil, and southern China) have set up economic zones for intensive forestry practices [10]. Especially in the subtropical region of China, many native forests have been transformed into single-species plantations over the past decades [22]. Among them, the Chinese fir (*Cunninghamia lanceolata*) plantation covers approximately 10.96 million ha [23], accounting for 6% of the world's forest plantations [24]. Given the large area of plantations, any small changes in these systems will bring a non-negligible impact on the whole terrestrial ecosystem. Afforestation may alter the structure and diversity of soil microbial communities by affecting the soil physicochemical properties, plant community compositions, litter input, and root exudates [14,25,26]. The long-term impact of the transformation from natural forests to plantations on soil microbial communities and functions remains poorly understood.

This study aims to explore the effect of the forest type transformation on the soil physicochemical characteristics, microbial community structure and diversity, and nitrogen cycling microbial functional groups through sequencing with 16S rRNA to the soil of natural broadleaved forests, mixed-species plantations, and Chinese fir plantations. Specifically, our objectives are to clarify: (i) the change in diversity and richness of bacterial and archaeal communities with the conversion from natural forests to plantations; (ii) the relationship between the change in microbial communities with the shift in soil physicochemical properties undergoing a transition of forest types; and (iii) the impact of the nitrogen functional community by the forest-type transformation.

2. Materials and Methods

2.1. Site Description

This study was conducted at the Changyuan forest farm (27°1′46″–27°1′55″ N, 118°24′41″–118°26′55″ E, 220–231 m a. s. l.) in Jianou city, Fujian Province, China. The site is located on the low mountain and hilly area of the south of the Wuyi Mountains, with relatively steep terrain and a slope ranging from 20° to 35°. It belongs to a subtropical monsoon climate, with a mean annual precipitation of 1650 mm and a mean annual temperature of 20 °C. The soil is developed from biotite granite with a more than 100 cm thick of the soil layer and is classified as red soil according to the Chinese Soil Classification System, which is equivalent to Ultisols in the United States Department of Agriculture (USDA) soil taxonomy [27]. Three forest-type stands were selected for this study, i.e., natural broadleaved forest (N), mixed-species plantation (M), and Chinese fir plantation (P) (Table S1). The natural broadleaved forest was dominated by Schima (*Schima superba* (Theaceae)), Japanese oak (*Lithocarpus glaber* (Thunb.)), Rubber tree (*Hevea brasiliensis* (Kunth. Muell.)), Chinese hemlock (*Tsuga chinenesis* (Franch.) Pritzel ex Diels.), Japanese zelkova (*Zelkova serrata* (Thunb.) Makino), and Chinese chinquapin (*Castanea henryi* (Skam) Rehder & Wilson). The mixed-species plantation and Chinese fir plantation were reconstructed from natural broadleaved forests more than 30 years ago. The mixed-species plantation was dominated by the tree species of *C. lanceolata* and *S. superba*. The main understory vegetation in these forests is listed in the Supplementary Materials.

2.2. Soil Sampling

For each type of forest, three sample plots (0.5 ha) were established and five pits (20 m × 20 m) were randomly selected within each plot. The distance between selected pits was maintained at more than 6 m. Given the transition zone for microbial communities occurring at 10–25 cm in natural forest soil [28] and 10–20 cm in poplar plantation soil [29], we collected soil samples from two depths (D1: 0–20 cm; D2: 20–50 cm) in this study. Ten soil samples from two depths in 5 pits were collected with soil drills (diameter: 2.5 cm), respectively, after removing the surface litter and herbs. To minimize deviations caused by sampling, we uniformly mixed soil samples at the same depth from the same plot and subsequently divided them into three composite samples. A total of 54 composite soil samples were collected, which were then subdivided into two portions. Samples for soil microbial analysis were transferred into sterilized centrifuge tubes (50 mL) and frozen in dry ice, then stored at −80 °C when returning to the laboratory. The rest of the samples were transferred into a container with ice bags and then stored at 4 °C when coming back to the laboratory. At the same time, we used the ring core (volume: 100 cm^3; diameter: 5.05 cm; height: 5 cm) to collect soil samples from the two depths at each pit, wrapped with fresh-keeping film, and transported to the laboratory for the determination of soil bulk density (BD).

2.3. Soil Physical and Chemical Properties Analyses

Prior to measurement, fresh soil samples for physicochemical property analysis were sieved through 2 mm meshes to discard the fine roots and stones. Soil BD was measured gravimetrically. Soil water content (SWC) was evaluated by drying (105 °C for 24 h) and weighing samples. Soil pH was measured using a pH probe (AB15 + Basic, Accumet, San Diego, CA, USA) by mixing soil and water at a 1:2.5 volume ratio. Total nitrogen (TN) and mineral nitrogen (e.g., ammonium nitrogen, NH_4^+–N; nitrate nitrogen, NO_3^-–N) were determined as described by Feng et al. [29]. SOC was measured according to standard methods for soil analyses [30]. Dissolved organic carbon (DOC) was measured with a TOC analyzer (TOC-L, Shimadzu, Tokyo, Japan) by mixing soil and water in a 1:5 volume ratio. Soil microbial biomass carbon (MBC) and nitrogen (MBN) were assessed with fresh soil samples using a chloroform fumigation–extraction method, as described previously [31,32]. The fumigated and nonfumigated soil samples were extracted with 0.5 mol·L^{-1} potassium sulfate for 30 min, and measurements of MBC and MBN were performed using a TOC analyzer (TOC-L, Shimadzu, Tokyo, Japan) [33]. Soil available

phosphorus (AP) was extracted with 0.5 mol·L^{-1} $NaHCO_3$ for 1 h, and then analyzed colorimetrically at a wavelength of 882 nm with a UV-VIS spectrophotometer (UV-2550; Shimadzu, Tokyo, Japan) [34].

2.4. DNA Extraction of Soil Samples

Soil DNA extraction was conducted using the E.Z.N.A® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. The concentration and purity from all extractions were determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The quality of DNA was checked with 1% agarose gel electrophoresis. Extracted DNA was stored at −20 °C for further analysis.

2.5. Quantitative PCR Assay of Functional Genes

The quantitative analysis of functional genes involved in nitrification, denitrification, and nitrogen fixation was conducted with the primer pairs amoA-1F/amoA-2R for the bacterial ammonia monooxygenase alpha subunit (*amoA*) [35], amoAF/amoAR for the archaeal *amoA* [36], cd3Af/R3cdR for cytochrome cd_1-containing nitrite (NO_2^-) reductase (*nirS*) [37], F1aCu/R3Cu for copper-containing NO_2^- reductase (*nirK*) [38], nosZ-F/nosZ-1622R for nitrous oxide (N_2O) reductase (*norZ*) [39], and nifHF/nifHR for nitrogenase reductase (*nifH*) [40], respectively. Detailed information on primers and reaction conditions used for amplification is presented in Table S2.

2.6. 16S rRNA Gene Amplicon Sequencing and Bioinformatics Analyses

Polymerase chain reaction (PCR) amplification was conducted by a thermocycler PCR system (GeneAmp 9700, ABI, Waltham, MA, USA) with the primer pair 515FmodF/806RmodR, the general primers for amplifying the V4 hypervariable region of the 16S rRNA gene in bacteria and archaea [29]. PCR amplification was performed in triplicate in a 20 μL reaction system: 4 μL of 5 × FastPfu buffer (Bioneer, Daejeon, Korea), 2 μL of 2.5 mM deoxyribonucleoside triphosphates (dNTPs; Larova, Teltow, Germany), 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase (TransGen AP221-02, Beijing, China), 0.2 μL of bovine serum albumin (BSA; New England Biolabs (NEB), Ipswich, MA, USA), and 10 ng of template DNA. PCR was carried out using the following program: denaturation at 95 °C for 3 min, 27 cycles at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 45 s, with a final extension at 72 °C for 10 min. PCR products were extracted using agarose gel (2%), purified with an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and further quantified by QuantiFluorTM-ST (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Purified amplicons were pooled in equimolar, and paired-end (2 × 300 bp) sequenced using an Illumina MiSeq platform (Illumina, San Diego, CA, USA) following the standard protocols by Majorbio Bio-pharm Technology Co. Ltd. (Majorbio, Shanghai, China). Raw fastq files were demultiplexed and quality-filtered with Trimmomatic, as well as merged using Fast Length Adjustment of Short reads (FLASH, https://ccb.jhu.edu/software/FLASH/, last accessed on 20 December 2021) with the following criteria: (i) reads were truncated when receiving a mean quality score less than 20 over a 50 bp sliding window; (ii) sequences that overlap length >10 bp were merged in terms of the overlap with mismatch <2 bp; (iii) primers were exactly matched allowing 2 nucleotide mismatching, and reads containing ambiguous bases were removed. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoffs using UPARSE with a "greedy" algorithm that performs chimera filtering and OTUs clustering simultaneously [41]. The taxonomy of the qualified sequence was analyzed with the Ribosomal Database Project (RDP) Classifier algorithm (http://rdp.cme.msu.edu/, last accessed on 22 December 2021) against Silva (SSU128) 16S rRNA database [42] using a confidence threshold of 70% [43]. Sequence data are available through the National Center for Biotechnology Information (NCBI) Sequence Read Archive database (SRA) under the identification code PRJNA633237. After that, we performed functional annotation with the Functional Annotation of the Prokaryotic Taxa

(FAPROTAX) database to explore the relevant potential microbial functions related to the nitrogen cycle [44].

2.7. Statistical Analyses

Shapiro–Wilk's and Levene's tests were employed to verify the normal distribution of residuals and the homogeneity of variance, respectively. One-way analysis of variance (ANOVA), followed by Tukey's test was used to analyze the statistical differences of soil physicochemical properties resulting from the different land-use types based on three forest stands. Alpha (α) diversity of microbial communities was evaluated with Chao 1 (richness) and Shannon indexes (diversity) determined by the genera-level taxonomic matrices using PAST software v.3.2 [45]. Hierarchical clustering was carried out using USEARCH [41] to clarify the similarity and dissimilarity of soil microbial communities between different forest types. Venn graphs were used to identify the number of shared and unique OTUs at different soil depths among the three forest types based on a similarity level of 97% OTUs. Pearson's correlation analysis and redundancy analysis (RDA) (CANOCO 5.0, http://www.canoco.com, last accessed on 23 December 2021) were applied to evaluate the relationship between soil factors and microbial communities. Two-way permutation multivariate analysis of variance (PERMANOVA) [46] was used to test variations of microbial community abundance at different soil depths from diverse forest-type habitats. Functional annotation of the nitrogen functional community in soil samples was carried out by the FAPROTAX database [44]. Pearson's rank correlation coefficients were calculated to explore the relationship between microbial groups and soil physicochemical properties using the "NetworkX" package (http://networkx.github.io/, last accessed on 25 December 2021) in Python, and the calculations were corrected using the Benjamini–Hochberg FDR [47].

3. Results

3.1. Variation of Soil Physicochemical Properties with Conversion of Forest Types

Compared with the natural forest, the content of SWC, SOC, DOC, MBC, and MBN in the surface soil of plantations (including mixed-species plantations and pure Chinese fir plantations) after conversion decreased significantly ($P < 0.05$), but the NH_4^+–N content increased significantly ($P < 0.05$; Table 1). In addition, soil pH and the content of NO_3^-–N, TN, and AP decreased significantly ($P < 0.05$), regardless of the surface or subsurface soil after the natural forest converted to the plantation, while soil BD showed the opposite ($P < 0.05$). Overall, the variation of soil properties in surface soil (D1) was larger than that of subsurface soil (D2). Among them, the content of NO_3^-–N, TN, SOC, and AP had the greatest variation, regardless of the surface or subsurface soil.

Table 1. Soil physicochemical properties of different soil depths from diverse forest types (mean ± standard deviation).

	N_D1	N_D2	M_D1	M_D2	P_D1	P_D2	CV_{D1} (%)	CV_{D2} (%)
BD	1.06 ± 0.06c	1.22 ± 0.05b	1.20 ± 0.04b	1.34 ± 0.04a	1.25 ± 0.05ab	1.37 ± 0.05a	7.88	6.07
SWC	176.5 ± 19.3a	169.2 ± 11.1ab	140.1 ± 6.9c	147.1 ± 4.3bc	144.9 ± 10.3bc	148.8 ± 2.1abc	13.38	7.90
pH	4.53 ± 0.04b	4.72 ± 0.05a	4.33 ± 0.06c	4.51 ± 0.03b	3.78 ± 0.06e	4.08 ± 0.08d	8.03	6.40
NO_3^-–N	9.30 ± 0.95a	5.36 ± 0.95b	1.98 ± 0.12c	0.95 ± 0.08c	1.35 ± 0.24c	0.91 ± 0.06c	91.71	94.04
NH_4^+–N	6.76 ± 0.22b	3.47 ± 0.40c	8.80 ± 0.23a	4.19 ± 0.16c	8.89 ± 0.86a	4.56 ± 0.22c	14.21	13.19
TN	1.82 ± 0.04a	0.96 ± 0.06bc	1.02 ± 0.11b	0.64 ± 0.04de	0.81 ± 0.06cd	0.48 ± 0.07e	38.44	31.73
SOC	68.60 ± 3.73a	20.59 ± 2.04c	32.81 ± 5.85b	15.58 ± 1.72c	22.65 ± 2.40bc	12.63 ± 1.31c	51.72	22.80
DOC	34.60 ± 2.49a	18.67 ± 0.47cd	26.84 ± 1.13b	15.23 ± 0.78de	23.07 ± 2.35bc	12.74 ± 1.72e	19.16	17.74
MBC	173.22 ± 8.80a	84.21 ± 6.31c	133.62 ± 9.49b	73.33 ± 4.01c	120.41 ± 16.75b	66.63 ± 4.66c	18.29	10.89
MBN	34.07 ± 4.00a	16.76 ± 0.64cd	24.82 ± 2.14b	13.89 ± 0.54d	20.24 ± 2.13bc	11.78 ± 1.87d	25.02	16.93
AP	2.58 ± 0.15a	1.25 ± 0.06bc	1.56 ± 0.14b	0.81 ± 0.13d	1.05 ± 0.07cd	0.76 ± 0.12d	39.50	26.96

N: Natural broadleaved forest, M: Mixed-species plantation, P: Chinese fir (*Cunninghamia lanceolate* (Lamb.) Hook.) plantation; D1: 0–20 cm, D2: 20–50 cm. The lowercase letters refer the significant difference ($P < 0.05$) based on the Tukey test. BD: bulk density ($g \cdot cm^{-3}$); SWC: soil water content ($g \cdot kg^{-1}$); NO_3^-–N: nitrate nitrogen ($mg \cdot kg^{-1}$); NH_4^+–N: ammonium nitrogen ($mg \cdot kg^{-1}$); TN: total nitrogen ($g \cdot kg^{-1}$); SOC: soil organic carbon ($g \cdot kg^{-1}$); DOC: dissolved organic carbon ($mg \cdot kg^{-1}$); MBC: microbial biomass carbon ($mg \cdot kg^{-1}$); MBN: microbial biomass nitrogen ($mg \cdot kg^{-1}$); AP: available phosphorus ($mg \cdot kg^{-1}$). *CV*: coefficient of variation (standard deviation/mean*100).

3.2. Alpha Diversity Patterns and Microbial Community Abundance

The richness and diversity of soil bacteria and archaea decreased significantly ($P < 0.05$) with soil depths (Figure 1). At the same soil layer, almost all the richness and diversity of bacteria and archaea declined significantly when the natural forest converted to the plantations ($P < 0.05$). Moreover, compared with the mixed-species plantation, the soil bacterial diversity of pure Chinese fir plantation decreased significantly ($P < 0.05$).

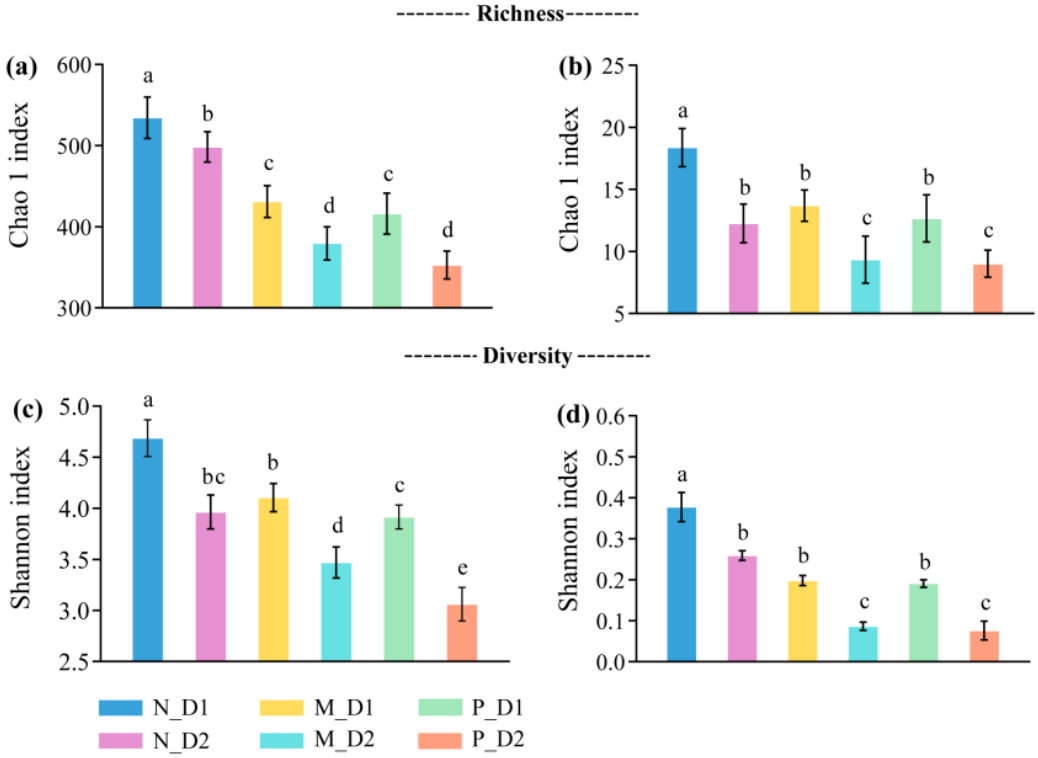

Figure 1. Richness and diversity of the bacteria, (**a**,**c**), and archaea, (**b**,**d**), at the genera-level. N: Natural broadleaved forest, M: Mixed-species plantation, P: Chinese fir (*Cunninghamia lanceolate* (Lamb.) Hook.) plantation; D1: 0–20 cm, D2: 20–50 cm. Error bars represent the standard deviation (SD, $n = 9$). The lowercase letters denote the significant difference ($P < 0.05$) based on the Tukey test.

Overall, thirty-two known bacterial phyla and five known archaea phyla were observed in all samples (Figure 2a,b). As main bacterial phyla, an abundance of Proteobacteria (31.38%), Acidobacteria (19.09%), Chloroflexi (14.80%), Actinobacteria (12.62%), Planctomycetes (7.69%), Verrucomicrobia (4.91%), and Gemmatimonadetes (1.23%) together made up more than 91% of all bacteria. The abundance of unclassified archaea accounted for 96.28%, while the known phyla, such as Thaumarchaeota, Crenarchaeota, Euryarchaeota, Diapherotrites, and Nanoarchaeaeota together only accounted for 3.72% of the archaeal community. Actinobacteria, Chloroflexi, GAL15, WPS-2, Nitrospirae, Patescibacteria, Crenarchaeota, and Thaumarchaeota were more abundant in the natural forest soil (Figure S1). However, Acidobacteria, Calditrichaeota, Cyanobacteria, Dependentiae, Elusimicrobia, Entotheonellaeota, Firmicutes, Latescibacteria, Rokubacteria, and Diapherotrites showed a higher abundance in the plantation soil.

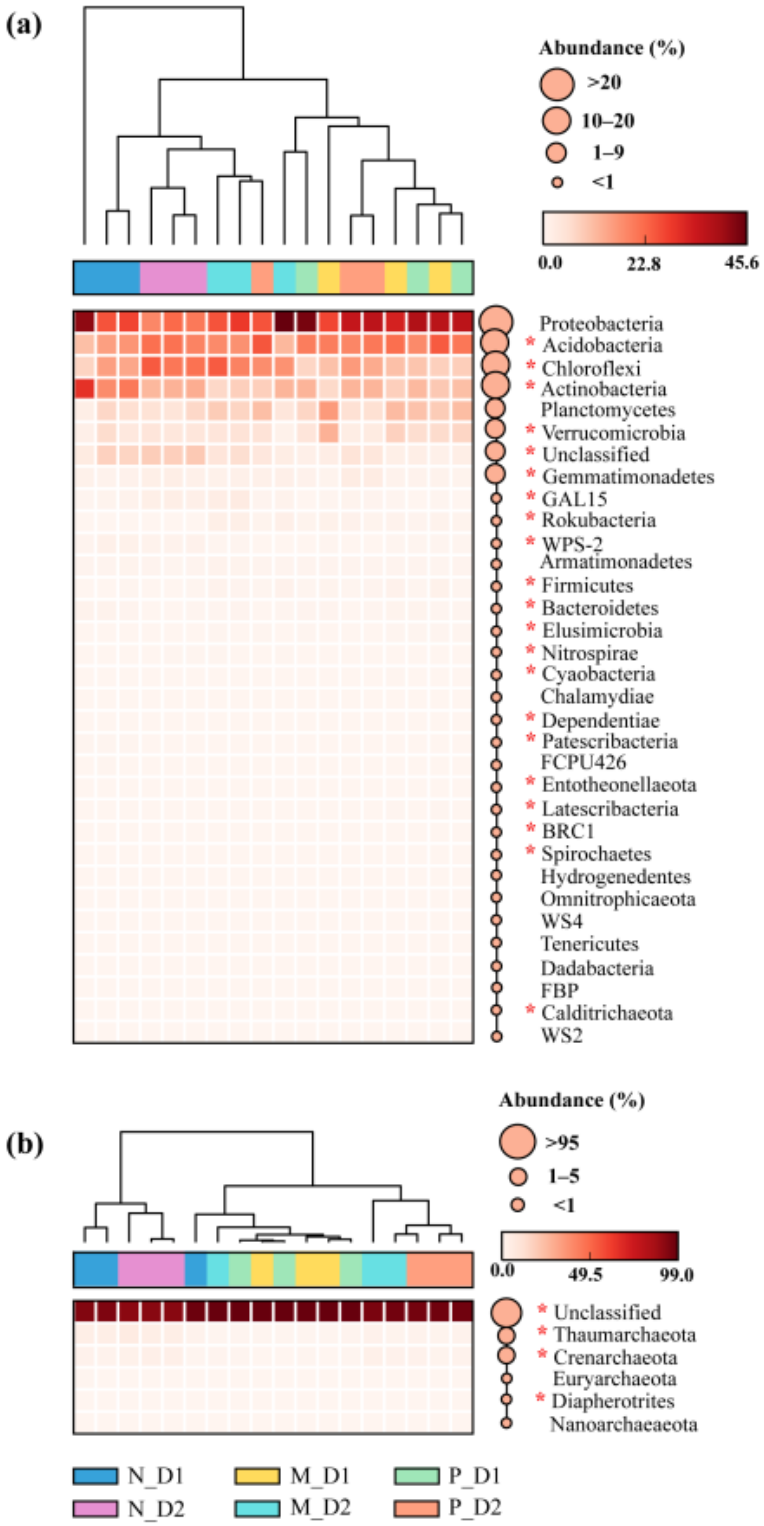

Figure 2. Variations in the relative abundance of bacteria (**a**) and archaea (**b**) at the phylum level. N: Natural broadleaved forest, M: Mixed-species plantation, P: Chinese fir (*Cunninghamia lanceolata*) plantation; D1: 0–20 cm, D2: 20–50 cm. The asterisk (*) represents a significant difference by post hoc Tukey–Kramer test ($P < 0.05$ after Benjamini–Hochberg correction).

3.3. Microbial Community Composition and RDA Analysis

There were 1499 shared OTUs in all samples, accounting for 26.19% of all observed OTUs (5723; Figure 3). There were 89, 48, 135, 252, 95, and 59 unique OTUs in the surface

(D1) and subsurface (D2) soil of the natural forest, mixed-species plantation, and Chinese fir plantation, respectively. The number of OTUs in the mixed-species plantation was higher than that of natural forests and pure Chinese fir plantations.

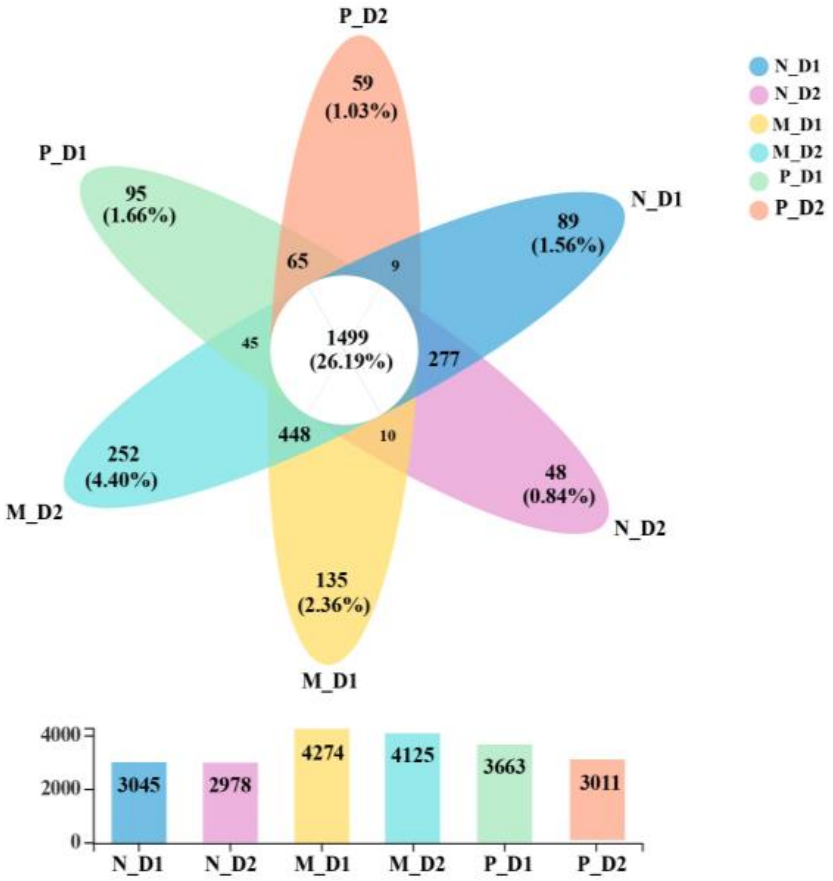

Figure 3. Venn diagram of the number of shared and unique operational taxonomic units (OTUs). Overlapping parts represent the shared OTUs, non-overlapping parts represent the specific OTUs of the groups, and the number indicates the corresponding number of OTUs. The corresponding numbers in the bar are the number of OTUs to different groups. N: Natural broadleaved forest, M: Mixed-species plantation, P: Chinese fir (*Cunninghamia lanceolata*) plantation; D1: 0–20 cm, D2: 20–50 cm.

Cluster analysis showed that communities of soil bacteria and archaea in the natural broadleaved forest were clearly distinguished with those in the mixed-species plantation and the Chinese fir plantation (Figure 4a,c). Communities of bacteria and archaea at the same depth in all types of forests clustered together as one group, respectively. RDA analysis showed that the first and second axes altogether explained more than 60% of the total variation in the soil bacterial community (Figure 4b) and 52% in the soil archaeal community (Figure 4d), respectively. SOC (R^2 = 0.74, P = 0.001), NO_3^-–N (R^2 = 0.71, P = 0.001), TN (R^2 = 0.33, P = 0.041), and AP (R^2 = 0.45, P = 0.014) showed a significant correlation with the bacterial community structure. Moreover, NO_3^-–N (R^2 = 0.60, P = 0.002), TN (R^2 = 0.36, P = 0.028), and AP (R^2 = 0.44, P = 0.01) were significantly correlated with the archaeal community structure.

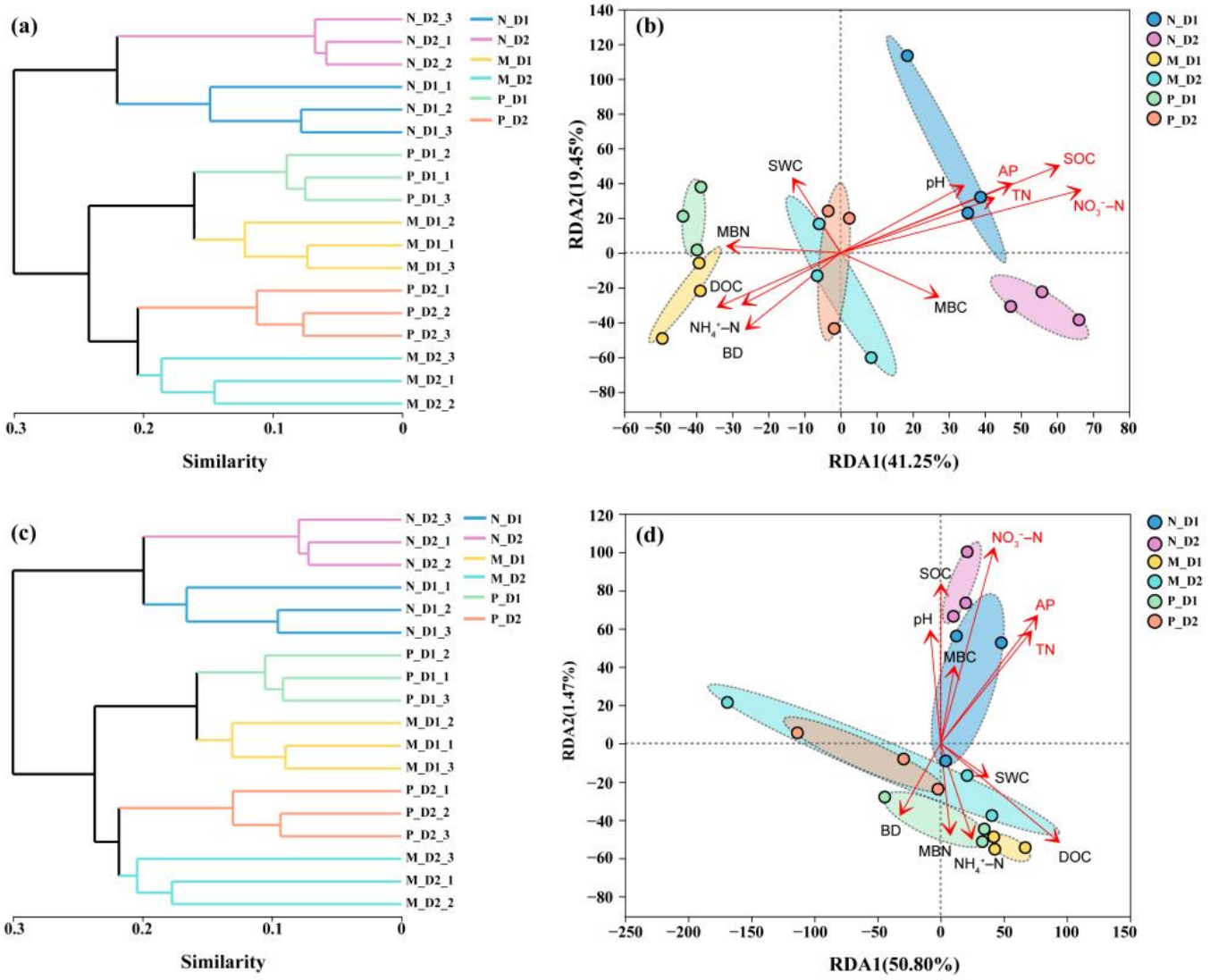

Figure 4. Clustering analysis for soil bacterial (**a**) and archaeal (**c**) communities based on the Bray–Curtis dissimilarity at the OTU level. Redundancy analysis (RDA) of the relationship between bacterial (**b**) and archaeal (**d**) communities and soil physicochemical properties. Arrow's length indicates correlated degree of soil physicochemical properties and bacterial and archaeal communities. The significant correlations are marked with red font ($P < 0.05$). Color ellipse indicates significant clusters based on permutation analysis ($P < 0.05$). N: Natural broadleaved forest, M: Mixed-species plantation, P: Chinese fir (*Cunninghamia lanceolata*) plantation; D1: 0–20 cm, D2: 20–50 cm. BD: bulk density; SWC: soil water content; NO_3^-–N: nitrate nitrogen; NH_4^+–N: ammonium nitrogen; TN: total nitrogen; SOC: soil organic carbon; DOC: dissolved organic carbon; MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; AP: available phosphorus.

Potential interaction between soil physicochemical properties and microbes was evaluated by Pearson's rank correlation (Figure S2). Rokubacteria (about seven properties) and Patescibacteria (about six properties) presented the highest number of interactions with soil factors. In addition, SOC (correlated with 16 phyla), NO_3^-–N (12 phyla), AP (11 phyla), and TN (9 phyla) were the soil factors most closely related to microbial groups.

3.4. Nitrogen Functional Genes Abundance

The number of gene copies of all nitrogen functional communities in the surface soil was significantly higher than that of the subsurface soil at the same forest types (Figure 5). All notable changes of gene copies caused by the conversion of forest types mainly occurred

at the surface soil. The gene copies of ammonia-oxidizing archaea (AOA; F = 113.25, $P < 0.001$) and ammonia-oxidizing bacteria (AOB; F = 163.26, $P < 0.001$) in the mixed-species plantation and Chinese fir plantation were significantly lower than that of the natural broadleaved forest (Figure 5a,b). On the contrary, the number of denitrification gene copies, such as *nirS* (F = 351.26, $P < 0.001$), *nirK* (F = 114.09, $P < 0.001$), and *nosZ* (F = 749.47, $P < 0.001$), significantly increased after the conversion from natural forests to plantations (Figure 5c–e). Moreover, the gene copies of *nifH* (nitrogen fixation function) in natural forests was significantly higher than that of other forests (Figure 5f).

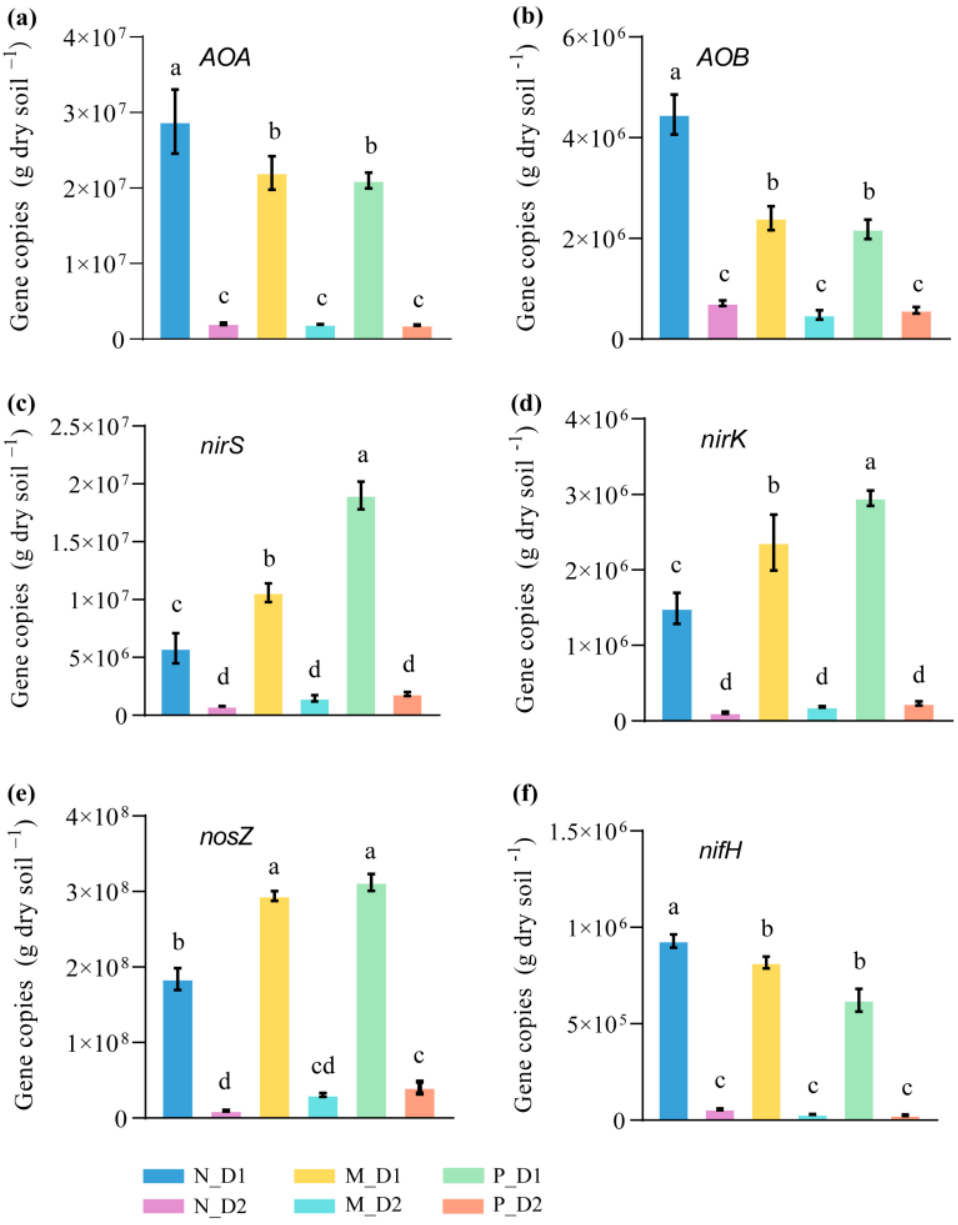

Figure 5. Abundance of functional genes of *amoA* of archaea (ammonia-oxidizing archaea, AOA) (**a**), *amoA* of bacteria (ammonia-oxidizing bacteria, AOB) (**b**), *nirS* (**c**), *nirK* (**d**), *nosZ* (**e**), and *nifH* (**f**) from the soil samples. N: Natural broadleaved forest, M: Mixed-species plantation, P: Chinese fir (*Cunninghamia lanceolata*) plantation; D1: 0–20 cm, D2: 20–50 cm. Error bars represent the standard deviation (SD, n = 9). Lowercase letters indicate significant differences between groups based on the Tukey test ($P < 0.05$).

3.5. Correlations of Soil Properties and Nitrogen Functional Genes

The abundance of AOA, AOB, and *nifH* showed a significantly negative correlation with BD, but a positive correlation with other soil properties (i.e., NH_4^+–N, TN, SOC, DOC, MBC, MBN, and AP) (Figure 6). The abundance of *nirS* was negatively correlated with SWC and pH but positively correlated with NH_4^+–N. The abundance of *nirK* was negatively correlated with SWC and pH but positively correlated with NH_4^+–N, DOC, and MBC. Moreover, the abundance of *nosZ* was negatively correlated with pH but positively correlated with NH_4^+–N, DOC, MBC, and MBN.

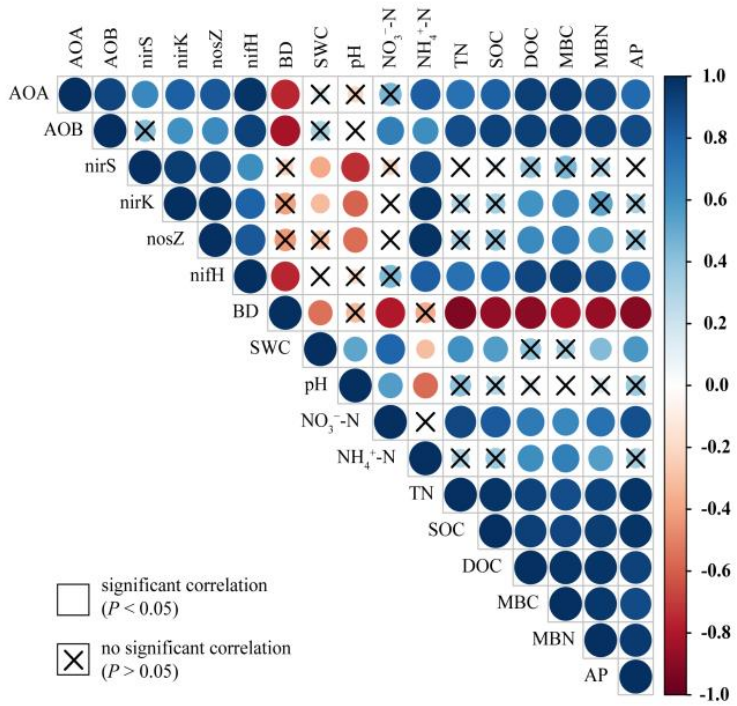

Figure 6. Correlations between soil physicochemical properties and the abundance of functional communities related to the nitrogen cycle. The variables include soil bulk density (BD), soil water content (SWC), nitrate nitrogen (NO_3^-–N), ammonium nitrogen (NH_4^+–N), total nitrogen (TN), soil organic carbon (SOC), dissolved organic carbon (DOC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and available phosphorus (AP). The functional communities include ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), denitrifiers (*nirS*, *nirK*, and *nosZ*), and nitrogen fixers (*nifH*). The colors show the Pearson's correlation coefficients and indicate a positive or negative correlation. The size of circles signifies the strength of the correlation. Non-significant correlations ($P > 0.05$) are marked.

3.6. Changes of Nitrogen Functional Communities with Forest Conversion

The relative abundance of nitrogen functional bacteria, including nitrate reduction, nitrification, aerobic nitrite oxidation, nitrogen respiration, nitrate respiration, and aerobic ammonia oxidation bacteria changed significantly with the conversion of forest types ($P < 0.05$; Figure 7). Specifically, nitrification and aerobic nitrite oxidation bacteria were more abundant in natural broadleaved forests and mixed-species plantations ($P < 0.05$; Figure S3). Aerobic ammonia oxidation bacteria were more abundant in pure Chinese fir plantations and surface soil of mixed-species plantations. However, nitrogen respiration and nitrate respiration bacteria were more abundant in the surface soil of pure Chinese fir plantations ($P < 0.05$). Finally, nitrate reduction bacteria only showed a significant difference between two soil depths in natural forests, i.e., the relative abundance of nitrate reduction bacteria in surface soil was higher than that of subsurface soil ($P < 0.05$).

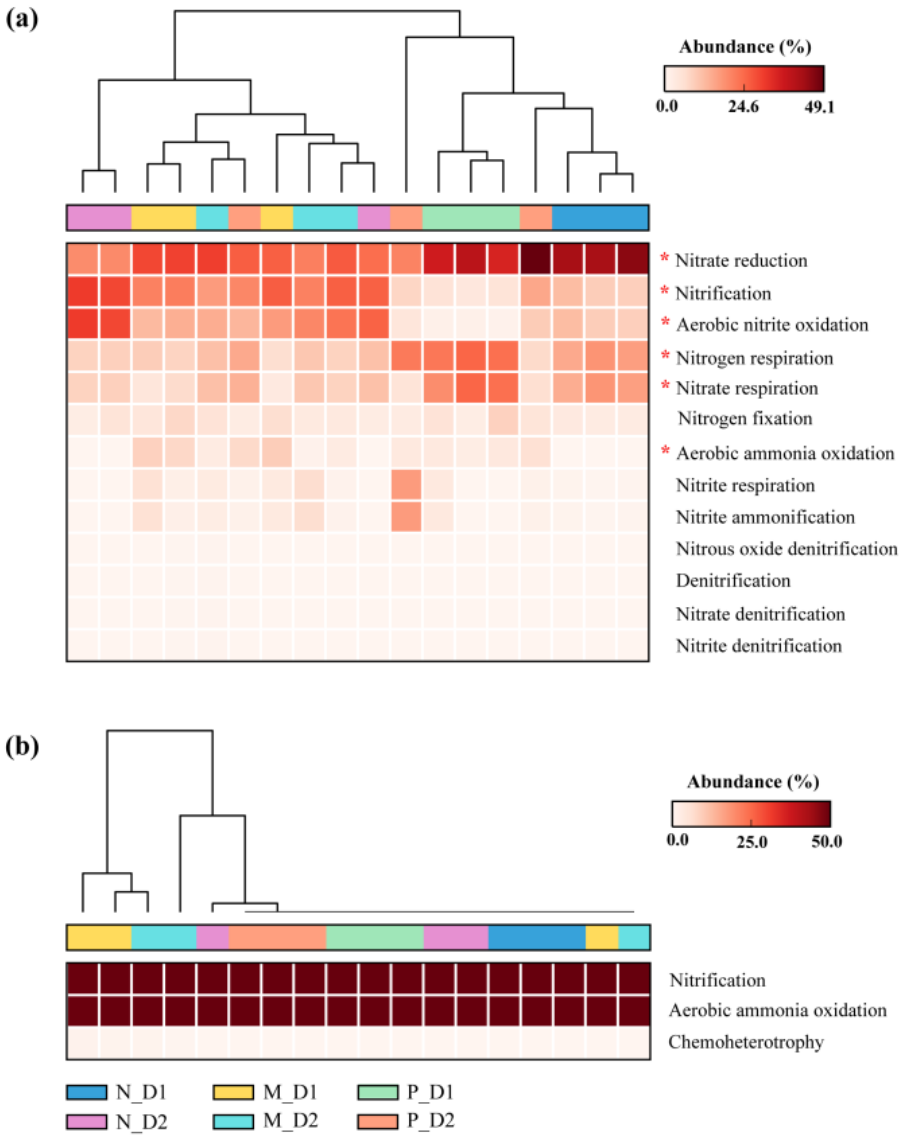

Figure 7. Variations in the relative abundance of the nitrogen functional bacteria (**a**) and archaea (**b**) identified by Functional Annotation of the Prokaryotic Taxa (FAPROTAX) database. N: Natural broadleaved forest, M: Mixed-species plantation, P: Chinese fir (*Cunninghamia lanceolata*) plantation; D1: 0–20 cm, D2: 20–50 cm. The asterisk (*) represents a significant difference determined by a post hoc Tukey–Kramer test ($P < 0.05$ after Benjamini–Hochberg correction).

4. Discussion

The structure of the soil microbial community is heavily determined by the overground populations of the ecosystem [48]. Changes in plant species have a significant influence on the composition of soil microbial communities [49]. Some previous studies reported an increase in the richness and diversity of microorganisms caused by the transition from natural forests to plantations [50,51]; however, we observed a converse result in this study, i.e., the conversion of natural forests to plantations led to a significant decline in the diversity and richness of bacteria and archaea ($P < 0.05$; Figure 1). This verifies our first objective regarding the changes in diversity and richness of bacteria and archaea communities with the forest conversion.

The decrease in bacteria may be due to the reduction of soil pH and soil organic carbon content [52,53]. A higher H^+ concentration under a low pH status could destroy the permeability and stability of bacterial cell membranes [51], and then limit the microbial activity and bacterial growth [54]. The decrease in soil organic carbon input, after conver-

sion from natural broadleaved forests to coniferous plantations [55], caused a decline of bacterial biomass [56]. Our observation of changes in soil physicochemical properties with a transformation of forest types confirmed these views. We found that soil pH, SOC, DOC, and MBC decreased significantly with the conversion from natural forests to plantations (Table 1). Soil pH in natural broadleaved forests is generally considered to be higher than in coniferous forests [57]. The decline of pH may be driven by the accumulation of nutrient cations in plant biomass and the increasing aluminum saturation of the soil exchange complex [58].

The reduction of archaea may be attributed to the change in ammonia concentrations with forest type conversion. On the one hand, Thaumarchaeota ammonia oxidizers tend to play a significant role in the nitrogen cycle when the ammonia concentration is too poor to sustain bacterial ammonia oxidizers in acidic forest soils [59]. On the other hand, archaea tend to be much less common and less active when the ammonia concentration is higher in acidic soil [60]. The soil of this study is a typical acid soil, and the pH ranged from 3.78 to 4.72 in the three forest types (Table 1). The concentration of NH_4^+–N increased significantly with the transformation from natural forest to plantation (Table 1). These findings reversely reflect the variation of archaea with the conversion of forest types.

Furthermore, microbial diversity and richness decreased significantly with soil depth ($P < 0.05$; Figure 1), which is consistent with previous studies [29,61]. The survival rate of some surface microorganisms in the underground soil declined due to the strong ecological filtration function of the vertical space [28]. Microbial communities in natural forests could be clearly distinguished from those in plantations (Figure 4a,c), which means the conversion of forest types has a significant effect on the composition of soil microbial communities. In underground vertical space, the microbial communities in the surface and subsurface soil presented an obvious stratification. This was supported by other studies. The transition zone for microbial communities usually occurred at a depth of 10–25 cm in natural forest soils [28]. In a poplar plantation, the transition zone for bacterial and archaeal communities was reported at a depth of 10–20 cm [29].

The 16S rRNA genes from soil bacteria belong to at least 32 phyla-level groups, and members of different phyla have various contributions to different soil bacterial communities [62]. The seven main bacterial phyla from this study were also observed in other ecosystems (Figure 2), such as forests [29,52], grasslands [63], and agricultural systems [64]. These findings suggested that soils from different ecosystems consist mainly of similar dominant bacterial communities, but the relative abundance of the taxa varied from those systems [65]. The abundance of archaea in soils is generally low [62]. In this study, the relative abundance of five known archaeal phyla was only 3.72%.

The relative abundance of bacteria and archaea in natural forests and plantations was significantly different ($P < 0.05$; Figure 2 and Figure S1). The main bacterial phylum—Actinobacteria—was more abundant in natural forests. As a copiotrophic taxon, the relative abundance of Actinobacteria was proven to positively correlate with soil pH [52], and increased with soil organic matter [65], total carbon, total nitrogen, and available nitrogen [66,67]. In this study, the higher content of SOC, DOC, MBC, TN, NO_3^-–N, MBN, and pH in natural forests than plantations supported these results. Interestingly, we found Chloroflexi was more abundant in subsurface soil of natural broadleaved forests and mixed-species plantations (Figure S1). This is consistent with the result of Frey et al. [68] who reported Chloroflexi to be more abundant in subsoils (110–155 cm) than in topsoil (0–25 cm). As an oligotrophic taxon, the presence of Chloroflexi in subsoils may indicate an adaptation of its members to the relatively low nutrient environment characteristics [68]. In addition, the relative abundance of Chloroflexi was reported to be positively correlated with soil pH [66]. In our study, a lower content of NH_4^+–N, SOC, DOC, MBC, MBN, and AP but a higher pH in subsurface soil (20–50 cm) of natural broadleaved forests and mixed-species plantations than surface soil (0–20 cm) from all forest types could be used for explaining these results. Moreover, the member of Chloroflexi includes autotrophic, heterotrophic, and mixotrophic taxa [69]. Therefore, different physiological strategies might

be responsible for them coping with the conversion of forest types. Similarly, the main archaeal phylum—Crenarchaeota—has the same distribution pattern as Chloroflexi. We presumed that they adopted a similar oligotrophic survival strategy. The abundance of Crenarchaeota has been reported to be positively correlated with pH [70]. Crenarchaeal nitrifiers may have the potential ability to dominate the nitrifying community, although those Crenarchaeota seem to be oligotrophic nitrifiers, being relatively more abundant in soils with low nitrogen levels [71]. In addition, the natural forest has a more abundant Thaumarchaeota, which is consistent with the study of Pedrinho et al. [72]. These two archaeal phyla usually act as an important role in N cycling, and most of the members are involved in the process of ammonia oxidation in acidic soils [73]. The existence of an abundance of these microorganisms may help in the mineralization process and contribute to N availability [74].

On the contrary, some communities, such as the main bacterial phyla Acidobacteria have a higher abundance in plantations. This may be attributed to a relatively low soil pH in plantations. Previous studies have demonstrated that lower pH is beneficial to Acidobacteria, its distribution being negatively correlated with soil pH [75]. The Acidobacteria exhibit a slow metabolic rate under low nutrient conditions and are versatile heterotrophs [76] and oligotrophs [77]. However, other rare bacterial and archaeal communities with low relative abundance could not be ignored, and they have their preferred environments. Some of them may have unique traits or affect other species, thus affecting ecosystem function [78]. The strategies of these rare taxa have close interactions with abundant species, which may be attributed to the complex relationship with soil properties, especially the availability of carbon, nitrogen, and phosphorus (Figure S2). However, their ecological functions remain to be further explored. All these results demonstrate our second objective concerning the close relationship between microbial communities and soil physicochemical properties undergoing a transition of forest types.

There were more than 70% shared OTUs within three forest types (Figure 3). This indicates that the presence of core microbial communities could resist or adapt to the interference due to changes from forest tree species. We also found that the total and unique number of OTUs in the mixed-species plantation were all higher than that of natural broadleaved forests and pure Chinese fir plantations (Figure 3). As a result, mixed forests might provide a more suitable habitat and higher survival possibilities for microorganisms, compared with the natural forest and pure forest. Soil bacterial structure in the natural broadleaved forest is different from that in the coniferous forest [65]. Changes in tree species affect soil properties, and act on microbial communities [79]. It has been previously reported that soil nutrient availability is correlated with the microbial community [80]. Among numerous soil properties, we found that SOC, TN, NO_3^-–N, and AP have a significant influence on bacterial and archaeal community structures (Figure 4b,d), which may be attributed to the larger variation of these properties (Table 1). The content of these soil nutrients decreased significantly after the conversion of forests (Table 1), which will bring a negative influence on microbial growth. This result is consistent with previous studies on the conversion from natural forests to plantations [51,81].

The nitrogen cycle is one of the most essential ecological processes in which microorganisms participate [82]. Sequences linked to Proteobacteria and Acidobacteria were the most abundant in all three types of forests (Figure 2). Members of these phyla have wide metabolic and physiological diversity, which is very important for the soil carbon and nitrogen cycles [83,84]. It was reported that these two phyla are the predominant groups in acidic soil, and they are adversely affected by forest conversion and fertilizer use [85,86]. Previous studies have demonstrated that members of Alphaproteobacteria (especially the order Caulobacterales and Rhodospirillales) are responsible for the decomposition and recycling of organic compounds and play an important role in the denitrification process [87,88]. In this study, we observed that the conversion from natural forests to Chinese fir plantations, firstly, did not cause a reduction in the abundance of Proteobacteria and Acidobacteria, and secondly, brought a large increase in the abundance of Alphaproteobacteria. The growth

of these taxa is projected to increase the denitrification potential of the soil nitrogen cycle in Chinese fir plantations. The increased number of denitrification gene copies, such as *nirS*, *nirK*, and *nosZ* (Figure 5c–e), and the more abundant nitrogen respiration and nitrate respiration bacteria (Figure S3) after the conversion from natural forests into plantations supported this hypothesis. Therefore, the conversion from natural broadleaved forests into Chinese fir plantations may increase the potential risk of N_2O emissions in this area.

AOA and AOB could oxidize ammonium/ammonia into other forms of inorganic and reactive nitrogen in the N cycle [89]. In this study, the gene copies of AOA and AOB decreased after the conversion from natural forests into plantations (Figure 5). Interestingly, aerobic ammonia oxidation bacteria from the functional annotation of nitrogen functional communities showed a higher relative abundance in plantations (Figure S3). This seems to be contradictory. However, the low relative abundance of nitrification and aerobic nitrite oxidation bacteria in plantations may partly explain this contradiction (Figure S3). As a result, it may be the reduction of nitrification and aerobic nitrite oxidation bacteria rather than the decline of AOA and AOB abundances after conversion from natural forests to Chinese fir plantations, to be responsible for the weakening of soil nitrification potentials. The fade of nitrification may limit the production of N_2O, since AOA and AOB are responsible for the production of N_2O in the aerobic soil. In addition, members of Actinobacteria, such as the order Acidimicrobiales, Micromonosporales, Rubrobacterales, and Solirubrobacterales, can perform certain soil functions, including nitrogen fixation and organic material decomposition [90]. In this study, nitrogen fixation function (*nifH*) gene copies decreased with the conversion from natural forests to plantations (Figure 5f), which indicated a negative influence on soil nitrogen fixation processes. These results confirmed our third objective concerning the deep influence of forest-type transformations on nitrogen-functional communities.

5. Conclusions

The transformation of natural broadleaved forests into Chinese fir plantations could directly impact soil physicochemical properties and, in turn, affect the abundance, composition, and structure of soil bacterial and archaeal communities. Soil pH and nutrient quantity (content) and quality (availability), especially C, N, and P, significantly acted on the change of microbial communities. Among numerous soil properties, we observed that SOC, NO_3^-–N, TN, and AP were essential soil factors closely linked to the structure and composition of bacterial and archaeal communities. The rare taxa have a close relationship with soil physicochemical properties and abundant taxa, and thus their ecological functions need to be further determined. The functional group of denitrifies increased, while the functional group of nitrifiers and nitrogen fixation decreased after transforming from natural broadleaved forests into Chinese fir plantations. This suggests an enhancement of denitrification but a weakening of nitrification and nitrogen fixation in Chinese fir plantations. The enhancement of denitrification may increase the risk of N_2O emission, but the fade of nitrification may limit the production of N_2O. However, how the dynamic of N_2O is affected by forest-type conversions needs to be further explored by combining with the measurement of N_2O flux. In all, these findings can provide valuable insights into the role of forest conversions on the soil nitrogen cycle, in order to cope with global climate change.

Supplementary Materials: The following supporting information can be downloaded at: http://www.mdpi.com/article/10.3390/f13020158/s1, Figure S1: Comparison of the relative abundance of bacteria (a) and archaea (b) at the phyla level in different soil depths (D1: 0–20 cm, D2: 20–50 cm) from natural broadleaved forests (N), mixed-species plantations (M), and Chinese fir (*Cunninghamia lanceolata*) plantations (P); Figure S2: Pearson's rank correlation coefficients and statistical significance between phyla of bacteria and archaea to soil physiochemical properties. Left figures (a,c) show the difference at 0.05 level, and right figures (b,d) show the difference at 0.01 level; Figure S3: Comparison of the relative abundance of nitrogen functional bacterial communities in different soil depth (D1: 0–20 cm, D2: 20–50 cm) from natural broadleaved forests (N), mixed-species plantations (M), and Chinese fir (*Cunninghamia lanceolata*) plantations (P); Table S1: Basic status of natural broadleaved

forest (N), mixed-species forest (M), and Chinese fir (*Cunninghamia lanceolata*) plantation (P) (mean ± Standard deviation); Table S2: Primers and PCR amplification conditions used in qPCR analysis of nitrogen cycle functional genes.

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Conflicts of Interest: The authors declare no conflict of interest.

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